

# **Community-Acquired Pneumonia in Indonesia**

Helmia Farida

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# **Community-Acquired Pneumonia in Indonesia**

## **Thuis-opgelopen Pneumonie in Indonesië**

**Thesis**

to obtain the degree of Doctor from  
the Erasmus University Rotterdam  
by command of the  
Rector Magnificus

Prof.dr. H.A.P. Pols  
and in accordance with the decision of the Doctorate Board

The public defense shall be held on  
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by

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*Untuk yang tercinta dan tersayang  
Bapak dan almh Ibu  
Suamiku Anang Achmadi dan anak-anakku Faris, Hana, Azky*

The studies in this thesis were performed on behalf of the Community-Acquired Pneumonia in Indonesia (CAPSIN) study group :

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# Chapter 1

## Introduction and outline of the thesis





## INTRODUCTION

Community-acquired pneumonia (CAP) remains a significant cause of morbidity, mortality, and an economic burden [1]. In low - to middle - income countries, it is one of the most important causes of death and loss of years of life [2,3]. Of the 15 million deaths reported for the year 2004 in Southeast Asian countries (as defined by WHO), 1.5 million (10 %) were due to respiratory infections, mostly lower respiratory tract infections, including 0.5 million deaths due to tuberculosis. Likewise, these infections were responsible for over 40/440 million disability adjusted life years lost in this area of the world [3].

Knowledge about the epidemiology, etiology and pathogenesis of CAP is essential for patient management in order to achieve the optimum patients' outcomes [4]. As antibiotic therapy is one of the cornerstones in the management of patients with infections including CAP, the quality of care, in particular the quality of the use of antibiotics, is also crucial in the management of CAP cases [5]. Although host factors such as age and gender, and severity of disease on presentation, are the most important determinants of outcome of CAP, patients with CAP also need to be identified and empirically treated at an early stage of their illness since delay in the timely instigation of appropriate empirical antimicrobial therapy for CAP has been independently associated with poor patient outcomes [6].

CAP is often caused by pathogens that have colonized the upper airways prior to causing lower respiratory disease. This has been proven for *Streptococcus pneumoniae*. Therefore, the commensal microflora of the upper respiratory tract, especially the nasopharyngeal space needs to be studied in detail.

Indonesia is the world's fourth most populous country, with a population of close to 250 million inhabitants. In Indonesia, CAP ranks the first as the cause of mortality in children [7] and the sixth in adults [8]. However, there are still very few data available with regard to CAP in Indonesia. Data are lacking on the etiology, antimicrobial management, and supportive patient care, leading to difficulties in controlling this disease, and, consequently, to a potentially enhanced burden from this disease. Also, the normal nasopharyngeal microflora of Indonesian inhabitants has received little attention so far. These are in sharp contrast to the many experiences with this disease and with nasopharyngeal carriage reported from western countries, and even from some countries neighbouring Indonesia [9-11]. Consequently, the management of CAP patients in Indonesia has been, so far, based on experience gained elsewhere and presented in international guidelines [8] without evaluation of their appropriateness for the Indonesian setting. There is also, to our knowledge, no proper evaluation of the outcomes of patients with CAP in this country.

Therefore, the general aim of the studies presented in this thesis was to address issues necessary to improve the outcomes for patients with CAP requiring hospital admission in Indonesia. More specifically we aimed to better delineate the spectrum of etiologies of CAP in adult patients in Indonesia, to evaluate the level of appropriateness of antibiotic management of patients with CAP, and of other supporting measures which can be implemented in the Indonesian setting. Nasopharyngeal carriage of pneumococci and of Gram-negative enteric bacilli among healthy children and adults was also addressed, since these bacterial species were the commonest agents of bacterial CAP in this area of the world.

## **OUTLINE OF THE THESIS**

The main questions to be answered in this thesis were addressed in two major studies, i.e. the CAPSIN (Community-Acquired Pneumonia Study in Indonesia) project and the Nasopharyngeal Colonization Study (NASCO), and included:

1. What are the pathogens causing CAP in Indonesia? What is the frequency of antimicrobial resistance of clinical bacterial isolates of CAP in Indonesia? Is there an association between the etiology and the underlying diseases, severity of disease, and mortality?
2. What is the frequency of nasopharyngeal carriage of the most common respiratory pathogens in healthy people in Indonesia? What is the frequency of antimicrobial resistance of strains carried by healthy individuals? What are the risk factors for throat carriage of the major respiratory pathogens?
3. What are the characteristic differences between respiratory pathogens isolated from colonized but otherwise healthy individuals versus those obtained from infected individuals?
4. How should the management of patients with CAP (in particular, with regard to antibiotic therapies) be evaluated? What are the results of such an evaluation in an Indonesian hospital?

To address these questions, the content of the thesis is presented in five sections.

### **Part I: Background**

In **chapter 2**, a review of the published literature on CAP in Southeast Asia is presented. This review focuses on the most recent data.

### **Part II: Etiology of community-acquired pneumonia in Indonesia**

In **chapter 3**, the etiology of CAP in a large cohort of adult patients hospitalized with a clinical diagnosis of CAP in two acute care hospitals in the city of Semarang, the capital of

Central Java province, Indonesia, is presented. The etiological diagnosis was determined based on a comprehensive range of diagnostic laboratory tests and interpretation of these test results by integrative panel discussions involving clinicians, radiologists, and clinical microbiologists. One of the implicated pathogenic species, *Leptospira spp.*, is discussed separately in **chapter 4**.

### **Part III: Characteristic differences between isolates from colonized and infected individuals**

Pneumonia, especially bacterial pneumonia, is usually preceded by colonization of the upper respiratory tract by potential lower respiratory pathogens. A population-based survey of nasopharyngeal carriage of *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and other Gram-negative bacilli among healthy individuals is described in **chapter 5** and **chapter 6**. The risk factors for carriage of these potential pathogens are analyzed. The heterogeneity among isolates from the major bacterial Gram-negative respiratory pathogen, i.e. *K. pneumoniae*, isolated from healthy and infected individuals is presented in **chapter 7**.

### **Part IV: Management of community-acquired pneumonia in Indonesia**

**Chapter 8** describes the development of a set of quality indicators for the assessment of the medical management of CAP patients in the Indonesian setting.

### **Part V: Discussion**

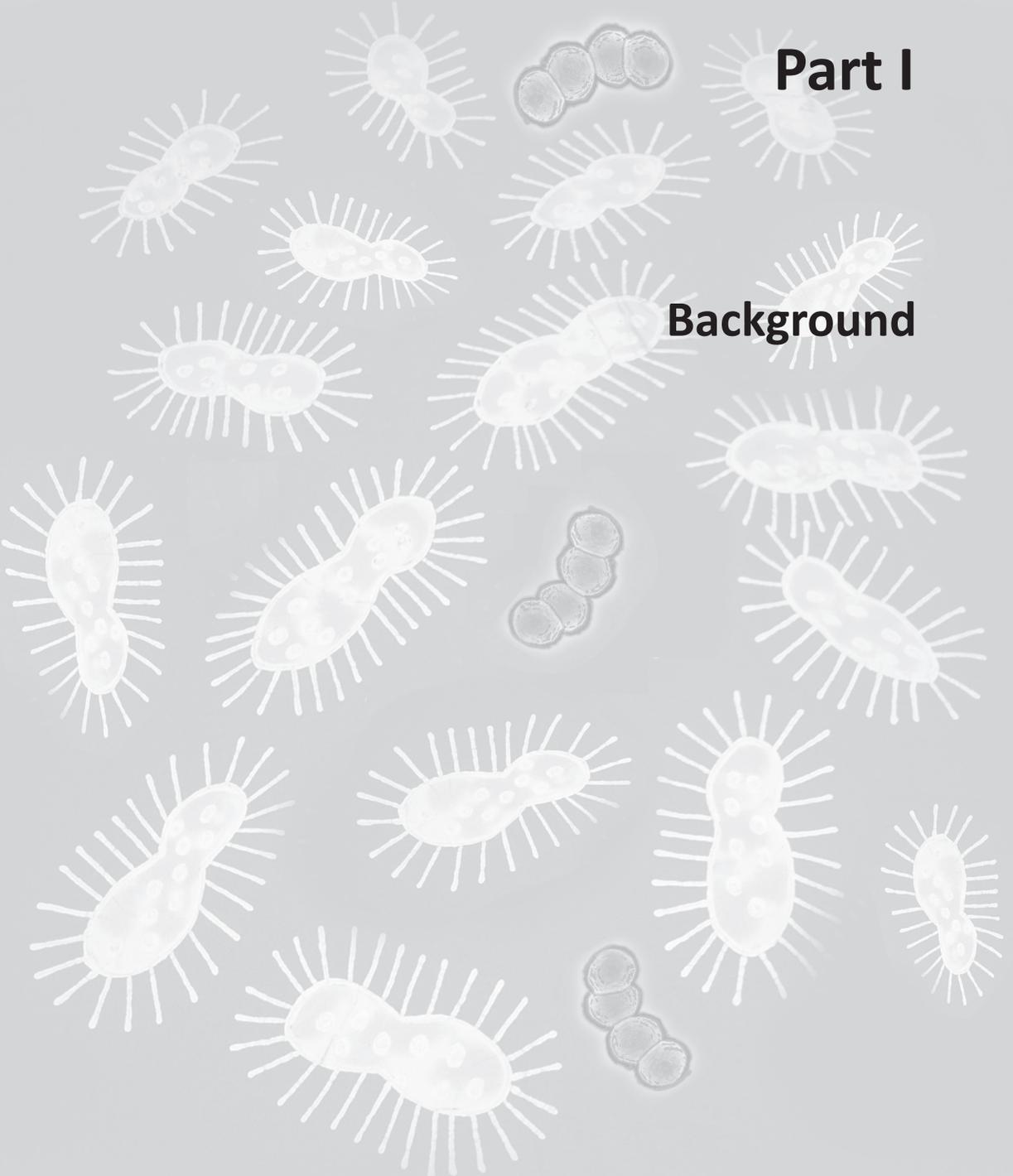
The main findings of the studies in this thesis are discussed and suggestions for further research regarding the management of CAP in Indonesia are given in **chapter 9** and summarized in **chapter 10**.

## REFERENCES

1. Olsen SJ, Laosiritaworn Y, Siasiriwattana S, Chunsuttiwat S, Dowell SF (2006) The incidence of pneumonia in rural Thailand. *Intl J Infect Dis* 10: 439-445.
2. Gray D, Zar HJ (2010) Childhood pneumonia in low and middle income countries: Burden, prevention and management. *Open Infect Dis J* 4: 74-84.
3. World Health Organisation (2008) The global burden of disease: 2004 update. Geneva: World Health Organization.
4. Charles PGP, Whitby M, Fuller AJ, Stirling R, Wright AA, et al. (2008) The etiology of community-acquired pneumonia in Australia: Why penicillin plus doxycycline or a macrolide is the most appropriate therapy. *Clin Infect Dis* 46: 1513-1521.
5. Schouten JA, Hulscher ME, Wollersheim H, Braspenning J, Kullberg BJ, et al. (2005) Quality of antibiotic use for lower respiratory tract infections at hospitals: (how) can we measure it? *Clin Infect Dis* 41: 450-460.
6. Garnacho-Montero J, Garcia-Cabrera E, Diaz-Martin A, Lepe-Jimenez JA, Iraurgi-Arcarazo P, et al. (2010) Determinants of outcome in patients with bacteraemic pneumococcal pneumonia: importance of early adequate treatment. *Scand J Infect Dis* 42: 185-192.
7. The Ministry of Health Republic of Indonesia (2009) Pneumonia, penyebab kematian utama balita.
8. Perhimpunan Dokter Paru Indonesia (2003) Pneumonia komuniti: Pedoman diagnosis & penatalaksanaan di Indonesia. Jakarta.
9. Hara K, Yahara K, Gotoh K, Nakazono Y, Kashiwagi T, et al. (2011) Clinical study concerning the relationship between community-acquired pneumonia and viral infection in northern Thailand. *Intern Med* 50: 991-998.
10. Mustafa MIA, Al-Marzooq F, How SH, Kuan YC, Ng TH (2011) The use of multiplex real-time PCR improves the detection of the bacterial etiology of community acquired pneumonia. *Tropical Biomedicine* 28: 531-544.
11. Poulouse V (2008) Severe community-acquired pneumonia requiring intensive care: a study of 80 cases from Singapore. *Singapore Med J* 49: 458-461.

# Part I

## Background





## Chapter 2

# Community-acquired pneumonia in adults in Southeast Asia: A review

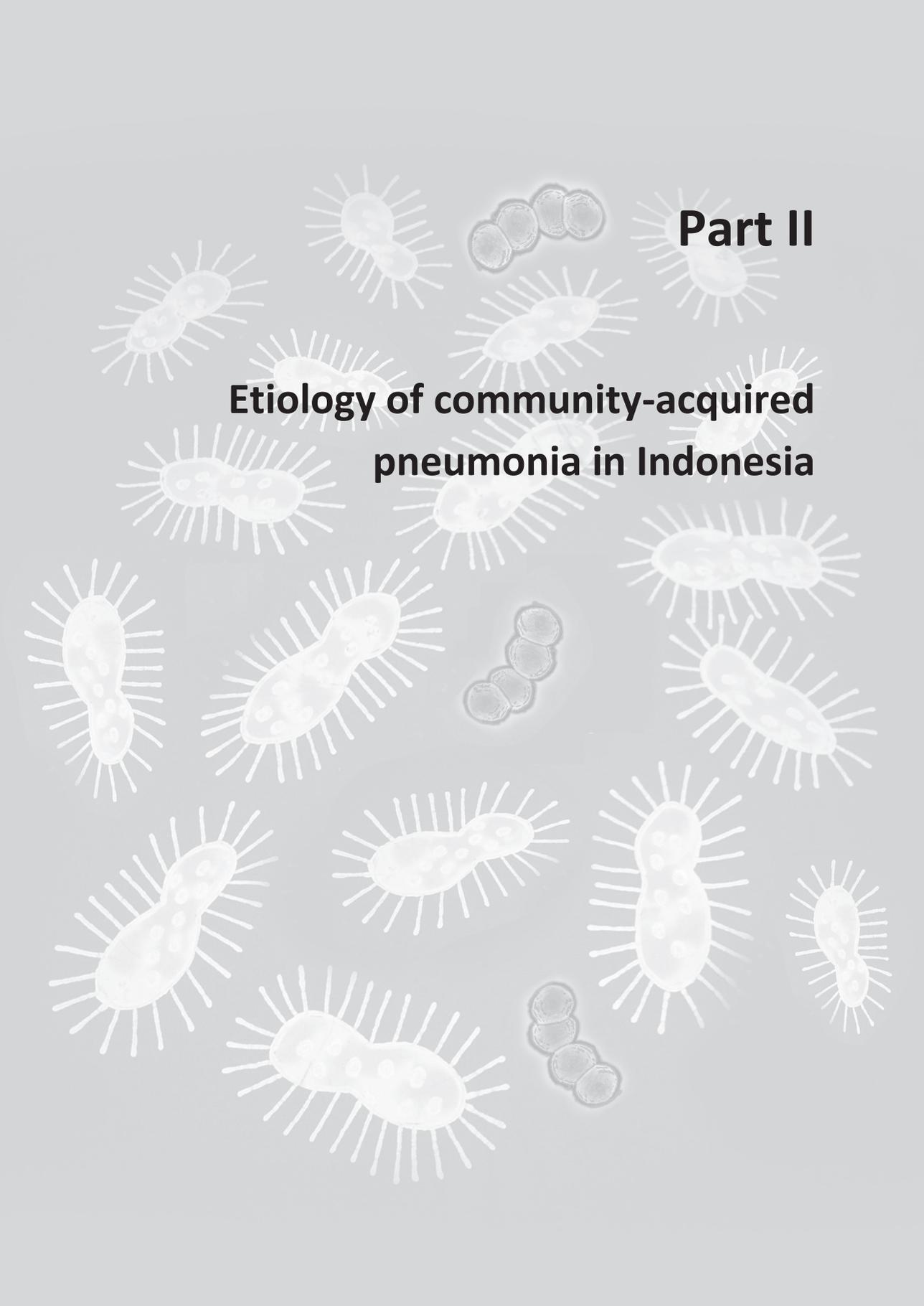
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## Part II

# Etiology of community-acquired pneumonia in Indonesia



# Chapter 3

## Viruses and Gram-negative bacilli dominate the etiology of community-acquired pneumonia in Indonesia

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## ABSTRACT

### Background

Knowledge about the etiology of community-acquired pneumonia (CAP) is essential for adequate management. Presently, only few studies about CAP are available from Southeast Asia.

### Method

From October 2007 to April 2009, adult patients admitted with CAP to two hospitals in Semarang, Indonesia, were included in our study to detect the bacterial and viral etiology using a wide range of advanced diagnostic methods. The severity of disease was classified according to the Pneumonia Severity Index (PSI) and the outcome was assessed as 30-day mortality.

### Results

In total, 148 patients were included. Influenza virus (18%), *Klebsiella pneumoniae* (14%), and *Streptococcus pneumoniae* (13%) were the most common agents. Mixed infections were common (28%). Other Gram-negative bacilli, *Mycobacterium tuberculosis*, *Chlamydia pneumoniae* each accounted 5%. The bacteria presented wild type antibiotic susceptibility profiles. Forty-four percent of included subjects were high-risk patients (PSI class IV-V), and 52% had at least one underlying disease. The mortality rate (30%) was significantly associated with severity ( $P < 0.001$ ), and with failure to establish an etiological diagnosis ( $P = 0.027$ ). No associations were found between etiology and underlying diseases, PSI class, nor mortality ( $P > 0.05$ ).

### Conclusions

Viruses and Gram-negative bacilli are dominant causes of CAP in this region, more so than *S. pneumoniae*. Most of the bacteria have wild type susceptibility to antimicrobial agents. Patients with severe disease and those with unknown etiology had higher mortality.

## INTRODUCTION

Knowledge about the etiology of community-acquired pneumonia (CAP) is essential for patient management [1]. However, only few studies on CAP are available from Southeast Asia; and most used limited microbiological methods [2-5]. This study aimed to describe the current etiology of CAP using full-range diagnostic methods, antimicrobial susceptibility, underlying diseases, severity and outcome of CAP patients in Indonesia.

## MATERIAL AND METHOD

### Study design

A prospective cohort study was performed in Dr. Kariadi Hospital (an 850-bed academic hospital) and in Semarang Municipal Hospital (a 130-bed secondary hospital).

### Subjects

From October 2007 until April 2009 hospitalised patients >13 years were included if CAP was diagnosed within 24 hours of admission. CAP was defined as radiological evidence of an infiltrate on chest X-ray and  $\geq 2$  of 6 criteria (cough, purulent sputum, temperature  $>38.5^{\circ}\text{C}$ , abnormal chest auscultation, white blood cells  $>10$  or  $<4 \times 10^9/\text{l}$ , positive culture of blood or pleural fluid). Subjects were excluded if they had received parenteral antibiotic before inclusion, had been hospitalised within four weeks of admission, were severely immunocompromised (HIV infection, chemotherapy, neutropenia  $<1000/\text{uL}$ , steroid treatment  $>20\text{mg}/\text{day}$  for more than two weeks), had terminal stage of malignancy, or evidence of other causes of abnormalities on the X-ray. Subjects with missing data of 30-day mortality were excluded for the analysis for mortality but not for the etiology, severity and underlying diseases.

### Data collection

All patients were assessed upon admission, followed daily during hospitalisation, and on day-30 according to a standardised protocol. Age, gender, cigarette and alcohol use, and clinical presentations were recorded. The admission chest X-rays were assessed by a radiologist in-charge, and later re-assessed to develop standardised X-ray descriptions. Complete blood cell-count, blood chemistry, and blood-gas analysis were performed on admission. Underlying diseases were assessed from clinical, laboratory and radiology data. Severity score was determined using the Pneumonia Severity Index (PSI) [6]. The outcome

was assessed as 30-day mortality. Management of patients was leaved to the discretion of the physicians in charge.

### The microbiological evaluation

Sputum, throat swab, blood, paired sera with a 4-week interval, and urine were sent to Clinical Microbiology Laboratory of Dr. Kariadi Hospital, Semarang. Gram-stained smears were evaluated to assess sputum quality and to help culture interpretation. Ziehl–Neelsen staining was done to screen for acid fast bacilli. Sputum and blood cultures (four BACTEC bottles [Becton-Dickinson, Rochester, UK] per patient) were performed on blood agar, chocolate agar, chocolate agar with gentamicin 5mg/L, and MacConkey agar (Oxoid, Basingstoke, UK). Crystal violet colistine broth (CVCB) and Ashdown agar [7] were used for *Burkholderia pseudomallei* isolation. Bacteria isolated from the initial cultures, sputa, throat swabs, sera, and urine were stored at  $-80^{\circ}\text{C}$ , and transported to Rotterdam, for further analyses.

*Streptococcus pneumoniae* was identified with optochin disk (Oxoid) and, in case of doubt, DNA probe (Accuprobe, San Diego, USA). Gram-negative bacilli (GNB) were identified using the Vitek-2 system (bioMérieux, l'Etoile, France). *Acinetobacter baumannii* was identified using PCR (*bla*<sub>OXA-51-like</sub>) [8]. *Moraxella catarrhalis* was confirmed using tributyrin test (Rosco Diagnostics-Taastrup, Denmark). X-V factors (Becton, Dickinson and Company - Sparks, USA) were used for *Haemophilus influenzae* identification. Slidex Staph Plus latex agglutination (bioMérieux) and Vitek-2 were used for *Staphylococcus aureus* identification. Ziehl-Neelsen positive sputa were cultured on MGIT media (Becton-Dickinson, Rochester, UK) and proceeded to PCR. Antimicrobial susceptibility tests were performed with disk diffusions (for *S. pneumoniae*), Vitek-2 (for *S. aureus* and GNB), and E-test (for *M. catarrhalis*). When extended-spectrum  $\beta$ -lactamase (ESBL)-production was suspected, isolates were further analyzed with with cefotaxime-clavulanate or ceftazidime-clavulanate E-test (bioMérieux). Reference strains from ATCC were used as controls and CLSI guidelines were applied.

Serology tests were performed with ELISA for *Chlamydia pneumoniae* (Medac Diagnostika, Germany), *Mycoplasma pneumoniae* (Virion\Serion, Würzburg, Germany), and *Legionella pneumophila* (Wampole Laboratories, Princeton, USA). Leptospira serology was performed using microscopic agglutination test (MAT) and ELISA [9] in the Royal Tropical Institute (KIT), Amsterdam, the Netherlands, if the patient had a clinical syndrom of leptospirosis. ELISA for respiratory viruses (Virion\Serion) was performed in the Laboratory of Virology, Erasmus MC. Real- time PCRs (RT-PCRs) were done on sputa and throat swabs for *C. pneumoniae* and *L. pneumophila* as described elsewhere [10]. RT-PCR for *M. pneumoniae*, rhinovirus, human corona virus (HCoV) 229E, OC43, and NL63, influenza A and B virus, human metapneumovirus (hMPV), RSV A and B, parainfluenza 1- 4

virus, adenovirus, and bocavirus were performed on throat swabs. Validation of PCR procedures was described before [11,12]. PCR for *Leptospira* and for *Chlamydia psittaci* were done as described elsewhere [13,14]. PCR for *Mycobacterium tuberculosis* was performed in Erasmus MC. Urinary antigen tests (BinaxNOW, Portland, USA) were performed to detect *S. pneumoniae* and *L. pneumophila* serogroup-1 antigen.

### Diagnostic criteria for microbial etiological agents

Sputum cultures were considered positive if sputum quality was good (leukocytes: epithelial-cells ratio was >2.5: 1, leukocyte count was  $\geq 10$ /low power field (LPF), epithelial-cell count was <10/LPF), and bacteria growing predominantly on agar were compatible with sputum microscopy. Blood cultures were considered positive if bacteria presented in  $\geq 1$  bottle for established pathogens or  $\geq 2$  bottles for potential skin contaminants.

Serology was considered positive if there was a four-fold titer increase of any immunoglobulin class. For influenza A and B, since re-infection in one year is unlikely, single sera with positive IgA and negative IgG during the first week of illness were also considered positive. Immunoglobulin classes measured were IgM, IgA, and IgG for *C. pneumoniae*; total immunoglobulin for *L. pneumophila*; IgA and IgG for respiratory viruses; IgM and IgG for *M. pneumoniae*; IgM and total immunoglobulin for *Leptospira*.

RT-PCR were considered positive if the CT value was <50 for *M. pneumoniae*,  $\leq 35$  for *Leptospira*, and <40 for other pathogens. A positive influenza A PCR was followed with another PCR to distinguish H5N1 from other influenza A viruses. Any positive reaction of the urinary antigen tests was considered positive.

A comprehensive review on clinical, radiology, and laboratory data was performed by panel discussions involving a pulmonologist, infectious disease specialists, radiologists, and clinical microbiologists to draw the final conclusions on the etiology of CAP. Etiology was considered if microbiology test results were compatible with the clinical presentation and radiology.

### Statistical analyses

Chi-Square or Fisher's exact test where appropriate was performed using SPSS 17 (SPSS Inc., Chicago, USA). The Kaplan-Meier method and the log-rank test were used to perform survival analysis. *P* value of <0.05 was considered significant.

### Ethics

The study was approved by The Ethical Committee of Faculty of Medicine Diponegoro University- Dr. Kariadi Hospital (36EC/FK/RSDK/2007). A written informed consent was obtained from each patient or guardian.

## RESULTS

### Patient characteristics

Of the 156 patients enrolled; 135 were hospitalised in Dr. Kariadi hospital and 21 in Semarang Municipal hospital. Eight patients were excluded because of non-confirmed CAP diagnosis (acute respiratory distress syndrome (3 patients), urosepsis (2 patients), no infiltrate on the X-ray on re-assessment (2 patients)), or preceding parenteral antibiotics (1 patient)). No 30-day mortality data was missing.

**Table 1.** Characteristics of patients with community-acquired pneumonia in Semarang, Indonesia

Characteristic	Number (%) of Patients, N=148
Age years, median; Inter quartile range (IQR)	58; 45-69
Male/female	73 (49) / 75 (51)
Length of stay, median; IQR days	6; 4-8
30-day mortality	44 (30)
Underlying diseases	
Hypertension	25 (17)
Congestive heart disease	16 (11)
Diabetes mellitus	14 (10)
Chronic obstructive pulmonary disease	12 (8)
Stroke	27 (18)
Asthma	3 (2)
Malignancy	5 (3)
Chronic kidney disease	4 (3)
Pneumonia Severity Index (PSI) *	
Class I	6 (4)
Class II	45 (30)
Class III	32 (22)
Class IV	45 (30)
Class V	20 (14)
Chest X-ray	
Bronchopneumonia	103 (70)
Alveolar pneumonia	32 (21)
Interstitial pneumonia	13 (9)
Specimens obtained	
Sputum, total/good quality**	103 (70) / 57 (39)
Blood culture	148 (100)
Paired sera/single sera	83 (56) / 64 (43)
Throat swabs	148 (100)
Urine	145 (98)

\* according to Fine et al, 1997 [6]

\*\* sputum was considered of good quality if the leukocytes : epithelial cells ratio was >2.5, the leukocyte count was  $\geq 10$ /LPF and the epithelial cell count <10/LPF

Patients' ages ranged 14–88 years, with 32% aged >60 years. Most patients (52%) had underlying diseases, with 19% having two or more underlying diseases. Severe pneumonia (PSI IV-V) was found in 44% patients. Most (70%) of the chest X-rays showed bronchopneumonia (Table 1).

Only 15% of patients had not received oral antibiotics prior to hospital admission, while 50% were unsure whether they had received antibiotics or not. Thirty eight (26%) patients had a complete set of test specimens (Table 1). No data were missing for underlying diseases, severity, and 30-day mortality.

### Microbiological results

One-hundred and fifty respiratory pathogens were identified from 100 (68%) patients. The microbiological methods by which pathogens were identified are described in Table 2. Final conclusions regarding the CAP etiology are summarised in Tables 3 and 4.

A single causative agent was found in 58 (39%) patients, of which 33 (22%) were viruses and 25 (17%) were bacteria. Polymicrobial infection was found in 42 (28%) patients, including viral infections with superimposed bacterial infection, tuberculosis with superimposed secondary infection, and other mixed-infections (multiple viruses, multiple bacteria, and combinations of virus(es) and bacteria) (Table 3).

Seventy viruses were identified in 67 (45%) patients, with influenza A as commonest, identified in 20 (13.5%) patients. PCR detected 16 (80%) cases, while serology detected 10 (50%) cases (Table 2). All influenza A viruses were H3N2. In 12 patients it was the single pathogen found (Table 3); in eight patients other pathogens were also involved (Table 4). Together, influenza A and B viruses caused 26 (18%) CAP in our patients either as single or as co-pathogen.

The second commonest virus was rhinovirus, which in all 13 (9%) cases were detected by PCR. In six patients this virus was the single pathogen; in seven it was a co-pathogen. Parainfluenza virus was the third commonest virus, detected in 11 (7%) patients, mostly by PCR; five episodes as single pathogen and six as co-pathogen. Other viruses found were HcoV OC43, adenovirus, hMPV, and RSV; most were as co-pathogens, and detected by PCR.

Eighty bacteria were found in 65 (44%) patients, with *Klebsiella pneumoniae* and *S. pneumoniae* as the most frequently diagnosed. *K. pneumoniae* was isolated from 21 (14%) patients; most (95%) from sputum cultures, and 5% from both blood and sputum cultures. *S. pneumoniae* was diagnosed in 20 (13%) patients; most (75%) by urinary antigen tests (Table 2). *C. pneumoniae* and *M. tuberculosis* were the third commonest bacterial species identified; each was diagnosed in 5% of patients. Other GNB (*Enterobacter cloacae*, *Klebsiella oxytoca*, *Achromobacter xylosoxidans*, and *Burkholderia cepacia*) were identified in 7 (5%) patients; most were isolated from sputum cultures. No *Burkholderia pseudomallei* was found. Blood cultures were positive only in 4 (2%) patients.



**Table 2.** Pathogens isolated from laboratory specimens obtained from patients with community-acquired pneumonia in Semarang, Indonesia

Pathogens	Blood Culture	Sputum Culture	Urinary Antigen Test	Blood + Sputum Culture	Sputum Culture + UAT	Blood and Sputum Culture + UAT	Serology	PCR	Serology + PCR	Culture + PCR	Total
<i>C. pneumoniae</i>							8				8
<i>M. pneumoniae</i>								1			1
<i>L. pneumophila</i>			1								1
<i>M. tuberculosis</i>		1								7	8
<i>Leptospira</i>							1	2	2		5
<b>Total bacteria</b>	<b>1</b>	<b>36</b>	<b>16</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>9</b>	<b>3</b>	<b>2</b>	<b>7</b>	<b>80</b>
<b>Total pathogens</b>											<b>150</b>

UAT = urinary antigen test, PIV = parainfluenza virus, HcoV= human coronavirus, hMPV = human metapneumovirus, RSV = respiratory syncytial virus  
 Other GNB = Other Gram-negative bacilli, i.e. *E. cloacae*, *S. maltophilia*, *A. xylosoxidans*, *B. cepacia*

**Table 3.** Final etiological diagnosis in patients presenting with community-acquired pneumonia in Semarang, Indonesia

Etiology	Patients (N =148) Number (%)
<b>Single pathogen</b>	<b>58 (39)</b>
virus	
Influenza A/B	12 (8) / 5 (3)
Rhinovirus	6 (4)
Parainfluenza virus	5 (3)
Adenovirus	2 (1)
RSV	1 (1)
Coronavirus OC43	1 (1)
human Metapneumovirus	1 (1)
<b>Subtotal</b>	<b>33 (22)</b>
bacteria	
<i>K. pneumoniae</i>	5 (3)
<i>S. pneumoniae</i>	5 (3)
<i>M. tuberculosis</i>	5 (3)
<i>Leptospira spp</i>	4 (3)
<i>C. pneumoniae</i>	2 (1)
<i>S. aureus</i>	2 (1)
<i>M. catarrhalis</i>	1 (1)
<i>B. cepacia</i>	1 (1)
<b>Subtotal</b>	<b>25 (17)</b>
<b>More than one pathogen*</b>	
Viral infection with superimposed bacterial infection	24 (16)
Tuberculosis with superimposed infection	3 (2)
Other mixed infections	15 (10)
<b>Subtotal</b>	<b>42 (28)</b>
<b>Unknown etiology (no pathogen detected)</b>	<b>48 (32)</b>

\*for further details, see Table 4

Combinations of pathogens in 42 patients are presented in Table 4. Frequent combinations of pathogens were *K. pneumoniae* + *S. pneumoniae* (n=5), adenovirus + *K. pneumoniae* (n=4), and influenza or coronavirus + *S. pneumoniae* (n=3 each).

**Table 4.** Combinations of respiratory pathogens observed among patients with community-acquired pneumonia

Pathogen	Rhinovirus		<i>M. tuberculosis</i>	<i>S. pneumoniae</i>	<i>K. pneumoniae</i>	<i>C. pneumoniae</i>	<i>S. aureus</i>	<i>M. catarrhalis</i>	Other GNB	<i>Leptospira sp</i>	Total
	Rhinovirus	hMPV									
Influenza A/B				3	2		1	1	2		9
PIV	1		1	1	2	1		1			7
Adenovirus				1	4				1		6
Rhinovirus	1			2	1	1	1			1	6
Coronavirus	1	1		3	1			2	2		10
hMPV		1				1		1	2		4
RSV				1	1						2
<i>K. pneumoniae</i>			1	5	1	2		1	1		10
<i>C. pneumoniae</i>			1			1					1
<i>M. pneumoniae</i>				1							1
<i>L. pneumophila</i>				1							1
<i>S. pneumoniae</i>				1			1				1
<i>M. catarrhalis</i>								1	1		1
<b>Total</b>	<b>2</b>	<b>1</b>	<b>3</b>	<b>18</b>	<b>11</b>	<b>5</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>1</b>	<b>59</b>

PIV= parainfluenza virus, hMPV= human metapneumovirus, RSV= respiratory syncytial virus, GNB= Gram - Negative Bacilli. Note: 42 patients with multiple infection, of which 35 had 2 pathogens, 6 had 3 pathogens (Rhinovirus + *S. pneumoniae* + *S. aureus*, Adenovirus + *K. pneumoniae* + *S. pneumoniae*, Adenovirus + *K. pneumoniae* + other GNB, Coronavirus+ *K. pneumoniae* + *M. catarrhalis*, RSV type A + *K. pneumoniae* + *S. pneumoniae*, Parainfluenza + *K. pneumoniae* + *S. pneumoniae*) and 1 patient had 4 pathogens (Coronavirus + hMPV + *K. pneumoniae* + *M. catarrhalis*)

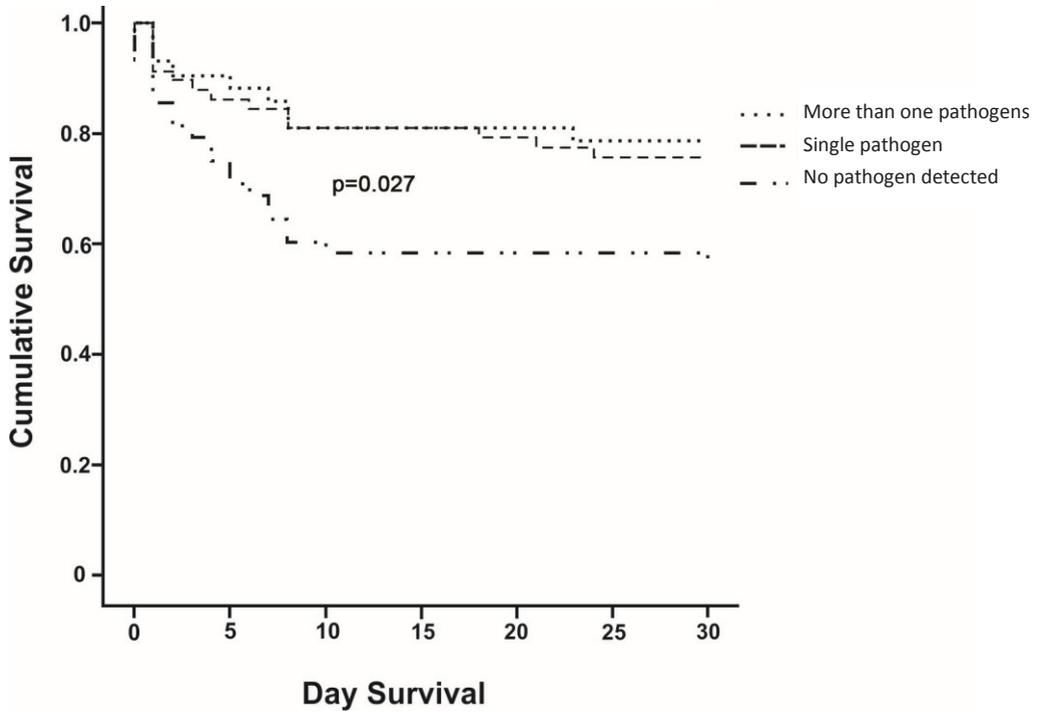
The etiology of CAP could not be elucidated in 48 (32%) patients. Pathogen detection was, predictably, related to the availability of diagnostic specimens. Out of 48 cases with unknown etiology, only one patient had complete laboratory specimens from which all tests results were negative. The other 47 had incomplete laboratory specimens; 46 of them had no positive results, and one had so many positive serology results that no conclusion could be drawn. Patients with severe disease (PSI class IV–V) were less likely to receive an etiological diagnosis compared to those with non-severe disease (PSI class I-III,  $P=0.01$ ).

One isolate of *K. pneumoniae* was an ESBL-producer, while the others were susceptible to antibiotics except to piperacillin (81% resistant) and nitrofurantoin (76% resistant). Of four regrowable *S. pneumoniae*, all were susceptible to penicillin, erythromycin, and vancomycin; one was intermediately resistant to co-trimoxazol, and three were resistant to tetracyclin.

### Clinical outcome

There was no significant difference in underlying diseases, PSI class, length of stay, and mortality among patients with single viral, single bacterial, and mixed viral-bacterial infections ( $P>0.05$ ). Patients with mixed-viral and mixed-bacterial infections were not analysed due to small numbers. The overall 30-day mortality rate was high (30%) and significantly associated with severity of disease ( $P<0.001$ ). The mortality rate ranged from 17% in PSI class I patients to 80% in PSI class V patients. Interestingly, survival analysis showed a significantly lower survival rate in patients with unknown etiology (Figure 1).

Early death ( $\leq 48$  hours of hospitalization) happened in 18 patients (41% mortality), and was associated with respiratory failure or irreversible septic shock; most of the early deceased patients (14 subjects) had delayed or no access to ICU care because of limited availability of the service. Late death ( $>48$  hours of hospitalization) occurred in 14 patients (32% of mortality), and was associated with worsening conditions from CAP (7 patients) or with their underlying diseases (7 patients). Despite clinical improvement, 12 patients (27% of mortality) died after discharge from hospital for in-home recovery within 30 days of admission. The circumstance of these latter death remained unknown.



**Figure 1.** Survival of patients with Community-acquired pneumonia according to diagnostic yield.

## DISCUSSION

This is the first CAP etiology study conducted in Indonesia applying a full-range of microbiology tests. Influenza viruses, *K. pneumoniae*, and *S. pneumoniae* were the commonest agents with no correlation to the underlying diseases, severity, and outcome of CAP.

Viral infections were surprisingly common, either as single or combined etiology. PCR was instrumental in detecting most respiratory viruses. A recent report from Thailand found viruses in 9% of hospitalised adult CAP patients in Northern Thailand [2] and in 25% of adult CAP patients admitted from a pneumonia surveillance in two other provinces [15]. Incidence of influenza A infection was 7.4% in hospitalised CAP [15] and 7% in severe CAP [5]. The use of PCR may, thus, enhance the appropriate use of antimicrobial agents [16]. No other CAP studies in Southeast Asia so far have attempted to diagnose viruses.

*K. pneumoniae* and other GNB were frequently observed in our study, similar to reports from other Southeast Asian countries [2,3,17], but different from those from Europe, North America, or Australia [1,18,19]. The high prevalence of *K. pneumoniae* and other GNB might, in part, be explained by the common carriage of these bacterial species in the nasopharynx of healthy individuals in the same area [20]. The tropical climate with higher temperature and humidity in Semarang may increase the incidence of GNB infection, as those conditions promote the growth and virulence of GNB [21,22].

*S. pneumoniae* infection was detected in only 13% of the patients, mostly by urinary antigen tests. Cultures frequently failed to grow this species, possibly due to the common use of antibiotics in the community, underlining the importance of alternative methods to detect pneumococci, as by PCR [3] or urinary antigen test [23]. However, a positive finding with the latter test should be interpreted carefully particularly in patients with COPD [23]. Previous studies in Southeast Asia reported various numbers for the relative contribution of *S. pneumoniae*, from 2% [17] through 52% [24] depending on the study population and the laboratory methods, while studies from Europe, North America, and Australia consistently reported it as the most common cause of CAP [1,18,19].

Aside from the climate, the differences in CAP etiology in our study compared to above studies might also relate to differences in patients age, underlying diseases, and radiological type of pneumonia. Our patients were relatively younger, had more often cerebrovascular underlying diseases, and had more often bronchopneumonia in stead of lobar or alveolar pneumonia. The wide spread use of influenza vaccination in other parts of the world may help explain the differences in the incidence of influenza in CAP. Taken together, these differences imply that a guideline of CAP management construed and implemented in Europe, North America, and Australia might not be directly applicable in Indonesia.

Most CAP studies distinguished the definite from probable etiology based on microbiology results. We did not use this distinction, instead, we interpreted microbiological, clinical and radiological findings in a multidisciplinary discussion to make a final diagnosis, since in our opinion, clinical data can not be omitted in doing so.

Extending the range of laboratory diagnostics applied to include viral as well as bacterial and fungal pathogen will, predictably increase the number of pathogens detected, and, thereby, increase the chance of finding polymicrobial infections [4,25]. We found mixed infection in a sizable proportion of the cohort of patients included in the study. Some of this mixed infections are common, e.g. viral infection with superimposed bacterial infection [26] and tuberculosis with superimposed infection. Other mixed infections, e.g. by multiple bacteria or multiple viruses, are more difficult to understand and pose the problem of discriminating between infection and carriage.

Most bacteria causing CAP in our study were susceptible to early generation antibiotics. These findings are consistent with other observation from Indonesia [27,28].

Hence, the choice of antibiotics for the initial empirical treatment of CAP should not include the later generation of antibiotics, including a third generation cephalosporin which is recommended by the local guideline [29]. A more appropriate choice to consider would be amoxicillin-clavulanic acid or ampicillin-sulbactam.

The crude mortality rate was high, and related to severity of the disease. However, even in low risk patients, the mortality in our study was still quite high, not only compared to that in North-America and Europe, but also to other studies from Southeast Asian countries [2,5]. The high mortality observed in our study suggests problems in the patient care process which needs further analysis. Determinants of poor outcome may at least in part, include delay in presenting to the hospital, inappropriate initial treatment, limited access to mechanical ventilators, inappropriate antibiotic therapy, underlying diseases, and the severity of the disease on admission (to be published elsewhere). Interestingly, mortality was significantly higher among patients with unknown etiology. This finding may be explained by limitations in acquiring appropriate laboratory samples due to the severity of the disease at the time the patients were admitted. This is rather unfortunate, since these patients are those who need most to be managed based on an established etiology. This finding implies the need to perform all diagnostic procedures early, without delay, especially in patients with severe disease, so that the etiology of CAP in this group is established early, at a time its management can still be optimised.

Since this study observed one city in Indonesia, involving only two hospitals, our data might not be representative for Java or Indonesia. The incomplete laboratory specimens in some patients might limit the opportunity to reveal complete etiology of CAP.

## CONCLUSIONS

Multicenter CAP studies which include viral etiologies, should be carried out in Indonesia and other countries in this region to delineate the full spectrum of etiological agents in CAP patients and to generate local guidelines for the management of CAP.

Bacteria causing CAP in this area of the world continue to present wild type antibiotic susceptibility profiles so that early generation antibiotics can be used as initial empiric treatment, thereby avoiding the selection pressure exerted by newer generations of antibiotics, especially third generation cephalosporins. The frequent incidence of GNB infection requires initial therapy to cover GNB in addition to pneumococcus. Optimal bacteriological diagnostics are required to be able to adapt initial therapy, including alternative tests for detecting *S. pneumoniae*. Performing all diagnostic procedures early, without delay, should be prioritized since establishing an etiological diagnosis is likely to improve clinical outcomes.

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## Potential conflict of interest

All authors declare no conflict of interest

## REFERENCES

1. Charles PGP, Whitby M, Fuller AJ, Stirling R, Wright AA, et al. (2008) The etiology of community-acquired pneumonia in Australia: Why penicillin plus doxycycline or a macrolide is the most appropriate therapy. *Clin Infect Dis* 46: 1513-1521.
2. Hara K, Yahara K, Gotoh K, Nakazono Y, Kashiwagi T, et al. (2011) Clinical study concerning the relationship between community-acquired pneumonia and viral infection in northern Thailand. *Intern Med* 50: 991-998.
3. Mustafa MIA, Al-Marzooq F, How SH, Kuan YC, Ng TH (2011) The use of multiplex real-time PCR improves the detection of the bacterial etiology of community acquired pneumonia. *Tropical Biomedicine* 28: 531-544.
4. Song J-H, Oh WS, Kang C-I, Chung DR, Peck KR, et al. (2008) Epidemiology and clinical outcomes of community-acquired pneumonia in adult patients in Asian countries: a prospective study by the Asian network for surveillance of resistant pathogens. *Intl J Antimicrob Agents* 31: 107-114.
5. Apisarnthanarak A, Puthavathana P, Mundy LM (2007) Risk factors and outcomes of influenza A (H3N2) pneumonia in an area where avian influenza (H5N1) is endemic. *Infect Control Hosp Epidemiol* 28: 479-482.
6. Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, et al. (1997) A prediction rule to identify low-risk patients with community-acquired pneumonia. *N Engl J Med* 336: 243-250.
7. Ashdown L, Clark S (1992) Evaluation of culture techniques for isolation of *Pseudomonas pseudomallei* from soil. *Appl Environ Microbiol* 4011-4015.
8. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, et al. (2006) Identification of *Acinetobacter baumannii* by detection of the bla OXA-51-like carbapenemase gene intrinsic to this species. *J Clin Microbiol* 44: 2974-2976.
9. Goris MGA, Leeflang MMG, Boer KR, Goeijenbier M, Gorp ECMv, et al. (2012) Establishment of valid laboratory case definition for human leptospirosis. *Bacteriol Parasitol* 3.
10. Templeton KE, Scheltinga SA, Eeden WCJFMvd, Graffelman AW, van den Broek PJ, et al. (2005) Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction. *Clin Infect Dis* 41: 345-351.
11. Stadhouders R, Pas SD, Anber J, Voermans J, Ted H.M. Mes, et al. (2010) The effect of primer-template mismatches on the detection and quantification of nucleic acids using the 5' nuclease assay. *J Mol Diagn* 12: 109-117.
12. Hoek RAS, Paats MS, Pas SD, Bakker M, Hoogsteden HC, et al. (2013) Incidence of viral respiratory pathogens causing exacerbations in adult cystic fibrosis patients. *Scandinavian Journal of Infectious Diseases* 45: 65-69.
13. Ahmed AA, Engelberts MFM, Boer KR, Ahmed N, Hartskeerl R (2009) Development and validation of a real-time PCR for detection of pathogenic *Leptospira* species in clinical materials. *PLoS ONE* 4: e7093.
14. Heddema ER, Hannen EJv, Duim B, Jongh BMD, Kaan JA, et al. (2006) An outbreak of psittacosis due to *Chlamydophila psittaci* genotype A in a veterinary teaching hospital. *J Med Microbiol* 55.
15. Olsen SJ, Thamthitawat S, Chantra S, Chittaganpitch M, Fry AM, et al. (2010) Incidence of respiratory pathogens in persons hospitalized with pneumonia in two provinces in Thailand. *Epidemiol Infect* 138: 1811-1822.
16. Gaydos CA (2013) What is the role of newer molecular tests in the management of CAP? *Infect Dis Clin North Am* 27: 49-69.
17. Abdullah S, Darus Z, Hashim CWA (2011) The outcome of hospitalized patients with community-acquired pneumonia in Hospital Universiti Sains Malaysia. *Intl Med J* 18: 121 - 127.
18. Reissig A, Mempel C, Schumacher U, Copetti R, Gross F, et al. (2013) Microbiological diagnosis and antibiotic therapy in patients with community-acquired pneumonia and acute COPD exacerbation in daily clinical practice: Comparison to current guidelines. *Lung*: 1-8.
19. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, et al. (2012) Etiology of community-acquired pneumonia among hospitalized adults in the United States: Preliminary data from the CDC etiology of pneumonia in the community (EPIC) study. *ID Week 2012*. San Diego, CA.

20. Farida H, Severin JA, Gasem MH, Keuter M, Broek Pvd, et al. (2013) Nasopharyngeal carriage of *Klebsiella pneumoniae* and other Gram-negative bacilli in pneumonia-prone age groups in Semarang, Indonesia. *J Clin Microbiol* 51: 1614-1616.
21. Eber MR, Shardell M, Schweizer ML, Laxminarayan R, Perencevich EN (2011) Seasonal and temperature-associated increases in Gram-negative bacterial bloodstream infections among hospitalized patients. *PLoS one* 6: e25298.
22. Raetz CRH, Reynolds CM, Trent MS, Bishop RE (2007) Lipid modification systems in Gram-negative bacteria. *Annu Rev Biochem* 76: 295-329.
23. Tzeng DH, Lee YL, Lin YH, Tsai CA, Shi ZY (2006) Diagnostic value of the Binax NOW assay for identifying a pneumococcal etiology in patients with respiratory tract infection. *J Microbiol Immunol Infect* 39: 39-44.
24. Reechaipichitkul W, Lulitanond V, Sawanyawisuth K, Lulitanond A, Limpawattana P (2005) Etiologies and treatment outcomes for out-patients with community-acquired pneumonia (CAP) at Srinagarind Hospital, Khon Kaen, Thailand. *Southeast Asian J Trop Med Public Health* 36: 1261-1267.
25. Johansson N, Kalin M, Annika TL, Giske CG, Hedlund J (2010) Etiology of community-acquired pneumonia: Increased microbiological yield with new diagnostic methods. *Clin Infect Dis* 50: 202-209.
26. Pavia AT (2013) What is the role of respiratory viruses in community-acquired pneumonia? What is the best therapy for influenza and other viral causes of community-acquired pneumonia? *Infect Dis Clin North Am* 27: 157-175.
27. Lestari ES, Severin JA, Filius PMG, Kuntaman K, Duerink DO, et al. (2008) Antimicrobial resistance among commensal isolates of *Escherichia coli* and *Staphylococcus aureus* in the Indonesian population inside and outside hospitals. *Eur J Clin Microbiol Infect Dis* 27: 45-51.
28. Severin JA, Lestari ES, Kloezen W, Toom NL-d, Mertaniasih NM, et al. (2012) Faecal carriage of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae among humans in Java, Indonesia, in 2001–2002. *Trop Med Int Health* 17: 455-461.
29. Perhimpunan Dokter Paru Indonesia (2003) *Pneumonia komunitas: Pedoman diagnosis & penatalaksanaan di Indonesia*. Jakarta.

## Chapter 4

# Are pathogenic *Leptospira sp.* agents of community-acquired pneumonia in Indonesia? A case series

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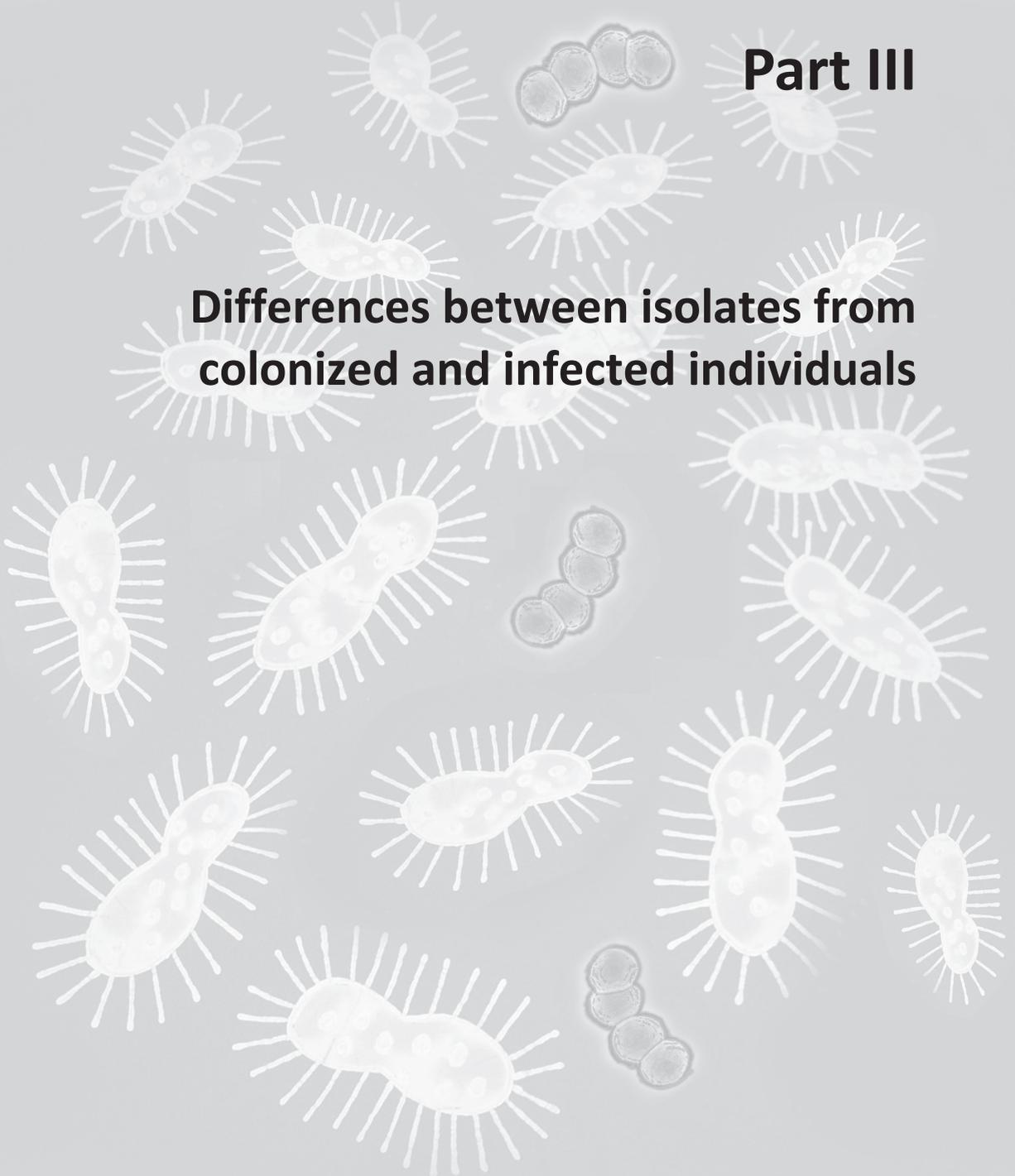
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## Part III

### Differences between isolates from colonized and infected individuals





# Chapter 5

## Nasopharyngeal carriage of *Streptococcus pneumoniae* in pneumonia-prone age groups in Semarang, Java island, Indonesia

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## ABSTRACT

### Introduction

Nasopharyngeal carriage of *Streptococcus pneumoniae* precedes pneumonia and other pneumococcal diseases in the community. Little is known about *S. pneumoniae* carriage in Indonesia, complicating strategies to control pneumococcal diseases. We investigated nasopharyngeal carriage of *S. pneumoniae* in Semarang, Indonesia.

### Methods

A population-based survey was performed in Semarang, Indonesia. Nasopharyngeal swabs and questionnaires were taken from 496 healthy young children (6-60 month-old) and 45-70 year-old adults.

### Results

Forty-three percent of children aged 6-60 months and 11% of adults aged 45-75 years carried *Streptococcus pneumoniae*. Determinants of carriage were being a child (OR 7.7; 95% CI = 4.5-13.0), passive smoking (OR 2.1; 95% CI = 1.3-3.4), and contact with toddler(s) (OR 3.0; 95% CI = 1.9-4.7). The most frequent serotypes found were 6A/B and 15B/C. The current commercially available vaccines cover <50% serotypes found in children. Twenty-four percent of *S. pneumoniae* strains were penicillin non-susceptible, and 45% were resistant to co-trimoxazol.

### Conclusions

The limited coverage of commercially available vaccines against the serotypes found in this population, and the high proportion of non-susceptibility to penicillin and co-trimoxazol suggest the need for region-specific strategies to control *S. pneumoniae*.

## INTRODUCTION

*Streptococcus pneumoniae* is a worldwide occurring pathogen [1]. Data on this species are abundantly available in developed countries, but still scarce in low-to-middle-income countries, leading to difficulties in designing national strategies to control pneumococcal diseases.

Since pneumococcal pneumonia is preceded by nasopharyngeal colonization with *S. pneumoniae* [2], it is relevant to study the nasopharyngeal carriage pattern in humans, particularly in those at higher risk of pneumonia. This has already been extensively studied in many parts of the world, but only few data are available from Indonesia [3], the fourth most populated country in the world. We investigated the nasopharyngeal carriage of *S. pneumoniae* in Indonesia, to learn the prevalence, risk factors, serotypes, and antimicrobial susceptibility.

## METHODS

### Ethics statement

The study was approved by The Ethical Committee of the Faculty of Medicine, Diponegoro University, Semarang. Written informed consent was given by the subjects or their caregivers.

### Subjects

A population-based survey was performed in Semarang, a city with 1.5 million residents in Central Java, among healthy children aged 6-60 months and healthy adults aged 45-70 years as described before [4]. Exclusion criteria were the presence of respiratory symptoms and antibiotic consumption within the last three days. Cluster random sampling was done from February to April 2010 to recruit subjects from all 16 districts of Semarang.

### Specimen collection and Laboratory testing

Nasopharyngeal swabs were obtained using rayon-tipped swabs and transported in Amies-charcoal media (COPAN, Italy). Swabs were inoculated on 5% sheep blood agar with gentamicin (5 mg/liter) and incubated at 35°C in 5% CO<sub>2</sub> for 48 hours. Identification of *S. pneumoniae* was performed using the optochin test (Oxoid, Basingstoke, UK) and, in case of doubt, a DNA hybridization test (Accuprobe, Gen-Probe Inc., San Diego, CA, USA). Disk diffusion antimicrobial susceptibility tests were performed and interpreted

according to EUCAST 2012. Serotyping of *S. pneumoniae* was done with a multiplex-PCR which covers 36 serotypes [5,6]. Control strains were included in all analyses.

Using a questionnaire that was developed to identify determinants of carriage, data on demography, house sanitation (crowding, smoke exposure from cigarette and mosquito coils), and water and food hygiene were recorded. Crowding was defined present if the ratio of total bedroom space to the number of family members was less than 4 m<sup>2</sup> [7]. Water hygiene was defined as poor when water other than tap or bottled water was used by the family. Food hygiene was considered poor if the family consumed street food.

### **Statistical Analysis**

Univariate analysis was done with Chi-square or Fisher's exact tests when appropriate, followed by backward stepwise logistic regression for variables with *P* value <0.2 using SPSS 17 (SPSS Inc, Chicago, USA). *P* value of <0.05 was considered significant.

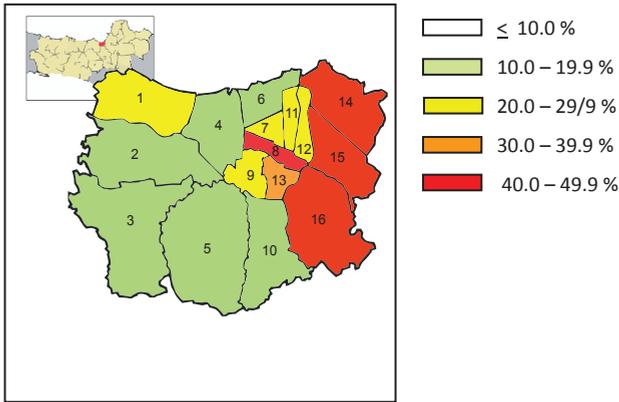
## **RESULTS**

### **Subjects**

Two hundred and fifty-three adults aged 45–70 years and 243 children aged <6 years participated in the study. The characteristics of the participants have been shown previously [4]. Crowding was common, as was exposure to smoke.

### **Carriage prevalence and determinants**

Overall carriage of *S. pneumoniae* was 27% (95% CI = 20-32), 43% in children, and 11% in adults. The proportion carrying *S. pneumoniae* varied significantly across the districts of Semarang, and tended to be higher in the suburban and eastern parts of the city (Figure 1).



**Figure 1.** Distribution of nasopharyngeal carriage of *S. pneumoniae* in the districts of Semarang

Serotype distribution significantly differed between the isolates from adults and children ( $p < 0.05$ , table 1). Serotype 6A/6B was the most frequently isolated from both adults and children.

**Table 1.** Serotype of *S. pneumoniae* isolated from healthy people in Semarang

	Children n (%)	Adults n (%)	Total n (%)	<i>p</i>
6A/6B	21 (19)	12 (39)	33 (23)	0.029
15B/C	11 (10)	4 (13)	15 (11)	
11A	11 (10)	1 (3)	12 (8)	
23F	10 (9)	1 (3)	11 (8)	
19F	9 (8)	0 (0)	9 (6)	
23A	5 (5)	0 (0)	5 (4)	
15A	2 (2)	3 (10)	5 (4)	
Others	22 (20)	2 (6)	24 (17)	
Untypeable	20 (18)	8 (26)	28 (20)	
Total	111 (100)	31 (100)	142 (100)	

Multivariate analysis showed that being a child (OR 7.7, 95% CI, 4.5-13.0), passive smoking (OR 2.1, 95% CI, 1.4-3.4), and contact with toddler(s) at home (OR 3.0, 95% CI, 1.9-4.7) were independent determinants of carriage.

### Antimicrobial susceptibility and serotypes

One hundred and forty-two strains were isolated from 133 subjects. In total, 34 (24%) strains were penicillin non-susceptible (MIC ranged 0.047-1.5), including 25 (23%) from children and 9 (29%) from adults ( $P=0.25$ ). Forty-five percent of the strains were resistant to co-trimoxazol, 1% to erythromycin, and 5% to tetracycline. There was no significant difference in the susceptibility pattern between isolates from children and those from adults ( $P>0.1$ ). No strain was resistant to penicillin or to vancomycin.

Capsular type 6A/B was the most prevalent serotype in all age groups (19% in children and 39% in adults). The most common capsular serotypes in children, comprising 61% of strains, were 6A/B, 15B/C, 11A, 23F, 19F, 23A. Those in adults, were 6A/B, 15B/C, and 15A. These two serotype patterns differed significantly ( $P=0.029$ ). Other serotypes were less frequently found (Table 1). Nineteen percent were untypeable with the multiplex-PCR employed.

### DISCUSSION

The carriage prevalence of *S. pneumoniae* among children in our study was comparable to those previously found among healthy children in Lombok, Indonesia [3], and in the Netherlands [8], but lower than those in Gambia [9], Poland [10], Australia [11], Thailand [12], and higher than those reported from Iran [13] and Korea [14]. The carriage prevalence among adults in our study was 11%; higher than that in Alaska [15], but lower than that among Australian Aboriginals of the same age [11]. The prevalence differences among populations may be related to sampling or laboratory methods (i.e. nasopharyngeal swab versus throat swab, the use of selective media), to certain characteristics of the population studied (i.e. the age of the subjects, household characteristics – especially the presence of toddlers, presence of upper respiratory tract infection, vaccination status), or to seasonal variation. Our samples were taken in the rainy season, during which the incidence of respiratory tract infection is likely to be somewhat increased.

The prevalence of *S. pneumoniae* with reduced susceptibility to penicillin and co-trimoxazole was high. The national and local guidelines for empirical antibiotics for community-acquired pneumonia in children recommend these two antibiotics as the first choices [16], and those for meningitis recommend ampicillin for the second line [17].

The commercially available 13-valent pneumococcal conjugate vaccines (PCV13) [18], which was introduced in 2011, provides in average 45% strain coverage for the infant population in this study, ranged from 13-100% in each district. PCV10 provides even lower coverage. However, the coverage of the PCV13 over the serotype repertoire in

other island in Indonesia in the past [3] was 60% and in other Southeast Asian countries [19] was higher (63%-97%). Our results may not be taken to reflect the serotype distribution throughout Indonesia, since the study was performed in a specific geographic location. The PCVs have not been included in the national vaccination program, rather, it is introduced in private clinics as they are still probably considered very expensive for Indonesian setting, and no enough information regarding with pneumococcal disease and carriage in Indonesia. Studies failed to reveal the burden of pneumococcal diseases in Indonesia probably due to technical problems [20,21]. This underscores the importance of surveillance of pneumococcal disease in Indonesia using implementable methods.

This study provides further evidence that passive smoking is an independent determinant of *S. pneumoniae* carriage among children [10,13]. The mechanism by which passive smoking influences the microbial ecology of the upper respiratory tract remains to be elucidated, however.

## **CONCLUSION**

In conclusion, nasopharyngeal carriage of *S. pneumoniae* was common among healthy children and adults in this part of the world, determined, at least in part, by the presence of toddlers in the household and smoking habit of the adults. The low coverage of commercially available vaccine against the serotypes found among children in the population, and the high proportion of non-susceptibility to penicillin and co-trimoxazol suggest the need for region-specific strategies to control *S. pneumoniae*. There is a need for a large epidemiological study on pneumococcal disease throughout Indonesia.

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## **Conflict of interest.**

All authors declare that they have no conflict of interest.

## REFERENCES

1. Linares J, Ardanuy C, Pallares R, Fenoll A (2010) Changes in antimicrobial resistance, serotypes and genotypes in *Streptococcus pneumoniae* over a 30-year period. *Clin Microbiol Infect* 16: 402-410.
2. Cardozo DM, Nascimento-Carvalho CM, Andrade AL, Silvany-Neto AM, Daltro CH, et al. (2008) Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents. *J Med Microbiol* 57: 185-189.
3. Soewignjo S, Gessner BD, Sutanto A, Steinhoff M, Prijanto M, et al. (2001) *Streptococcus pneumoniae* nasopharyngeal carriage prevalence, serotype distribution, and resistance patterns among children on Lombok Island, Indonesia. *Clin Infect Dis* 32: 1039-1043.
4. Farida H, Severin JA, Gasem MH, Keuter M, van den Broek PJ, et al. (2013) Nasopharyngeal carriage of *Klebsiella pneumoniae* and other Gram-negative bacilli in pneumonia-prone age groups in Semarang, Indonesia. *J Clin Microbiol* 51: 1614-1616.
5. Center for Disease Control and Prevention (CDC) (2011) PCR Deduction of Pneumococcal Serotypes.
6. Saha SK, Darmstadt GL, H.Baquai A, Hossain B, Arifeen SE, et al. (2008) Identification of serotype in culture negative pneumococcal meningitis using sequential multiplex PCR : Implication for surveillance and vaccine design. *PLoS One* 3: e3576.
7. The Ministry of Health of Republic Indonesia (1999) Peraturan Menteri Kesehatan Republik Indonesia No 829/1999 tentang Persyaratan Rumah Sehat (The Decree of The Minister of Health Republic of Indonesia Number 829/ 1999 regarding with Health Requirements of Housing). In: The Ministry of Health Republic Indonesia, editor.
8. Bogaert D, Engelen MN, Timmers-Reker AJM, Elzenaar KP, Peerbooms PGH, et al. (2001) Pneumococcal carriage in children in the Netherlands: A molecular epidemiological study. *J Clin Microbiol* 39: 3316-3320.
9. Hill PC, Townend J, Antonio M, Akisanya B, Ebruke C, et al. (2010) Transmission of *Streptococcus pneumoniae* in rural Gambian villages: A longitudinal study. *Clin Infect Dis* 50: 1468-1476.
10. Korona-Glowniak I, Niedzielski A, Malm A (2011) Upper respiratory colonization by *Streptococcus pneumoniae* in healthy pre-school children in south-east Poland. *Int J Pediatr Otorhinolaryngol* 75: 1529-1534.
11. Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS (2010) Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. *BMC Infect Dis* 10: 304.
12. Levine S, Dejsirilert S, Sangsuk L, Chantra S, Feikin DR, et al. Serotypes and antimicrobial resistance of *Streptococcus pneumoniae* in Thailand 2002–2004. *The Paed Infect Disease J* 25: 176-178.
13. Bakhshae M, Naderi HR, Ghazvini K, Sotoudeh K, Amali A, et al. (2012) Passive smoking and nasopharyngeal colonization by *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in daycare children. *Eur Arch Otorhinolaryngol* 269: 1127-1132.
14. Kuo CY, Hwang KP, Hsieh YC, Cheng CH, Huang FL, et al. (2011) Nasopharyngeal carriage of *Streptococcus pneumoniae* in Taiwan before and after the introduction of a conjugate vaccine. *Vaccine* 29: 5171-5177.
15. Rudolph KM, Parkinson AJ, Reasonover AL, Bulkow LR, Debra J. Parks, et al. (2000) Serotype distribution and antimicrobial resistance patterns of invasive isolates of *Streptococcus pneumoniae*: Alaska, 1991–1998. *J Infect Dis* 182: 490-496.
16. Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan - Kementerian Kesehatan Republik Indonesia (Directorate General Disease Control and Environmental Health- the Ministry of Health Republic Indonesia) (2010) Modul tatalaksana standar pneumonia (Module for standard management of pneumonia). Jakarta: Kementerian Kesehatan RI (the Ministry of Health RI).
17. Tim Adaptasi Indonesia (2008) Buku saku pelayanan kesehatan anak di rumah sakit : Pedoman bagi rumah sakit rujukan tingkat pertama di kabupaten/kota Kementerian Kesehatan RI - World Health Organization - Ikatan Dokter Anak Indonesia.

18. Pfizer (2011) Pneumococcal 13-valent conjugate vaccine (diphtheria CRM197 protein). In: Inc WP, editor. USA.
19. Jauneikaite E, Jefferies JM, Hibberd ML, Clarke SC (2012) Prevalence of *Streptococcus pneumoniae* serotypes causing invasive and non-invasive disease in South East Asia: A review. *Vaccine* 30: 3503-3514.
20. Yuliarti K, Hadinegoro SR, Supriyatno B, Karuniawati A (2012) Invasive pneumococcal disease among hospitalized children aged 28 days to 60 months in Jarkarta. *Southeast Asian J Trop Med Public Health* 43: 136-144.
21. Purniti PS, Subanada IB, Kari IK, Arhana B, Iswari IS, et al. (2011) Surveilans pneumokokus dan dampak pneumonia pada anak balita. *Sari Pediatri* 12: 359-364.



# Chapter 6

## Nasopharyngeal carriage of *Klebsiella pneumoniae* in pneumonia- prone age groups in Semarang area, Indonesia

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## ABSTRACT

Gram-negative bacilli (GNB) cause many pneumonia cases in Indonesia. We investigated nasopharyngeal carriage of GNB in Semarang, Indonesia. *K. pneumoniae* carriage in adults (15%) was higher than in children (7%,  $P=0.004$ ), while that of other-GNB was comparable. Poor food and water hygiene were determinants of carriage.

## INTRODUCTION

Worldwide, *Streptococcus pneumoniae* is considered the most common cause of community-acquired pneumonia (CAP). However, in tropical low-to-middle-income countries, *Klebsiella pneumoniae* and other Gram-negative bacilli (GNB) are reported as frequent causes of CAP, more often than in high-income countries [1]. In a recent study on CAP etiology in Indonesia, *K. pneumoniae* found as the most frequent agent (own observation, to be published elsewhere).

Since nasopharyngeal colonization may precede pneumonia, as demonstrated for *S. pneumoniae* [2], we investigated the prevalence of nasopharyngeal carriage of *K. pneumoniae* and other-GNB among young children and adults in Indonesia, and that of *S. pneumoniae* as benchmark.

## METHODS

Subjects were healthy children aged 6-60 months and healthy adults aged 45-70 years, free from respiratory symptoms, had no antibiotic consumption within the last three days, and living in Semarang, a city with 1.5 million residents in Central Java, Indonesia. Cluster random sampling was done from February to April 2010 to recruit subjects from all 16 districts of Semarang.

Nasopharyngeal swabs were obtained using rayon-tipped swabs and transported in Amies media (COPAN, Italy). Swabs were inoculated on 5% sheep blood-gentamycin 5µmg/mL agar and on MacConkey agar. Plates were incubated at 35°C in 5%CO<sub>2</sub> for 48 hours. All GNB and putative *S.pneumoniae* isolates were subcultured and stored in -80°C, and sent to Erasmus MC Rotterdam for identification using Vitek<sup>®</sup> 2 (bioMérieux, Marcy l'Etoile, France) and optochin test (Oxoid, Basingstoke, UK). Antimicrobial for the GNB susceptibility tests were performed using Vitek<sup>®</sup> 2, interpreted according to CLSI 2010. Control strains were included in all analyses.

A questionnaire was developed to identify determinants of carriage. Data on demography, sources of water used for family needs (drinking, preparing food, bathing), food hygiene and house sanitation (crowding, flies, smoke exposure from cigarette and mosquito coils), were collected. Water hygiene was considered poor when river water, well water, water sold by mobile vendors, or water from mobile tanks was used. Food hygiene was considered poor if bought from street vendors. Crowding was considered if the ratio of total bedroom space to the number of family members was <4 m<sup>2</sup> [3]. Univariate analysis was done with Chi-square or Fisher's exact tests when appropriate, followed by backward stepwise logistic regression for variables with *P* value <0.2 using SPSS 17 (SPSS Inc, Chicago, USA). Confidence interval (CI) was calculated on 95% level and

*P* values <0.05 were considered significant. The study was approved by The Ethical Committee of the Faculty of Medicine-Diponegoro University, Semarang. Written informed consent was given by the subjects or their caregivers.

## RESULTS

Two hundred and fifty-three adults aged 45–70 years and 243 children aged < 6 years participated in the study; approximately one fourth resided in suburban area, and the majority was living under hygienically restricted circumstances regarding their food and water supplies. Crowding was common, as was exposure to smoke (Table 1).

**Table 1.** Characteristics of the study participants

		Children N=243 n (%)	Adults N=253 n (%)	Total N=496 n (%)	<i>p</i>
Gender	Male	118 (49)	94 (37)	212 (43)	0.01
	Female	125 (51)	159 (63)	284 (57)	
Age	Median	2.1 yrs	55 yrs		
	Range	0.5-5 yrs	45-75 yrs		
Residence	Urban	180 (74)	186 (74)	366 (74)	0.9
	Suburban	63 (26)	67(26)	130 (26)	
Food hygiene	Good	81 (33)	107 (42)	188 (38)	0.04
	Poor	162 (67)	146 (58)	308 (62)	
Water hygiene	Good	114 (47)	119 (47)	233 (47)	0.9
	Poor	129 (53)	134 (53)	263 (53)	
Crowding *)	No	129 (56)	157 (66)	286 (61)	0.03
	Yes	100 (44)	80 (34)	180 (39)	
Mosquito coils use	No	184 (76)	170 (67)	354 (73)	0.04
	Yes	59 (24)	83 (33)	142 (29)	
Active smoking	No	243 (100)	200 (79)	443 (89)	0.01
	Yes	0 (0)	53 (21)	53 (11)	
Passive smoking	No	97 (40)	106 (42)	203 (41)	0.7
	Yes	146 (60)	147 (58)	293 (59)	
Contact with toddler(s)	No	111 (46)	116 (46)	227 (46)	0.9
	Yes	132 (54)	137 (54)	269 (54)	

\*) Only 366 subjects responded to this question

Overall, the carriage rate of *K. pneumoniae* and other-GNB was 11% (95% CI, 7-14) and 19,5 % (95% CI, 12-23) respectively. *K. pneumoniae* carriage in adults (15%) was higher than in children (7%,  $P=0.004$ ), while that of other-GNB was comparable (20% vs 19%,  $P=0.7$ ) (Table 2). As benchmark, overall carriage of *S. pneumoniae* was 27 % (95% CI, 20-32), 43% in children, and 11% in adults. The carriage of these bacteria together in combination was common. The presence or the absence of carriage of one species was not correlated to that of other species ( $P >0.1$ ). The proportion of carriage of these bacteria was not similar across the districts of Semarang, rather, carriage rates varied significantly and tended to be higher in the suburban and eastern parts of the city.

Fifty-four *K. pneumoniae* strains were isolated from 54 subjects. Only one strain was resistant to cefotaxime and ceftriaxone, but the presence of an extended-spectrum beta-lactamase (ESBL) could not be confirmed using ESBL confirmatory E-test. The remaining strains were all susceptible to the common antibiotics, including cefuroxime, but not to nitrofurantoin (43% being resistant or intermediate).

Table 2. Distribution of nasopharyngeal colonization among young children and adults in Semarang, Java, Indonesia

Carriage type	Children N = 243 n (%)	Adults N = 253 n (%)	Total N = 496 n (%)	P
Non-carrier	107 (44)	157 (62)	264 (53)	0.000
Total of <i>K. pneumoniae</i> carrier	16 (7)	38 (15)	54 (11)	0.004
Total of other-GNB*) carrier	49 (20)	47 (19)	96 (19)	0.7
Total of <i>S. pneumoniae</i> carrier	105 (43)	28 (11)	133 (27)	0.000
<i>K. pneumoniae</i> only	5 (2)	25 (10)	30 (6)	0.000
Other-GNB only	22 (9)	39 (15)	61 (12)	
<i>S. pneumoniae</i> only	78 (32)	17 (7)	95 (19)	
<i>K. pneumoniae</i> and <i>S. pneumoniae</i>	4 (2)	7 (3)	11 (2)	
Other-GNB and <i>S. pneumoniae</i>	20 (8)	2 (1)	22 (4)	
<i>K. pneumoniae</i> and Other-GNB	4 (2)	4 (2)	8 (2)	
<i>K. pneumoniae</i> , Other-GNB, and <i>S. pneumoniae</i>	3 (1)	2 (1)	5 (1)	

\*) GNB = Gram-negative bacilli

One hundred and seven GNB strains other than *K. pneumoniae* were found in 96 (19 %) subjects, of whom 49 were children and 47 were adults. The three most frequently isolated other-GNB were *Pseudomonas* spp, *Enterobacter* spp and *Acinetobacter* spp (Table 3). Antimicrobial susceptibility test for Enterobacteriaceae other than *K. pneumoniae* showed sensitivity of 100% to carbapenem, and of >95% to piperacillin-tazobactam, 3rd generation cephalosporins, gentamicin, ciprofloxacin. All *Pseudomonas* spp

isolates were susceptible to tobramycin, meropenem, ciprofloxacin, 67% were susceptible to ceftazidime, and 87% were susceptible to colistin.

Multivariate analysis showed that being an adult (OR 2.2, 95% CI, 1.1-4.2), poor water hygiene (OR 2.1, 95% CI, 1.1-3.9), poor food hygiene (OR 2.2, 95% CI, 1.1-4.3), and frequent use of mosquito coils (OR 1.8, 95% CI, 1.1-4.3) were independent determinants for *K. pneumoniae* carriage. Poor water hygiene (OR 1.8, 95% CI, 1.1-2.9), poor food hygiene (OR 1.8, 95% CI 1.1-2.7) and being a female (OR 1.9, 95% CI, 1.2-3.1) were independent risk factors for carriage of other-GNB.

**Table 3.** Nasopharyngeal carriage of Gram-negative bacilli (GNB)

Species	Children N = 243 n (%)	Adults N = 253 n (%)	Total N = 496 n (%)
<i>K. pneumoniae</i>	16 (7)	38 (15)	54 (11)
Other-GNB	56 **	51 **	107**
<i>Pseudomonas spp</i>	16 (7)	9 (4)	25 (5)
<i>Enterobacter spp</i>	11 (5)	10 (4)	21 (4)
<i>A.baumannii complex</i>	11 (5)	3 (1)	14 (3)
<i>Citrobacter spp</i>	0 (0)	11 (4)	11
<i>Aeromonas spp</i>	6 (2)	3 (1)	9
<i>Pantoea spp</i>	6 (2)	3 (1)	9
<i>Serratia spp</i>	0 (0)	4 (2)	4 (1)
<i>E.coli</i>	0 (0)	3 (1)	3 (1)
<i>S.maltophilia</i>	1 (1)	1 (1)	2 (1)
Other non-lactose fermenting bacilli *	5 (2)	4 (2)	9

\* Other non-fermenting bacilli were *C. testosteroni*, *L. adecarboxylata*, *R. radiobacter*, *P. vulgaris*, *B. militensis*, *S. algae*, *S. spiritivorum*

\*\* six children carried 2 other-GNB, 4 adults carried 2 other-GNB

## DISCUSSION

This is the first population-based study of nasopharyngeal carriage of GNB in Indonesia, a large low-to-middle-income Southeast-Asia country. The carriage rate of GNB was surprisingly high, exceeding that of *S. pneumoniae* in adults. Overall 30% of the 496 participants carried GNB, with *K. pneumoniae* as the commonest GNB species (11%) which is significantly higher among adults as compared to children. Few publications are available regarding nasopharyngeal carriage by *K. pneumoniae* or GNB, since these

bacteria usually are not considered as commensals in the nasopharyngeal space of healthy individuals. *K. pneumoniae* and other-GNB carriage is usually related to specific conditions, such as preceding antibiotic therapy, underlying diseases or long hospital stay [4]. A study performed in Brazil, a high middle-income country (World Bank 2011) found that the carriage rate of GNB among 1192 children attending day care was 9%, but *K. pneumoniae* was found in only 1.4% of the subjects [5]. Our study showed that carriage of *K. pneumoniae* and other-GNB in healthy individuals in Semarang is much more common. *K. pneumoniae* carriage was related to poor hygiene, particularly water and food hygiene, and to mosquito coil smoke exposure. The determinants of carriage by other-GNB were also related to poor water and food hygiene.

*K. pneumoniae* and many other-GNB present in the enteric micro-flora may serve as a source of water contamination particularly in countries with poor sanitation [6]. People who use contaminated water daily could, thus, become colonized. Food preparation using contaminated water or by contaminated persons may increase transmission to the oronasopharynx. A study in Malaysia, a neighbour country of Indonesia, found that 32% of street food in Malaysia were contaminated by *K. pneumoniae* [7].

Mosquito coils were also found as a *K. pneumoniae* carriage determinant. It had been demonstrated that one mosquito coil would release the same amount of particulate matter mass as burning 75-137 cigarettes [8].

The frequent carriage of *K. pneumoniae* and other-GNB may explain the frequent finding of these potential pathogens as etiology of pneumonia in previously healthy individuals in this part of the world [1]. However, the virulence profile of these isolates requires further study. Importantly in this respect, almost all strains of *K. pneumoniae* were found to have the wild type susceptibility to antimicrobial agents used for routine treatment of pneumonia, similar to those found in CAP patients in Semarang (own observation, will be published elsewhere). This finding also confirms findings from previous surveillance studies in the same community, where only few acquired resistance was found among enteric bacilli isolated from healthy people living in the community [9,10].

In conclusion, nasopharyngeal carriage of *K. pneumoniae* and other-GNB is common among healthy people in Indonesia, and is associated in part with exposure to external sources of these organisms in water and food. However, the pool of GNB found in the nasopharyngeal spaces of healthy individuals represents wild type strains in that they remain susceptible to antibiotics.

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**Conflict of interest.**

All authors declare that they have no conflict of interest

## REFERENCES

1. Liam CK, Pang YK, Poosparajah S (2006) Pulmonary tuberculosis presenting as community-acquired pneumonia. *Respirology* 11: 786-782.
2. Cardozo DaM, Nascimento-Carvalho CM, Andrade A-LcSS, Silvany-Neto AbM, Daltro CHC, et al. (2008) Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents. *J Med Microbiol* 57: 185-189.
3. The Ministry of Health of Republic Indonesia (1999) Peraturan Menteri Kesehatan Republik Indonesia No 829/1999 tentang Persyaratan Rumah Sehat (The Decree of The Minister of Health Republic of Indonesia Number 829/ 1999 regarding with Health Requirements of Housing). In: The Ministry of Health Republic Indonesia, editor.
4. Parm Ü, Metsvaht T, Sepp E, Ilmoja M-L, Pisarev H, et al. (2011) Risk factors associated with gut and nasopharyngeal colonization by common Gram-negative species and yeasts in neonatal intensive care units patients. *Early Hum Dev* 87: 391-399.
5. Lima ABM, Leão LSNdO, Oliveira LSdC, Pimenta FC (2010) Nasopharyngeal Gram-Negative Bacilli Colonization In Brazilian Children Attending Day-Care Centers. *Braz J Microbiol* 41: 24-27.
6. Panda SK, Patra AK, Kar RN (2012) Monitoring of multiple drug-resistant pathogens in a selected stretch of Bay of Bengal, India. *Environ Monit Assess* (2012) 184: 193-200.
7. Haryani Y, Noorzaleha AS, Fatimah AB, Noorjahan BA, Patrick GB, et al. (2007) Incidence of *Klebsiella pneumoniae* in street foods sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD-PCR analysis. *Food Cont* 18: 847-853.
8. Liu W, Zhang J, Hashim JH, Jalaludin J, Hashim Z, et al. (2003) Mosquito coil emissions and health implications. *Environ Health Perspect* 111: 1454-1460.
9. Lestari ES, Severin JA, Filius PMG, Kuntaman K, Duerink DO, et al. (2008) Antimicrobial resistance among commensal isolates of *Escherichia coli* and *Staphylococcus aureus* in the Indonesian population inside and outside hospitals. *Eur J Clin Microbiol Infect Dis* 27: 45-51.
10. Severin JA, Lestari ES, Kloezen W, Toom NL-d, Mertaniasih NM, et al. (2012) Faecal carriage of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae among humans in Java, Indonesia, in 2001-2002. *Trop Med Int Health* 17: 455-461.



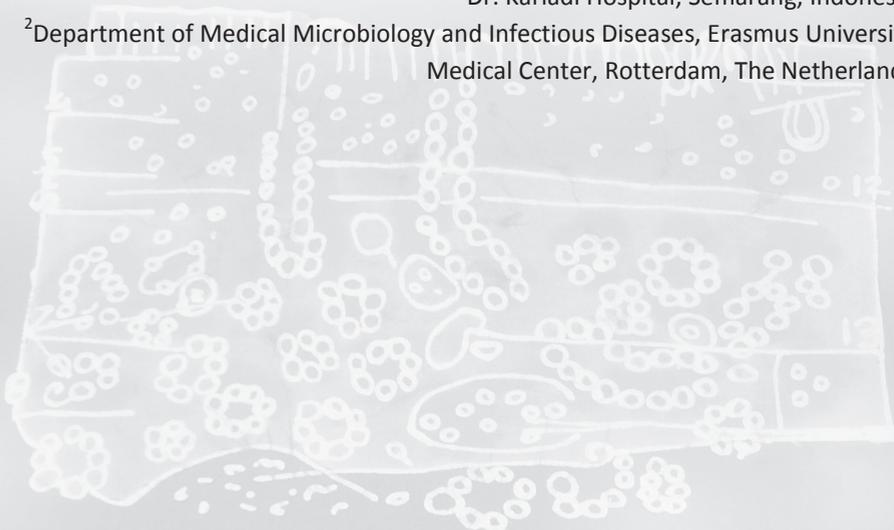
# Chapter 7

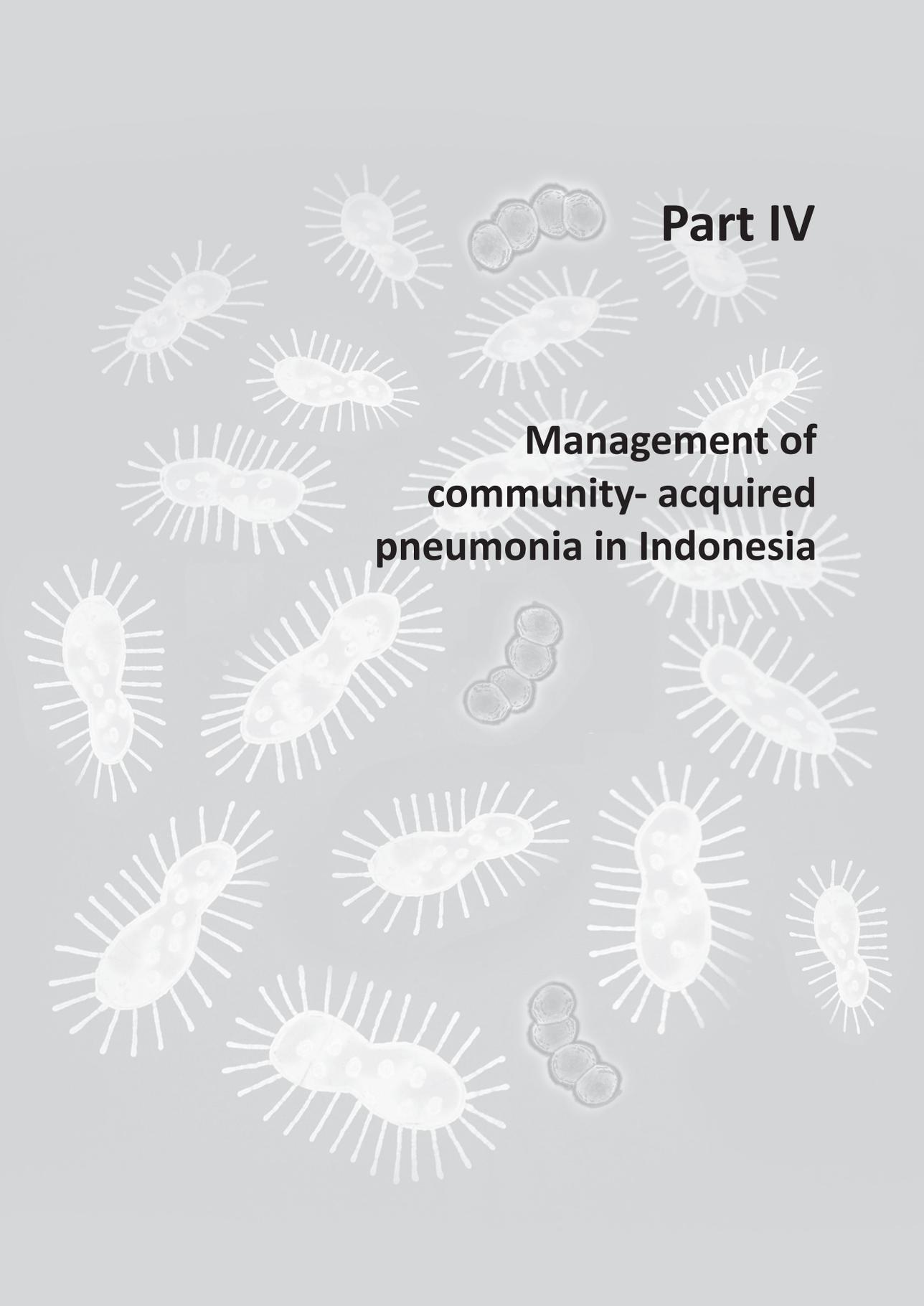
## Diversity among *Klebsiella pneumoniae* isolated from healthy subjects and pneumonia patients in a tropical country

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## Part IV

# Management of community- acquired pneumonia in Indonesia



# Chapter 8

## Development of quality indicators to evaluate antibiotic treatment of patients with CAP in Indonesia

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## **ABSTRACT**

### **Objective**

Appropriate antibiotic therapy is very important in the management of patients with community-acquired pneumonia (CAP). To evaluate the quality of care for CAP patients in particular antibiotic management, a qualified instrument is needed. This study aimed to develop such an instrument, which is applicable in a middle-income developing country setting.

### **Methods**

A previous study and Indonesian guidelines were reviewed to derive potential quality of care indicators (QIs). An expert panel performed a two-round Delphi consensus procedure on the QI's relevance to patient recovery, reduction of antimicrobial resistance, and cost containment. Applicability in practice including reliability, feasibility, and opportunity for improvement were determined in a data set of 128 hospitalized CAP patients in Semarang, Indonesia.

### **Results**

Fifteen QIs were selected by the consensus procedure. Five QIs did not pass feasibility criteria, because of inappropriate documentation, inefficient laboratory services, or patient factors. Three QIs provided minor opportunity for improvement. Two QIs contradicted each other; one of which was considered not valid and excluded. A final set of six QIs was defined for use in the Indonesian setting.

### **Conclusions**

Using the Delphi method, we defined a list of QIs for assessing the quality of care, in particular antibiotic treatment, for CAP in Indonesia. For further improvement, a modified Delphi method which includes discussion, a sound medical documentation system, improvement of microbiology laboratory services, and multi-center applicability tests are needed to develop a valid and applicable QI list for the Indonesian setting.

## INTRODUCTION

Community-acquired pneumonia (CAP) is a significant cause of morbidity and mortality, and an economic burden. In low-to middle-income countries, it is one of the most important causes of death [1]. A study done in Semarang, Indonesia, during 2007-2009 showed a high overall mortality of 30% in patients hospitalized with CAP, increasing from 17% in non-severe to 70% in severe CAP (personal data, unpublished). The mortality in this study was much higher than in other studies in Southeast Asia [2,3], indicating that the quality of patient care needs evaluation.

Appropriate antibiotic therapy is one of the most important elements in the management of CAP [4]. To evaluate the quality of care for CAP patients and to give health care professionals feedback, in particular regarding antibiotic management, a qualified instrument is needed. Such an instrument must contain validated quality indicators (QIs), have good feasibility and reliability, and provide opportunities for improvement. This instrument can initiate intervention programs to improve the quality of care of patients with CAP [5].

If possible, QIs should be based on scientific evidence such as rigorously conducted (trial based) empirical studies [6]. However, like in many fields of medicine, the scientific basis for strong recommendations on antibiotic treatment in CAP, i.e. based on randomized controlled trials (RCTs), is limited. For some aspects of treatment, e.g. comparing early and late of antibiotic administration, RCTs are considered unethical [7]. Therefore, as substitution for development of valid QIs in case of insufficient, absent, or methodologically weak evidence, a systematic procedure that combines the available evidence and expert opinion should be used (Rubin et al., 2001). A method of combining evidence and expert opinion is the Delphi method [8]. Using this method, a large group of experts can be consulted in several rounds with feedback of the results between rounds.

This study aimed to develop a list of QIs for assessing the quality of care, especially antibiotic treatment, for patients with CAP which is applicable in Indonesia, a middle-income country, by adapting the methods applied in a previous study in a developed country [4].

## METHODS

### Development of the list of quality indicators

A list of potential QIs describing the process of care for CAP patients was made up from a previous study [4], and an Indonesian national guideline [9] which is mainly based on international guidelines with some local contents in 2007. Levels of evidence as described by Guyatt et al. (1995) were assigned to indicators derived from the Indonesian

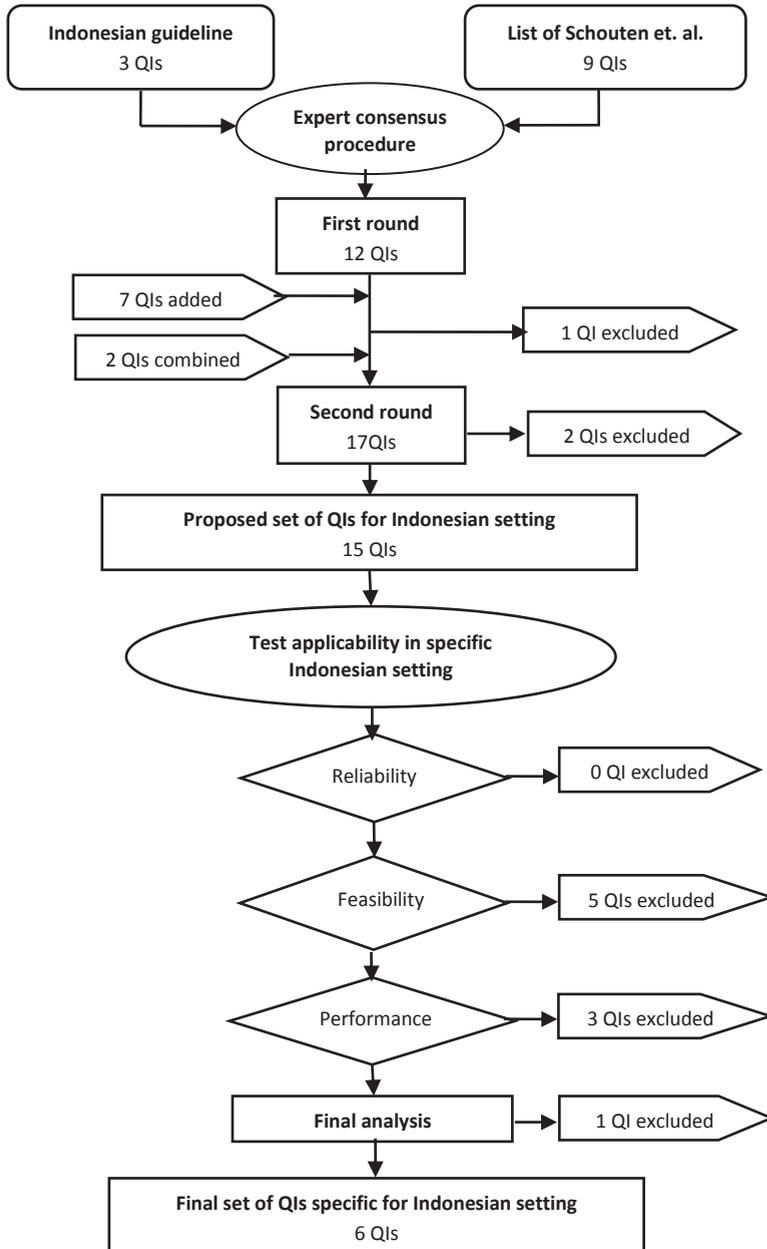
guideline by three investigators (KL, MK, MHG) using literatures obtained from a search in PubMed using the MeSH term [quality indicators] and [pneumonia]. Since there was no MeSH term for "community acquired pneumonia", the complete term was used to narrow down the search a little bit more. To specify more, the search terms "treatment" and "diagnosis" were added. Literature search was not done for indicators derived from the previous study as this has been systematically performed [4].

### **Rating and adding procedure by expert panel**

The list was presented as a questionnaire to a panel of eighteen specialists from different fields of medicine (ten internists, three internist-pulmonologists, two pharmacists, three clinical microbiologists) working in four different hospitals in Semarang, Indonesia. Using the Delphi method, in a two-round consensus procedure, the panel judged the potential indicators on (1) relevance for clinical recovery of the patient, (2) relevance for reduction of antimicrobial resistance, and (3) relevance for cost-effectiveness. A five-category Likert-scale was used that ranged from "completely agree" (category 1) to "completely disagree" (category 5). An extra answer category could be marked if the experts could not decide about a particular question.

In the first round, the experts had the opportunity to comment on the proposed indicators and to add or modify potential indicators for evaluation. An indicator was selected if >70% of the experts scored 1 ('completely agree') or 2 ('agree'). An indicator was rejected if > 70% of the experts scored 4 ('disagree') or 5 ('completely disagree'). Indicators which did not meet these criteria were reevaluated in the second round using the same criteria as applied in the first round, together with indicators added or modified by the experts during the first round [10]. Indicators that had met the selection and rejection criteria were also re-assessed in the second round to provide opportunity for the experts if they wanted to change their rating. This process could be repeated several times in order to achieve agreement for selection or rejection of all the proposed indicators among the experts. An indicator that still did not meet the selection or rejection criteria after several rounds will be removed as it is an indication that the indicator is weak or of low value.

The evidence level of indicators added by the experts during the consensus rounds were also assessed based on a search in PubMed. An indicator was considered not valid and excluded if contradictory evidence was identified.



**Figure 1.** Flowchart showing the steps in the development of quality indicators (QIs) for assessing and improving the quality of care related to antibiotic use in patients with community-acquired pneumonia in Semarang, Indonesia.

### **Assessment of applicability of quality indicators in a specific patient sample**

Study setting, population and data collection. Due to limited resource, the applicability study could only be done in 2012-2013. Data were retrieved retrospectively from all 128 medical files of patients involved in a CAP study from 2007-2009 in Dr. Kariadi Hospital, a university hospital in Semarang, Indonesia. Information was retrieved from admission sheets, medical and nursing records, and medication charts. Two reviewers (AR and AA) worked together to collect all the data and recorded them on printed standardized forms, then entered these data into one database.

*Reliability.* To test the inter-observer reliability of the data collection, 15 (11%) of the 128 patient medical files were randomly chosen. An independent group of two reviewers (HF and MHG) recorded the data from these files together on the same standardized forms. Inter-observer reliability was calculated as Kappa ( $\kappa$ ) scores. Scores of  $0.41 \leq \kappa \leq 0,6$  were considered to be moderate,  $0.61 \leq \kappa \leq 0.8$  good, and  $\kappa > 0.8$  very good. Scores of  $\kappa < 0.4$  were considered to be poor and led to elimination of the indicator (Landis and Koch, 1977).

*Feasibility.* Feasibility of data collection was defined as the percentage of missing values per indicator (i.e. the percentage of indicator values that could not be calculated because  $\geq 1$  element of the algorithm could not be retrieved from the available records). Indicators with feasibility  $\geq 25\%$  (meaning that  $\geq 25\%$  of the data were missing) were considered having poor feasibility and not selected (Schouten et al., 2005)

*Performance score.* The performance score is the rate of compliance toward a QI, which must be low enough to give the opportunity for improvement (Rubin et al., 2001). If the performance score for a QI is  $\geq 85\%$  (meaning the compliance is already high), the indicator is less sensitive to change or to make improvement, thus, not selected.

## **RESULTS**

### **Development of the set of quality indicators**

The process of the development of the list of QIs is summarized in Figure 1. Twelve QIs, nine derived from a Dutch study [4] and three from the Indonesian guideline, were presented to the expert panel. Seven new indicators were added by the panel. After the two round-consensus procedure three QIs were rejected: one ('urinary antigen test for *Legionella* spp upon clinical suspicion') because the tests were not available in Indonesia, one ('initiation of antibiotic treatment within 8 hours') because the experts thought that initiating antibiotic within four hours was better than eight hours, and one ('empirical antibiotic regimen according to local guidelines') because not all hospitals had a local

guideline. Two indicators, 'change broad-spectrum empirical into pathogen-directed therapy (streamlining)' and 'change empirical therapy according to culture if the patient does not improve or worsens after 24-72 hours of empirical therapy', were combined into one indicator: 'change broad-spectrum empirical into pathogen-directed antibiotic therapy (streamlining) once the culture result is available'.

The result was a final list of fifteen QIs (Table 1).

### **Assessment of the applicability of the QIs**

The reliability, feasibility, and performance score of the fifteen QIs were presented in Table 1.

*Reliability.* No indicator was rejected because of poor reliability (Table 1). One indicator scored  $\kappa = 0.412$  which means a moderate inter-observer reliability ('stop antibiotic therapy after three consecutive days of defervescence'). All other indicators scored  $\kappa > 0.6$ .

*Feasibility.* Five QIs did not pass feasibility criteria: 'transport sputum sample to the laboratory within 4 hours', 'obtain blood samples for culture from the left and the right arm', 'change broad-spectrum empirical into pathogen-directed therapy (streamlining) once culture result available', 'calculate unit cost' and 'evaluate albumin level before starting antibiotic treatment' (Table 1). For the QI 'evaluate albumin level before starting antibiotic treatment', feasibility was 100% because we were unable to make the indicator measurable. Although the time at which the albumin level is issued by the laboratory and time at which the antibiotic was started were usually recorded, we could not make sure that the doctors did evaluate the albumin level before starting the antibiotic. We could not rule out the possibility that the doctors order the albumin test for other purpose. For the other four QIs feasibility was poor because many data could not be retrieved from the medical records.

*Performance score.* Three QIs did not appear to provide opportunity for improvement, these are 'empirical antibiotic regimen according to national guidelines', 'adapt dose and dose interval of antibiotics to renal function', and 'make an X-ray at the emergency department' (Table 1).

Table 1. Summary of the process to develop the list of quality indicators (QIs) and assessment of applicability in Indonesian setting

Recommendation	Supporting evidence level <sup>a</sup>	Delphi method	Applicability tests			
			Reliability	Feasibility <sup>b</sup>	Performance <sup>c</sup>	
1. Make an X-ray at the emergency department	B [1,1]	Added	Selected	1	10.2	90.4 <sup>e</sup>
2. Take two sets of blood samples for culture	B [12,13]	Selected		1	0	0
3. Obtain blood samples for culture from the left and the right arm	D	Added	Selected	1	100 <sup>d</sup>	NA <sup>f</sup>
4. Obtain sputum samples for Gram stain and culture	D [14,15]	Selected		1	0	76.9
5. Transport sputum sample to the laboratory within 4 h	B [16]	Selected		0.7	73.0 <sup>d</sup>	46.7
6. Obtain sputum sample and start antibiotic therapy in the emergency department	D	Added	Selected	1	0.8	0
7. Urine antigen testing against <i>Legionella</i> spp. upon clinical suspicion	B [17]	Rejected <sup>f</sup>				
8. Timely initiation of antibiotic therapy (within 4 h after presentation)	B [18]	Selected		0.6	18.1	7.7
9. Timely initiation of antibiotic therapy (within 8 h after presentation)	B [9,19]	No decision	Rejected			

Table 1. Summary of the process to develop the list of quality indicators (QIs) and assessment of applicability in Indonesian setting

	Recommendation	Supporting evidence level <sup>a</sup>	Delphi method	Applicability tests		
				Reliability	Feasibility <sup>b</sup>	Performance <sup>c</sup>
10.	Empirical antibiotic regimen according to national guidelines	B [20-22]	Selected	1	0	90.6 <sup>e</sup>
11.	Empirical antibiotic regimen according to local guidelines	D	Added	Rejected <sup>g</sup>		
12.	Appropriate dose and dose interval of antibiotic with regard to renal function	D [4]	Selected	1	0	98.4 <sup>e</sup>
13.	Change broad-spectrum empirical into pathogen-directed antibiotic therapy (streamlining therapy)	C [14,15]	Combined into : Change broad-spectrum empirical into pathogen-directed antibiotic therapy (streamlining) once the culture result is available	1	41.2 <sup>d</sup>	4.0
14.	Change empirical therapy according to culture if the patient doesn't improve or worsens after 24-72 hours of empirical therapy	C [9]		Selected		
15.	Switching from iv to oral therapy, according to existing criteria and when clinically stable	B [23]	Selected	0,7	12.8	5.9

<sup>a</sup> According to the Evidence-Based Medicine Working Group, 1995 (Guyatt et al., 1995)

<sup>b</sup> Feasibility : % of patients with missing values

<sup>c</sup> Performance : % of patients in whom the indicator was performed

<sup>d</sup> Rejected because of the feasibility/ data missing (>25%)

<sup>e</sup> Rejected because of the performance (>85%)

<sup>f</sup> NA = Not applicable . The performance could not be calculated because of 100% data missing

<sup>g</sup> afterwards deleted due to lack of validity

\*CURB score: consciousness, blood ureum, respiratory rate, blood pressure

To summarize, seven QIs met all criteria for reliability ( $\kappa > 0.4$ ), feasibility (data missing  $< 25\%$ ) and performance ( $< 85\%$ ), indicating their applicability in (local) practice in Indonesia. These QIs are ‘take two sets of blood samples for culture’, ‘obtain sputum samples for Gram stain and culture’, ‘obtain sputum sample and start antibiotic therapy in the emergency department’, ‘timely initiation of antibiotic therapy within four hours after presentation’, ‘switching from iv to oral therapy when clinically stable’, ‘stop antibiotic therapy after three consecutive days of defervescence’, and ‘length of therapy is five days for uncomplicated CAP (CURB score  $\leq 2$ )’.

Though meeting all the applicability criteria, one QI (‘stop antibiotic therapy after three consecutive days of defervescence’) was judged not valid leaving a final set of six QIs.

## DISCUSSION

Using the Delphi method, we defined a list of QIs for assessing the quality of care, in particular antibiotic treatment, for patients with CAP in Indonesia. To our knowledge, this is the first study to develop QIs in Indonesia by using a scientifically sound method.

A two-round consensus procedure involving eighteen experts resulted in 15 QIs. In the applicability assessment in a specific Indonesian setting, five QIs were rejected because of poor feasibility. This was partly caused by the quality of medical records and the documentation of test results. Specific strategies need to be implemented to improve medical data documentation, so that important QIs will not be rejected solely based on the lack of data. The strategies range from relatively simple adjustments, such as simplifying and standardizing the format of the paper medical records, to a more complicated introduction of an electronic medical record system.

The absence of health insurance, or limited coverage of health insurance for diagnostic tests also determined the feasibility of data collection, as this was quite often the reason not to perform the recommended diagnostic tests, thereby limiting the availability of culture related data. Feasibility could also be poor as many doctors felt that the expensive cultures was of limited clinical value, for instance, patients had improved by the empirical antibiotic therapy, or the culture results frequently came too late: by the time the results came, the patient had already died or been discharged. Doctors also felt that the culture results were frequently difficult to interpret, in particular when the result was ‘sterile’, contaminant, or an uncommon respiratory pathogen. To improve the feasibility of the related QIs, microbiology laboratories need to improve their services, by shortening the turnaround time, and providing early and frequent clinical consultation to the clinicians.

Other causes of poor feasibility came from specific patient factors. Sputum cultures could not be obtained when patients were unable to produce sputum because of severe

CAP or of non-productive type cough, thereby, no data could be retrieved from the medical records to calculate the applicability. The proportion of patients with such specific factors may differ among different hospitals in different areas, underlining the importance of external validation of the QIs.

Three indicators were rejected because of providing minor opportunity for improvement. The indicator 'empirical antibiotic regimen according to national guidelines' achieved high performance since ceftriaxone, which is recommended by the national guideline as one of the first choices (aside  $\beta$ -lactam antibiotics +  $\beta$ -lactamase inhibitors), was cheap, covered by all available health insurance types, and had a relatively good susceptibility profile compared to other antibiotics. Therefore, ceftriaxone had already become the first choice for most infectious diseases including CAP in the studied hospital. Since ceftriaxone in routine dosing is safe for any kidney function level, the indicator 'appropriate dose and dose interval of antibiotic with regard to renal function' automatically achieved high performance. The indicator 'make an X-ray at the emergency department' was rejected because of high performance, possibly because the study was performed in an academic hospital that might not represent non-academic hospitals. A multicenter study is needed to increase generalizability of this QI list, as the performance may be lower in non-academic hospitals.

Two QIs appeared to contradict each other: 'length of therapy is five days for uncomplicated CAP (CURB score  $\leq 2$ )' which has a A2 level evidence [24,25], and 'stop antibiotic therapy after three consecutive days of defervescence' which was derived from expert opinion in a previous study (Schouten et al., 2005). The latter QI was accepted by the previous study because of no contradictory evidence in the year of the study (2005) and of its good applicability. However, we found that subsequent studies in 2006 have already used different parameters to make a decision to stop antibiotic, including fever, respiratory symptoms like dyspnea, coughing, and sputum production which are important clinical signs of CAP. Therefore, the decision to stop antibiotic therapy should rely not only on fever defervescence, but also on the improvement of respiratory symptoms and the general condition of the patient [26-28]. Thus, the indicator 'stop antibiotic therapy after three consecutive days of defervescence' had been not valid at the time of the Delphi rounds implementation, that should be removed despite its good applicability.

Contradiction between indicators in the QI list might come from the design of the Delphi method, in which each expert scored the questionnaires independently. The experts might have different perceptions on a proposed indicator because they consider different definitions, different patient groups, or different work conditions, which were specific for a particular expert but not generally applicable. The absence of a group discussion in Delphi method might raise the possibility of mis-perception between the experts with the observers who prepared, collected and analyzed the questionnaires rated

by the experts. This might happen with the indicators 'Evaluate albumin level before starting antibiotic treatment' and 'Calculate unit cost', for which the observers were unable to make them measurable. In addition, without a group discussion, the observers might miss the points why the experts agree or disagree or proposed specific indicators, which might not just include a scientific judgment, but also a local consideration. For instance, the QI 'Timely initiation of antibiotic therapy (within 4 h after presentation)' should have been removed since a stronger evidence published in 2005 [29] showed that early antibiotic therapy did not reduce mortality. However, this QI might also be kept when in the local setting, delayed giving antibiotic is common; thereby it is included as an indicator of care [30]. A modified Delphi method, such as the RAND method, which includes a group discussion between the consensus rounds, might be implemented to diminish the problems of contradiction or different perception [31].

The changing of validity status of an indicator from time to time, as happened with the indicator 'stop antibiotic therapy after three consecutive days of defervescence' underlines the necessity to always check its validity even after only few years of publication.

To keep in mind, methods for determining the quality of care of patients with CAP have also been developed by several other institutions [4,7]. The resulted QIs might differ in some points because of the difference in setting. For instance, there is no necessity to use 'give antibiotic according to the guideline' or 'appropriate dose and dose interval of antibiotic with regard to renal function' as a QI when the antibiotic is the first choice for most infectious diseases in this setting and does not need to be adjusted according to renal function. When a QI is invariably high with little inter-hospital variation, it becomes insensitive and less successful as an indicator for quality improvement [4], as the main goal of our list of QIs was to prioritize the indicators most needed for improvement of the patient care. In other hand, additional QIs might be proposed by local experts to improve problems which are specific to their setting. In our study, the experts added several QIs related to blood and sputum cultures ('Obtain blood samples for culture from the left and the right arm' or 'Transport sputum sample to the laboratory within 4 h', and 'Obtain sputum sample and start antibiotic therapy in the emergency department'), implying their hope to improve the problem of low clinical value of the cultures from contamination and failure to grow the important pathogens.

The use of valid and applicable QIs for CAP patients in Indonesia is emphasized by the fact that CAP is an important cause of mortality in this country. In addition, with the new Indonesian government policy to provide a national health insurance for all residents in Indonesia (BPJS Health Care/JKN) since January 2014 [32], it is urgent to implement appropriate QIs, as a measure to control the quality of health care and cost efficacy. Once implemented, the implication on health care and patient outcome need to be re-studied, as part of continuing improvement in the national health care system.

## **CONCLUSIONS**

This study provides a basis for further investigations for the development of a sound, valid set of QIs for the management of CAP in Indonesia. To improve the development of the QI list, a modified Delphi method which includes a discussion session may be more appropriate in the Indonesian setting. To improve the feasibility of the QIs, it is recommended that hospitals use a solid and sound medical record system and improve the services of microbiology laboratories to increase the clinical value of cultures. An external validation is needed to assess the generalizability of the QIs.

## **ACKNOWLEDGMENT**

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## **Conflict of Interest**

All authors declare no conflict of interest.

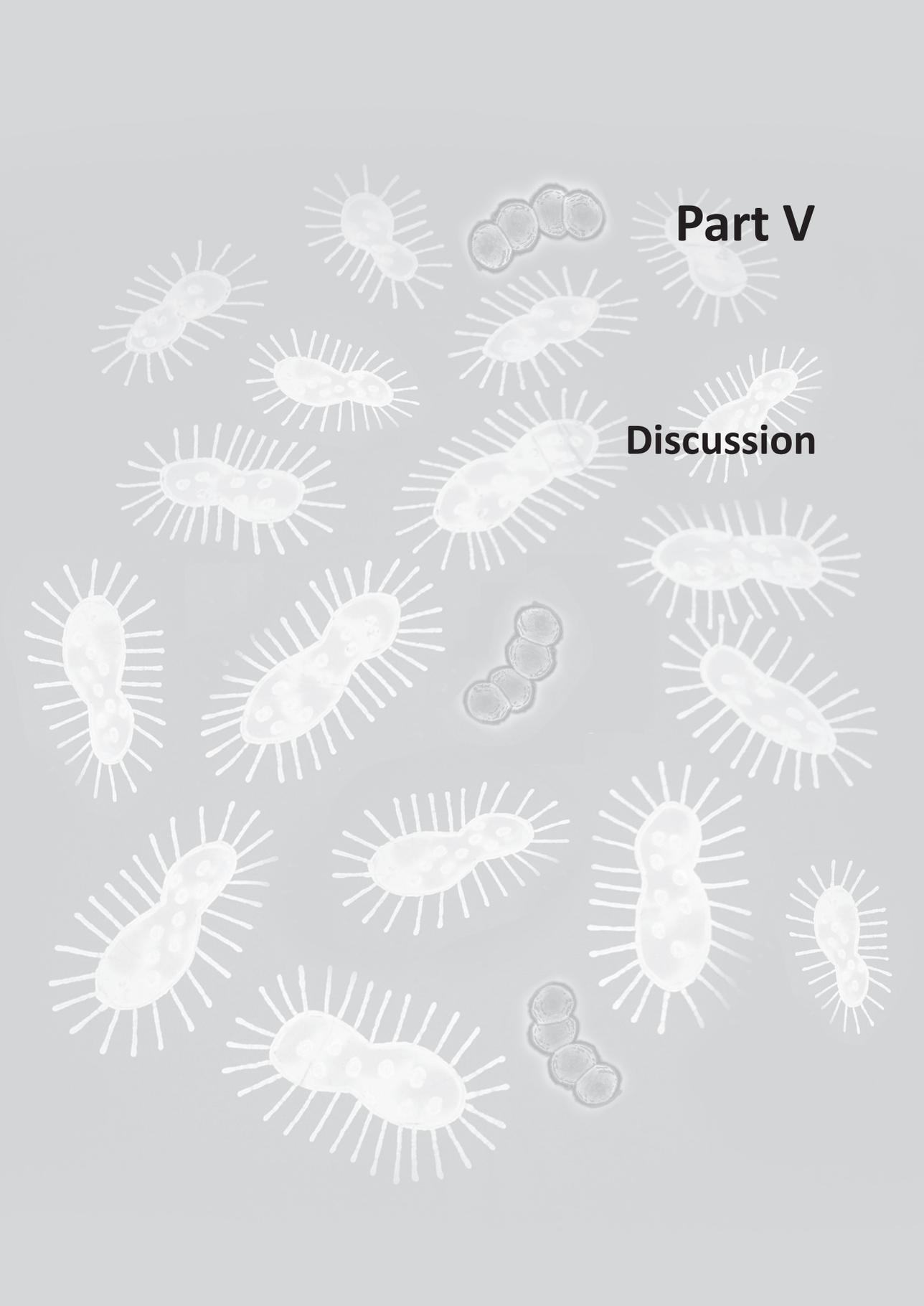
## REFERENCES

1. Gray D, Zar HJ (2010) Childhood pneumonia in low and middle income countries: Burden, prevention and management. *Open Infect Dis J* 4: 74-84.
2. Hara K, Yahara K, Gotoh K, Nakazono Y, Kashiwagi T, et al. (2011) Clinical study concerning the relationship between community-acquired pneumonia and viral infection in northern Thailand. *Intern Med* 50: 991-998.
3. Mustafa MIA, Al-Marzooq F, How SH, Kuan YC, Ng TH (2011) The use of multiplex real-time PCR improves the detection of the bacterial etiology of community acquired pneumonia. *Tropical Biomedicine* 28: 531-544.
4. Schouten JA, Hulscher ME, Wollersheim H, Braspenning J, Kullberg BJ, et al. (2005) Quality of antibiotic use for lower respiratory tract infections at hospitals: (how) can we measure it? *Clin Infect Dis* 41: 450-460.
5. Rubin HR, Pronovost P, Diette GB (2001) From a process of care to a measure: The development and testing of a quality indicator. *Int J Qual Health Care* 13 489-496.
6. Hearnshaw HM, Harker RM, Cheater FM, Baker RH, Grimshaw GM (2001) Expert consensus on the desirable characteristics of review criteria for improvement of health care quality. *Qual Health Care* 10: 173-178.
7. Seymann GB (2006) Community-acquired pneumonia: defining quality care. *J Hosp Med (Online)* 1: 344-353.
8. Hsu C-C, Sandford BA (2007) The Delphi technique: Making sense of consensus. *Practical Assessment Research and Evaluation*. pp. 1-8.
9. Perhimpunan Dokter Paru Indonesia (2003) *Pneumonia komunitas: Pedoman diagnosis & penatalaksanaan di Indonesia*. Jakarta.
10. Cantrill JA, Sibbald B, Buetow S (1998) Indicators of the appropriateness of long-term prescribing in general practice in the United Kingdom: consensus development, face and content validity, feasibility, and reliability. *Qual Health Care* 7: 130-135.
11. Marrie TJ, Majumdar SR (2005) Management of community-acquired pneumonia in the emergency room. *Respiratory Care Clinics of North America* 11: 15-24.
12. Campbell SG, Marrie TJ, Anstey R, Dickinson G, Ackroyd-Stolarz S (2003) The contribution of blood cultures to the clinical management of adult patients admitted to the hospital with community-acquired pneumonia: A prospective observational study. *Chest* 123: 1142-1150.
13. Waterer GW, Jennings SG, Wunderink RG (1999) The impact of blood cultures on antibiotic therapy in pneumococcal pneumonia. *Chest* 116: 1278-1281.
14. Lim WS, Baudouin SV, George RC, Hill AT, Jamieson C, et al. (2009) BTS Guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax* 64: iii1-iii55.
15. van Kasteren ME, Wijnands WJ, Stobberingh EE, Janknegt R, van der Meer J, et al. (1998) Optimization of the antibiotics policy in the Netherlands. II. SWAB guidelines for the antimicrobial therapy of pneumonia in patients at home and as nosocomial infections. *Ned Tijdschr Geneesk* 142: 952-956.
16. Jefferson H, Dalton HP, Escobar MR, Allison MJ (1975) Transportation delay and the microbiological quality of clinical specimens. *Am J Clin Pathol* 64: 689-693.
17. Lettinga KD, Verbon A, Weverling GJ, Schellekens JFP, Den Boer JW, et al. (2002) Legionnaires' disease at a Dutch flower show: Prognostic factors and impact of therapy. *Emerg Infect Dis* 8: 1448-1454.
18. Houck PM, Bratzler DW, Nsa W, Ma A, Bartlett JG (2004) Timing of antibiotic administration and outcomes for medicare patients hospitalized with community-acquired pneumonia. *Arch Intern Med* 164: 637-644.
19. Meehan TP, Fine MJ, Krumholz HM, Scinto JD, Galusha DH, et al. (1997) Quality of care, process, and outcomes in elderly patients with pneumonia. *JAMA* 278: 2080-2084.
20. Menendez R, Ferrando D, Valles JM, Vallterra J (2002) Influence of deviation from guidelines on the outcome of community-acquired pneumonia. *Chest* 122: 612-617.
21. Mortensen EM, Restrepo MI, Anzueto A, Pugh JA (2006) Antibiotic Therapy and 48-Hour Mortality for Patients with Pneumonia. *Am J Med* 119: 859-864.
22. Barlow GD, Lamping DL, Davey PG, Nathwani D (2003) Evaluation of outcomes in community-acquired pneumonia: A guide for patients, physicians, and policy-makers. *Lancet Infectious Diseases* 3: 476-488.
23. Ramirez JA, Vargas S, Ritter GW, Brier ME, Wright A, et al. (1999) Early switch from intravenous to oral antibiotics and early hospital discharge: A prospective observational study of 200 consecutive patients with community-acquired pneumonia. *Arch Intern Med* 159: 2449-2454.
24. Tellier G, Niederman MS, Nusrat R, Patel M, Lavin B (2004) Clinical and bacteriological efficacy and safety of 5 and 7 day regimens of telithromycin once daily compared with a 10 day regimen of clarithromycin twice daily in patients with mild to moderate community-acquired pneumonia. *J Antimicrob Chemother* 54: 515-523.

25. File TM, Jr., Mandell LA, Tillotson G, Kostov K, Georgiev O (2007) Gemifloxacin once daily for 5 days versus 7 days for the treatment of community-acquired pneumonia: a randomized, multicentre, double-blind study. *J Antimicrob Chemother* 60: 112-120.
26. el Moussaoui R, de Borgie CA, van den Broek P, Hustinx WN, Bresser P, et al. (2006) Effectiveness of discontinuing antibiotic treatment after three days versus eight days in mild to moderate-severe community acquired pneumonia: randomised, double blind study. *BMJ* 332.
27. Anzueto A, Niederman MS, Pearle J, Restrepo MI, Heyder A, et al. (2006) Community-Acquired Pneumonia Recovery in the Elderly (CAPRIE): efficacy and safety of moxifloxacin therapy versus that of levofloxacin therapy. *Clin Infect Dis* 42: 73-81.
28. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, et al. (2007) Infectious Diseases Society of America/American Thoracic Society. Consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 44: S27-S72.
29. Marrie TJ, Wu L (2005) Factors influencing in-hospital mortality in community-acquired pneumonia: a prospective study of patients not initially admitted to the ICU. *Chest* 127: 1260-1270.
30. Bordon J, Aliberti S, Duvvuri P, Wiemken T, Peyrani P, et al. (2013) Early administration of the first antimicrobials should be considered a marker of optimal care of patients with community-acquired pneumonia rather than a predictor of outcomes. *Int J Infect Dis* 17: e293-299.
31. Fitch K, Bernstein SJ, Aguilar MD, Burnand B, LaCalle JR, et al. (2001) RAND/UCLA Appropriateness method user's manual Santa Monica: RAND. pp. 123.
32. Menteri Hukum dan Hak Azasi Manusia Republik Indonesia, editor (2011) Undang - Undang Republik Indonesia Nomor 24 tahun 2011 tentang Badan Penyelenggara Jaminan Sosial Jakarta: Kementrian Sekretariat Negara.

**Appendix 1.** Supporting evidence level (Guyatt GH, 1995)

Evidence level	Definition	Example of a study providing the specified level of evidence
A1	A good systematic review of studies designed to answer the question of interest	Systematic review of randomized, controlled trials
A2	One or more rigorous studies designed to answer the question but not formally combined	Randomized, controlled trial
B	One or more prospective clinical studies that illuminate but do not rigorously answer the question	Prospective cohort study; underpowered or poor quality randomized controlled trial; nonrandomized, controlled trial
C	One or more retrospective clinical studies that illuminate but do not rigorously answer the question	Audit or retrospective case-control study
D	Formal combination of expert views or other information	Delphi study; expert opinion; informal consensus



**Part V**

**Discussion**



# Chapter 9

## General discussion and conclusions





## INTRODUCTION

In this thesis, we have studied the etiology and management of community-acquired pneumonia (CAP) in Indonesia in patients that were admitted to hospital, the characteristics of major respiratory pathogens carried by healthy individuals, the risk factors of carriage, and the differences between isolates from healthy individuals and those from patients with CAP. The development of a valid and applicable list of quality indicators (QIs) as a tool to evaluate the quality of care of CAP patients in Indonesia is described as well.

In this chapter, the main findings are summarized and discussed on the basis of the four research questions posed in chapter 1 of this thesis. Furthermore, recommendations for patient care and suggestions for further research on the different topics are presented.

### RESEARCH QUESTION 1 – THE ETIOLOGY OF CAP

*What are the pathogens causing CAP in Indonesia? What is the frequency of antimicrobial resistance of clinical isolates of CAP in Indonesia? Is there an association between etiology and the underlying diseases, severity of disease, and mortality ?*

From October 2007 until April 2009, 148 adult patients who were admitted to two hospitals in Semarang because of CAP were included in the study. Employing a wide range of the most advanced microbiological tests we found that influenza A and B viruses were the most common (18%) cause of CAP, either as single or co-pathogen, followed by *Klebsiella pneumoniae* (14%) and *Streptococcus pneumoniae* (13.5%). The next commonest pathogens (approximately 5% in incidence each) were *Mycobacterium tuberculosis*, *Chlamydia pneumoniae*, and other Gram-negative bacilli (GNB) including *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and *Burkholderia cepacia*. However, *K. pneumoniae* and other GNB together (19%) surpassed the incidence rate of influenza infection. Almost all bacterial pathogens had wild type susceptibility profiles, indicating that they remained susceptible to early generation antibiotics. The study also found that the crude 30-days mortality was very high (30%). The etiology did not relate to the underlying diseases, nor predicted the severity or mortality of CAP; however, the severity of CAP at the time of admission was predictive of mortality in this cohort of patients.

This study shows that the pattern of pathogens causing CAP in Indonesia is different from that in countries in North America, European countries, and Australia. The etiology

pattern found in this study is closer to the pattern previously observed in neighboring countries [1-4]. Therefore, a guideline for the management of CAP construed and implemented in North America, Europe, or Australia might not be appropriate in Indonesia. Multicenter CAP studies including viral diagnostics should be carried out in other cities in Indonesia or even in Southeast Asia to delineate the full spectrum of etiological agents in this region and, thus, yield the necessary information to develop or improve local guidelines for the management of CAP. An antibiotic which covers *K. pneumoniae* and other Gram-negative aerobic bacilli should be included in the empirical regimens for CAP in Indonesia. Because these bacterial agents still have wild type antibiotic susceptibility profiles, earlier generation antibiotics, for instance ampicillin-sulbactam or amoxicillin-clavulanate, would be the most appropriate choice, thereby avoiding the selection pressure exerted by newer generations and broad-spectrum antibiotics, especially third-generation cephalosporins and carbapenems [5]. Also, inclusion of an antiviral agent such as oseltamivir to treat influenza virus infection should be considered as part of an empirical regimen, especially in cases of severe CAP requiring intensive care. Our findings also unequivocally support seasonal influenza vaccination for risk groups to prevent severe CAP and CAP mortality due to influenza in Indonesia as has been implemented in many other countries [6,7].

The use of PCR is instrumental to detect viruses, particularly in an epidemiological study, though it might still be too expensive for routine patient care. However, implementation of PCR-based detection of influenza viruses in throat or sputum samples would be a highly desirable additional routine test in this area of the world. Alternatively, for centers without PCR facilities rapid immunochromatographic lateral flow and membrane-based immunoassays are available that have  $\pm$  90% predictive values at the prevalences of influenza found in the clinical setting of CAP admission in Indonesian hospitals [8].

Optimal bacteriological diagnostics are required to be able to adapt the initial therapy, including alternative tests for detecting *S. pneumoniae* such as antigen tests [9,10]. The implementation of accurate and rapid diagnostic procedures at patient's presentation should be prioritized, particularly in cases of severe CAP since establishing an etiological diagnosis is likely to improve outcomes [11].

Leptospirosis is a zoonotic disease that is endemic in Indonesia. The clinical manifestations are variable. Patients may not be icteric and initial presentation may resemble pneumonia. Indeed, in our study, we found four cases fulfilling the clinical criteria of CAP and having a positive *Leptospira spp* PCR on throat and serum. In this area of the world, *Leptospira spp* should be in the differential diagnosis of CAP in particular when the patient is of the risk group, and appropriate diagnostics should be performed. The possibility of a *Leptospira spp* PCR on throat swab as a non-invasive diagnostic test for leptospirosis needs further study.

## RESEARCH QUESTION 2 – NASOPHARYNGEAL CARRIAGE OF RESPIRATORY BACTERIAL PATHOGENS

*What is the frequency of nasopharyngeal carriage of the most common bacterial pathogens in the respiratory tract of healthy people in Indonesia? What is the frequency of antimicrobial resistance of strains carried by healthy individuals? What are the risk factors for throat carriage of the major bacterial respiratory pathogens?*

We studied the nasopharyngeal carriage of *S. pneumoniae*, *K. pneumoniae*, and other GNB in a population-based survey among healthy inhabitants of Semarang city. A total of 496 subjects were included. In healthy adults aged 45-70 years old, the carriage rate of GNB was surprisingly high (34%), exceeding that of *S. pneumoniae* (11%). In adults, *K. pneumoniae* was the single most common (15%) GNB species isolated from the nasopharynx, while in children *K. pneumoniae* and *Pseudomonas spp.* were the most common ones (7% each). The carriage rate of GNB other than *K. pneumoniae*, such as *E. coli*, *Enterobacter spp.*, *Pseudomonas spp.*, *A. baumannii complex*, was 19% in adults and 20% in children. The nasopharyngeal carriage rate of GNB in the community in Europe, North America, and Brazil was reported to be much lower, 1-6% [12,13]. High GNB carriage rates are usually related to specific conditions, such as preceding antibiotic therapy, underlying diseases or long hospital stay [14]. In our study, high GNB carriage rates were related partly to poor water and food hygiene, corresponding to reports by others that GNB are frequent contaminants in water and foods in this part of the world [15-17]. The antimicrobial susceptibility profile of *K. pneumoniae* and other Enterobacteriaceae showed low prevalence of resistance to early generation of antibiotics. Since the throat carriage rate of *K. pneumoniae* and other GNB is high, the finding of these pathogens in sputum culture of pneumonia patients should be interpreted carefully, for instance, by assessing the quality of sputum, or performing semiquantitative or quantitative cultures. It is also of interest to study whether a correlation between carriage and infection by *K. pneumoniae* or other GNB can be established as was shown for *S. pneumoniae* [18,19]. Studies have reported such a correlation in hospital settings [20,21], which suggests that this may happen in the community as well.

The carriage rate of *S. pneumoniae* was 43% in children 6-60 months old and 11% in adults 45-70 years old. The proportion of subjects carrying *S. pneumoniae* varied significantly across the districts of Semarang, and tended to be higher in the suburban and eastern parts of the city. Carriage was associated with being a child, presence of toddlers at home and passive smoking. The prevalence of *S. pneumoniae* with reduced susceptibility to penicillin and co-trimoxazole was high. However, the national and local guidelines for empirical antibiotics for CAP in children still recommend these two

antibiotics as the first choices [22], while those for meningitis still recommend ampicillin for the second line [23].

The most common capsular serotypes in children, comprising 61% of strains, were 6A/B, 15B/C, 11A, 23F, 19F, 23A, while those in adults were 6A/B, 15B/C, and 15A, comprising 75% of strains. The commercially available 13-valent pneumococcal conjugate vaccines (PCV) [24] provided overall only 45% strain coverage for pneumococci isolated in the infant and toddler population in this study. However, the coverage varied among districts, from 13 to 100%. The coverage of this vaccine in other Southeast Asian countries is higher (63%-97%) [25], implying the need to perform a nationwide epidemiological study, in which isolates from both invasive disease and carriage should be included.

### RESEARCH QUESTION 3 – BACTERIAL PATHOGEN CARRIAGE VERSUS INFECTION

*What are the characteristic differences between bacterial respiratory pathogens isolated from colonized but otherwise healthy individuals versus those obtained from infected individuals?*

The characteristics of *K. pneumoniae* strains from healthy children, healthy adults, and adults with CAP were compared, regarding the antimicrobial susceptibility profile, hypermucoviscosity phenotype, capsular types, the presence of known virulence factors *rmpA* (regulator of mucoid phenotype) and *wcaG* (a gene which encodes the conversion of mannose to fucose to produce a thicker capsule). These two genes provide *Klebsiella* capsules that protect them from phagocytosis by neutrophils [26]. *K. pneumoniae* strains isolated from children differed significantly from those isolated from healthy adults, i.e., they rarely expressed the hypermucoviscous phenotype, and had no *wcaG* gene. In contrast, strains from healthy adults and adult CAP patients were highly similar with regard to hypermucoviscosity, capsular types, and the presence of *rmpA* and *wcaG* genes, indicating that they come from closely related clones. We suggest that healthy adults in our study population are at a higher risk to develop infections by *K. pneumoniae*, compared to children since adults were colonized more frequently, and by more virulent strains.

The characteristics of *S. pneumoniae* from healthy and infected individuals could not be compared in our study, since not enough *S. pneumoniae* strains could be isolated from the infected individuals (CAP patients) to make a valid comparison.

#### RESEARCH QUESTION 4 – QUALITY INDICATORS FOR MANAGEMENT OF PATIENTS WITH CAP

*How should the management of patients with CAP be evaluated? What are the results of such an evaluation in an Indonesian hospital?*

The mortality rate in CAP patients in our study was much higher than that in other studies in Southeast Asia [2,9,27], indicating that the quality of patient care may need closer evaluation. Ready access to health care is one potential issue. However, once presented to health care with CAP, appropriate antibiotic therapy is one of the most important elements in the management of CAP patients [28]. To evaluate the quality of care for CAP patients and to provide health care professionals with feedback on their antibiotic management, a qualified instrument is needed. Such an instrument must contain validated quality indicators (QIs), have good feasibility and reliability, and provide opportunities for improvement. Such an instrument can be developed by a systematic procedure that combines scientific evidence and expert opinion [29], like the Delphi method offers. Using this method, we developed a list of 15 indicators by a two-round consensus procedure. The applicability in terms of reliability, feasibility, and opportunity for improvement of these QIs was assessed by retrieving 128 medical files of patients hospitalized for CAP from 2007-2009 in Dr. Kariadi hospital, Semarang, Indonesia.

The reliability of the QIs was moderate or good. Five indicators did not pass feasibility criteria, either because of inappropriate medical data documentation, insufficient microbiology laboratory services, or patient factors. Three QIs provided little opportunity for improvement. Two out of the remaining seven indicators contradicted each other, one of which was found to be no longer valid according to recent evidence. A final set of six indicators was defined for use in the Indonesian setting. These included 'take two sets of blood samples for culture', 'obtain sputum samples for Gram stain and culture', 'obtain sputum sample and start antibiotic therapy in the emergency department', 'timely initiation of antibiotic therapy within four hours after presentation', 'switching from iv to oral therapy when clinically stable', and 'length of therapy is five days for uncomplicated CAP (CURB score  $\leq 2$ )'. This list of QIs is proven to be valid and applicable in Indonesian hospitals, in particular of class A and B which are equipped with microbiology services, to evaluate the quality of care in the management of CAP. Use of this list in such hospitals and the subsequent evaluation of the findings will provide information needed to improve the quality of care in the hospital, in particular which relevant to the QIs. The application of this list will also give information on the generalisability of this QI list.

Our study also provides a basis for the development of valid sets of QIs for the management of patients with other important medical problems in hospitals in Indonesia in order to improve health care in Indonesia in general.

A problem with using the Delphi method as we did, is the absence of a meeting of the participants. This meeting was absent to keep the experts' independence in rating the proposed indicators, but might leave different views about the indicators unnoticed and undiscussed by the experts and the observers who prepared, collected and analyzed the questionnaires rated by the experts. The consequences were: some QIs could not be operationalized by the observers and some QIs contradicted each other. To avoid this, we suggest to implement a modified Delphi method, such as the RAND/UCLA Appropriateness method, a method to synthesize the scientific literature and expert opinion on health care topics which includes a discussion between consensus rounds, but still allows the experts to rate the proposed indicators independently and anonymously [30].

To increase the feasibility so that no important indicators are rejected solely because of lack of data, more standardized medical documentation systems need to be implemented. In addition, clinical microbiology laboratories should provide early and frequent clinical consultation to clinical wards, so that clinicians are willing to order cultures, thus, increasing the feasibility of using those indicators that rely on culture results.

The development and implementation of QIs for the care of CAP patients and patients with other medical problems in Indonesia is especially urgent because since January 2014 the Indonesian government has implemented a new policy which provides all Indonesian citizens with health insurance [31]. The use of valid and generally applicable QIs will help to maintain the highest standard of care and control the costs.

## **RECOMMENDATIONS FOR PATIENT CARE**

To optimize the outcome of patients and prevent problems of antimicrobial resistance in patients hospitalized with CAP, it is important to perform all diagnostic procedures early, i.e., on the day of admission. The provision of facilities to do culture at the emergency department might stimulate doctors to take culture before initiating antibiotics. This is particularly important for those patients that are admitted to hospitals for severe disease, so that the etiology of CAP in this group is established at a time when its management can still be adjusted to improve outcomes. Laboratories of Clinical Microbiology should also institute appropriate standards of services, provide valid and rapid tests for common pathogens, reduce the turnaround time, and give early and frequent clinical consultations to their fellow clinicians in the hospital wards. By this means, the results of microbiology tests will contribute to better patient outcomes and control the problems of antimicrobial resistance, and hence, increase the compliance of doctors to perform the relevant microbiological diagnostics for their patients.

Consequently, a more comprehensive set of valid and applicable quality indicators for the improvement and control of patient care can be developed.

The types of microbiology tests provided should refer to the prevailing etiologies of CAP in the area, which is derived from qualified epidemiological studies. Alternative tests for *S. pneumoniae* (such as PCR and urinary antigen test), *C. pneumoniae* (PCR, serology), and tests for influenza viruses (PCR, rapid test, serology) are some of the recommended tests (Table 1).

Since *K. pneumoniae*, other GNB and *S. pneumoniae* are commonly found in the nasopharynx of healthy individuals, specific measures to distinguish infection from carriage or colonization should be routinely performed. These include the assessment of sputum quality, the coherence between microscopy and cultures, and performing semiquantitative or quantitative cultures.

For *L. pneumophila*, given the incidence was low (only 1 case was detected), we do not recommend the tests for routine patient care. However, *Legionella longbeacheae* is probably an agent that needs to be tested in a future epidemiology study, provided the reports on its incidence in Southeast Asia are increasing [32,33].

**Table 1.** Microbiological tests to be provided by Indonesian hospitals (Class A and B) for evaluation of patients hospitalized for CAP

Pathogen	Routine diagnostics	Advanced diagnostics
Influenza A/ B virus	<p><i>Specimens</i> : throat swab OR nasopharyngeal swab/ aspirate</p> <p><i>Methods</i> : rapid lateral flow and membrane-based immunoassays</p>	<p><i>Specimens</i>: throat swab OR nasopharyngeal swab/aspirate, OR bronchial wash OR endotracheal aspirate OR sputum</p> <p><i>Methods</i> : real-time PCR</p>
<i>K. pneumoniae</i> and other GNB	<p><i>Specimens</i> : blood AND sputum OR endotracheal aspirate</p> <p><i>Methods</i>:</p> <p>Sputum or endotracheal aspirate: Gram-stain AND semi-quantitative/quantitative culture on elective and/or selective solid agar media (e.g. McConkey)</p> <p>Blood : commercial broth-based media</p>	

**Table 1.** Microbiological tests to be provided by Indonesian hospitals (Class A and B) for evaluation of patients hospitalized for CAP

<b>Pathogen</b>	<b>Routine diagnostics</b>	<b>Advanced diagnostics</b>
<i>S. pneumoniae</i>	<p><i>Specimens</i> : blood AND urine AND sputum OR endotracheal aspirate</p> <p><i>Methods</i>: Sputum OR endotracheal aspirate: Gram-stain and semiquantitative OR quantitative cultures on selective blood containing agar</p> <p>Urine : commercial rapid immunochromatographic assay</p> <p>Blood : commercial broth-based media</p>	<p><i>Specimens</i> : sputum OR endotracheal aspirate</p> <p><i>Methods</i> : real-time PCR</p>
<i>C. pneumoniae</i>	<p><i>Specimens</i> : serum</p> <p><i>Methods</i> : microimmunofluorescence</p>	<p><i>Specimens</i> : sputum OR endo-tracheal aspirates</p> <p><i>Methods</i> : real-time PCR</p>
<i>M. tuberculosis</i>	<p><i>Specimens</i> : sputum OR other respiratory samples</p> <p><i>Methods</i> : Acid-fast staining (Ziehl-Neelsen OR, better, Auramine)</p>	<p><i>Specimens</i> : sputum OR other respiratory samples</p> <p><i>Methods</i> : conventional culture on solid agars OR, better, commercial broth-based culture (M-GIT), PCR</p>
<i>Leptospira spp.</i>	<p><i>Specimens</i> : serum</p> <p><i>Methods</i> : commercial rapid immunochromatographic assay</p>	<p><i>Specimens</i> : serum OR urine</p> <p><i>Methods</i> : Serum : enzyme-linked immuno assay (ELISA), microscopic agglutination test (MAT)</p> <p>Urine : real-time PCR</p>

The implementation of our quality indicators (QIs) in hospitals in particular of class A and B in Indonesia will guide the doctors to select the clinically most useful diagnostic and therapy measures, thus improve patient care. Since the Indonesian government has been instituting the new policy of national health insurance, the development and implementation of such QIs is urgent as a measure to control the quality of care and prevent excess costs.

Although early initiation of antibiotic treatment (within 4 or 8 hours of admission) is no longer proven to reduce mortality, a long delay in initiating antibiotic therapy is an unexpected event from the perspective of the quality of care [34]. We assume this is the

reason why the experts recommended the QI ‘initiating antibiotic within 4 hours of admission’. This will need comprehensive efforts and coordination between doctors, emergency room staffs, and the pharmacy department. However, this QI potentially causes an increase of inappropriate use of antibiotics in the emergency room [35]. Careful evaluation and frequent feedback to the physicians is needed to achieve the goal of timely initial antibiotic therapy without increasing inappropriate antibiotic use [36,37].

The choice of first line empirical antibiotic therapy for CAP, which according to the national Indonesian guideline is ceftriaxone or a combination of a  $\beta$ -lactam with a  $\beta$ -lactamase inhibitor, needs evaluation. Since ceftriaxone exerts selective pressure for ESBL-producing GNB [38,39], the emergence and spread of these strains in the hospital should be monitored. The new guidelines should be developed to promote prudent antibiotic use behaviour among doctors. The best choice of empirical antibiotic therapy for an adult patient presenting with CAP requiring hospitalization in this setting would be ampicillin-sulbactam or amoxicillin-clavulanic acid. These antibiotics cover both *K. pneumoniae* and other GNB and *S. pneumoniae*, while limiting selective pressure for ESBL [40-42].

## RECOMMENDATIONS FOR FURTHER RESEARCH

Since Indonesia is a large and populous country consisting of 33 provinces with over 17,000 islands in total, the generalizability of our findings needs to be confirmed in larger, preferably multicenter studies. By this means, national and region-specific data are provided to inform the development of appropriate guidelines. Such data will also be important for the evaluation of the value of the guidelines in improving the quality of care and patient outcome.

Other research to be prioritized is a study of the correlation between nasopharyngeal carriage/colonization and infection by *K. pneumoniae* and other GNB among adults, as has been shown with *S. pneumoniae* [18]. The clonality of pathogens causing CAP also needs to be revealed using molecular techniques such as multilocus sequence typing (MLST) or whole genome sequencing. This information will improve our understanding on the pathogenesis and the transmission pattern of CAP pathogens, thereby directing prevention programs. The identification of capsular types from more pathogenic strains of *K. pneumoniae* might have implication for future vaccine development.

The possibility to develop throat swab PCR as an alternative diagnostic method for CAP caused by *Leptospira* spp. is another interesting topic for further research. The emergence and spread of ESBL-producing strains of GNB in the hospital and in the community should be monitored as ceftriaxone is routinely used as empiric antibiotic therapy.

## REFERENCES

1. Olsen SJ, Thamthitawat S, Chantra S, Chittaganpitch M, Fry AM, et al. (2010) Incidence of respiratory pathogens in persons hospitalized with pneumonia in two provinces in Thailand. *Epidemiol Infect* 138: 1811-1822.
2. Hara K, Yahara K, Gotoh K, Nakazono Y, Kashiwagi T, et al. (2011) Clinical study concerning the relationship between community-acquired pneumonia and viral infection in northern Thailand. *Intern Med* 50: 991-998.
3. Liam CK, Pang YK, Poosparajah S (2006) Pulmonary tuberculosis presenting as community-acquired pneumonia. *Respirology* 11: 786-782.
4. Poulouse V (2008) Severe community-acquired pneumonia requiring intensive care: a study of 80 cases from Singapore. *Singapore Med J* 49: 458-461.
5. Belmonte O, Drouet D, Alba J, Moiton MP, Kuli B, et al. (2010) Evolution of Enterobacteriaceae resistance to antibiotics in Reunion Island: emergence of extended-spectrum beta-lactamases. *Pathol Biol* 58: 18-24.
6. Seymann GB (2006) Community-acquired pneumonia: defining quality care. *J Hosp Med (Online)* 1: 344-353.
7. Members of the Western Pacific Region Global Influenza Surveillance and Response System (2013) Seasonal influenza vaccine policies, recommendations and use in the World Health Organization's Western Pacific Region. *WPSAR* 4: 1-9.
8. Center for Disease Control and Prevention (CDC) (2013) Rapid diagnostic testing for influenza: Information for clinical laboratory directors. *Seasonal Influenza (Flu)*. Atlanta: Center for Disease Control and Prevention.
9. Mustafa MIA, Al-Marzooq F, How SH, Kuan YC, Ng TH (2011) The use of multiplex real-time PCR improves the detection of the bacterial etiology of community acquired pneumonia. *Tropical Biomedicine* 28: 531-544.
10. Tzeng DH, Lee YL, Lin YH, Tsai CA, Shi ZY (2006) Diagnostic value of the Binax NOW assay for identifying a pneumococcal etiology in patients with respiratory tract infection. *J Microbiol Immunol Infect* 39: 39-44.
11. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, et al. (2007) Infectious Diseases Society of America/American Thoracic Society. Consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 44: S27-S72.
12. Podschun R, Ullmann U (1998) *Klebsiella spp.* as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 11: 589-603.
13. Lima ABM, Leão LSND, Oliveira LSdC, Pimenta FC (2010) Nasopharyngeal Gram-Negative Bacilli Colonization In Brazilian Children Attending Day-Care Centers. *Braz J Microbiol* 41: 24-27.
14. Parm Ü, Metsvaht T, Sepp E, Ilmoja M-L, Pisarev H, et al. (2011) Risk factors associated with gut and nasopharyngeal colonization by common Gram-negative species and yeasts in neonatal intensive care units patients. *Early Hum Dev* 87: 391-399.
15. Panda SK, Patra AK, Kar RN (2012) Monitoring of multiple drug-resistant pathogens in a selected stretch of Bay of Bengal, India. *Environ Monit Assess* (2012) 184: 193-200.
16. Haryani Y, Noorzaleha AS, Fatimah AB, Noorjahan BA, Patrick GB, et al. (2007) Incidence of *Klebsiella pneumoniae* in street foods sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD-PCR analysis. *Food Cont* 18: 847-853.
17. Vollaard AM, Ali S, van Asten HA, Ismid IS, Widjaja S, et al. (2004) Risk factors for transmission of foodborne illness in restaurants and street vendors in Jakarta, Indonesia. *Epidemiol Infect* 132: 863-872.
18. Bogaert D, De Groot R, Hermans PW (2004) *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *Lancet Infect Dis* 4: 144-154.
19. van der Poll T, Opal SM (2009) Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet* 374: 1543-1556.
20. Giannella M, Trecarichi EM, De Rosa FG, Del Bono V, Bassetti M, et al. (2014) Risk factors for carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection among rectal carriers: a prospective observational multicentre study. *Clin Microbiol Infect* 20: 1357-1362.

21. Lubbert C, Becker-Rux D, Rodloff AC, Laudi S, Busch T, et al. (2014) Colonization of liver transplant recipients with KPC-producing *Klebsiella pneumoniae* is associated with high infection rates and excess mortality: a case-control analysis. *Infection* 42: 309-316.
22. Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan - Kementerian Kesehatan Republik Indonesia (Directorate General Disease Control and Environmental Health- the Ministry of Health Republic Indonesia) (2010) Modul tatalaksana standar pneumonia (Module for standard management of pneumonia). Jakarta: Kementerian Kesehatan RI (the Ministry of Health RI).
23. Tim Adaptasi Indonesia (2008) Buku saku pelayanan kesehatan anak di rumah sakit : Pedoman bagi rumah sakit rujukan tingkat pertama di kabupaten/kota: Kementerian Kesehatan RI - World Health Organization - Ikatan Dokter Anak Indonesia.
24. Pfizer (2011) Pneumococcal 13-valent conjugate vaccine (diphtheria CRM197 protein). In: Inc WP, editor. USA.
25. Jauneikaite E, Jefferies JM, Hibberd ML, Clarke SC (2012) Prevalence of *Streptococcus pneumoniae* serotypes causing invasive and non-invasive disease in South East Asia: A review. *Vaccine* 30: 3503-3514.
26. Wu JH, Wu AM, Tsai CG, Chang XY, Tsai SF, et al. (2008) Contribution of fucose-containing capsules in *Klebsiella pneumoniae* to bacterial virulence in mice. *Exp Biol Med* 233: 64-70.
27. Song J-H, Oh WS, Kang C-I, Chung DR, Peck KR, et al. (2008) Epidemiology and clinical outcomes of community-acquired pneumonia in adult patients in Asian countries: a prospective study by the Asian network for surveillance of resistant pathogens. *Intl J Antimicrob Agents* 31: 107-114.
28. Schouten JA, Hulscher ME, Wollersheim H, Braspenning J, Kullberg BJ, et al. (2005) Quality of antibiotic use for lower respiratory tract infections at hospitals: (how) can we measure it? *Clin Infect Dis* 41: 450-460.
29. Rubin HR, Pronovost P, Diette GB (2001) From a process of care to a measure: The development and testing of a quality indicator. *Int J Qual Health Care* 13 489-496.
30. Fitch K, Bernstein SJ, Aguilar MD, Burnand B, LaCalle JR, et al. (2001) RAND/UCLA Appropriateness method user's manual Santa Monica: RAND. pp. 123.
31. Menteri Hukum dan Hak Azasi Manusia Republik Indonesia, editor (2011) Undang - Undang Republik Indonesia Nomor 24 tahun 2011 tentang Badan Penyelenggara Jaminan Sosial Jakarta: Kementerian Sekretariat Negara.
32. Phares CR, Wangroongsarb P, Chantra S, Paveenkitiporn W, Tondella M-L, et al. (2007) Epidemiology of severe pneumonia caused by *Legionella longbeachae*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*: 1-year, population based surveillance for severe pneumonia in Thailand. *Clin Infect Dis* 45: e147-155.
33. Whiley H, Bentham R (2011) *Legionella longbeachae* and legionellosis. *Emerg Infect Dis* 17: 579-583.
34. Bordon J, Aliberti S, Duvvuri P, Wiemken T, Peyrani P, et al. (2013) Early administration of the first antimicrobials should be considered a marker of optimal care of patients with community-acquired pneumonia rather than a predictor of outcomes. *Int J Infect Dis* 17: e293-299.
35. Wachter RM, Flanders SA, Fee C, Pronovost PJ (2008) Public reporting of antibiotic timing in patients with pneumonia: Lessons from a flawed performance measure. *Ann Intern Med* 149: 29-32.
36. Fee C, Metlay JP, Camargo CA, Maselli JH, Gonzales R (2010) ED antibiotic use for acute respiratory illnesses since pneumonia performance measure inception. *Am J Emerg Med* 28: 23-31.
37. Friedberg MW, Mehrotra A, Linder JA (2009) Reporting hospitals' antibiotic timing in pneumonia: adverse consequences for patients? *Am J Manag Care* 15: 137-144.
38. Skrlin J, Bacic Vrca V, Marusic S, Ciric-Crnec M, Mayer L (2011) Impact of ceftriaxone de-restriction on the occurrence of ESBL-positive bacterial strains and antibiotic consumption. *J Chemother* 23: 341-344.
39. Asensio A, Oliver A, Gonzalez-Diego P, Baquero F, Perez-Diaz JC, et al. (2000) Outbreak of a multiresistant *Klebsiella pneumoniae* strain in an intensive care unit: antibiotic use as risk factor for colonization and infection. *Clin Infect Dis* 30: 55-60.
40. Kolar M, Urbanek K, Latal T (2001) Antibiotic selective pressure and development of bacterial resistance. *Int J Antimicrob Agents* 17: 357-363.

41. Bantar C, Sartori B, Vesco E, Heft C, Saul M, et al. (2003) A hospitalwide intervention program to optimize the quality of antibiotic use: impact on prescribing practice, antibiotic consumption, cost savings, and bacterial resistance. *Clin Infect Dis* 37: 180-186.
42. Akova M (2008) Sulbactam-containing beta-lactamase inhibitor combinations. *Clin Microbiol Infect* 1: 185-188.

# Chapter 10

## Summary





Community-acquired pneumonia (CAP) is one of the most common infectious diseases worldwide. In low-to middle-income countries, it is one of the most important causes of mortality and morbidity. The aim of this thesis was to enhance our knowledge on clinical and microbiological aspects of CAP in Indonesia. This information is necessary for the development of evidence-based guidelines for the management of patients with CAP in Indonesia.

Part I provides background information on CAP in Southeast Asia. **Chapter 2** presents an overview of peer-reviewed literature on CAP in Southeast Asia from January 2000 to December 2011 with focus on the epidemiology, clinical presentation, etiology, risk factors, treatment, and outcome. As general conclusion one can state that in this part of the world the information on CAP is still lacking as only three Southeast Asian countries (Malaysia, Thailand, and Singapore) have published CAP studies. In addition, the research methods varied between the three countries that did study CAP, and the used methods were not frequently well standardized.

In part II, we explored the characteristics of CAP patients in two hospitals in Semarang city, Central Java, the pathogens causing CAP using up-to-date microbiological diagnostic tests, and the outcome of hospitalization. **Chapter 3** presents the results of the prospective, hospital-based study of 148 CAP patients. The etiological pattern of CAP proved to be quite different from that in developed countries in Europe, North America, or Australia. Respiratory viruses were the commonest agents, identified in 45% of the patients, with influenza A and B viruses as the most frequently detected (18%). Gram-negative bacilli (GNB), comprising of *Klebsiella pneumoniae* (14%) and other GNB (5%), were the commonest bacterial cause of CAP in patients admitted with this infection, followed by *Streptococcus pneumoniae* (13%). Mixed infections were common. The mortality rate among the 148 patients was high (30%) and significantly associated with severity, and with failure to establish an etiological diagnosis. **Chapter 4** reports leptospirosis in five cases of CAP based on several diagnostic tests including a rapid test for leptospirosis, serology with enzyme-linked immunosorbent assay (ELISA) and microscopic agglutination test (MAT), and polymerase chain reaction (PCR) on acute and convalescent sera, urine, sputum samples, and throat swabs. The finding that PCR on throat swabs was concordant with the PCR on acute serum samples was of interest, raising the possibility to employ throat swabs as alternative non-invasive samples for the diagnosis of acute leptospirosis.

As it is well known that pneumonia is preceded by nasopharyngeal carriage of respiratory pathogens, part III explores the carriage rate of *S. pneumoniae*, *K. pneumoniae*, and other GNB in healthy individuals and compares the characteristics of these strains to those isolated from pneumonia patients. **Chapter 5** and **chapter 6**

describe the results of our study on the nasopharyngeal carriage of *S. pneumoniae*, *K. pneumoniae*, and other GNB in healthy individuals and the analysis of the risk factors for carriage. The carriage rates of *S. pneumoniae* were 43% in children aged 6-60 months and 11% in adults aged 45-70 years, which are comparable to those in other developed countries such as the Netherlands and Japan. The carriage rates of *K. pneumoniae* were 7% in children and 15% in adults, while those for other GNB were 19% either in children or adults. These rates are much higher compared to those reported in developed countries. Throat carriage of GNB was associated with socio-economic circumstances, especially exposure to poor food and water hygiene.

**Chapter 7** presents the characteristic differences between *K. pneumoniae* isolated from healthy individuals and from CAP patients with regard to hypermucoviscosity, antimicrobial susceptibility profile, capsular types, and the presence of virulence factors *rmpA* and *wcaG*. Strains from healthy and pneumonic adults were more virulent than those from children, and seemed to come from closely related clones since they shared similarities with regard to hypermucoviscosity, capsular types, and the presence of *rmpA* and *wcaG* genes. Strains from healthy children seemed to be less virulent and to come from different clones as they differed from those from healthy adults with regard to hypermucoviscosity and the presence of *wcaG* genes. Thus, exposure to and throat carriage with GNB, specifically *K. pneumoniae*, may be especially important for the adult population, predisposing them to respiratory tract infections with such bacilli.

Part IV, chapter 8 presents the development of indicators to assess the quality of care, notably that related to the antibiotic treatment in CAP patients. Using Delphi methods, only 6 out of 15 suggested valid quality indicators (QIs) were accepted. Most indicators were rejected because of poor feasibility to employ them in the clinical setting in Indonesian health care. To improve the development of the QIs, it is recommended to implement a modified Delphi method which includes a discussion between the rounds, to institute a sound medical documentation, and to improve the services of clinical microbiology laboratories in Indonesia.

In part V, chapter 9 and 10, the main results are discussed and summarized. Suggestions for the improvement of health care practice and for further research are provided.

# **Nederlandse samenvatting**

## **Dutch Summary**

Thuis-opgelopen longontsteking (“community-acquired pneumonie” of CAP) is één van de meest voorkomende infectieziekten in de wereld. In lage- en middeninkomenslanden is het één van de meest voorkomende oorzaken van mortaliteit en morbiditeit. Het doel van dit proefschrift was om de kennis over de klinische en microbiologische aspecten van CAP in Indonesië te vergroten. Deze informatie is nodig om wetenschappelijk goed onderbouwde richtlijnen te kunnen ontwikkelen voor de behandeling van patiënten met CAP in Indonesië.

Deel I geeft achtergrondinformatie over CAP in Zuidoost-Azië. **Hoofdstuk 2** geeft een overzicht van de literatuur over CAP in Zuidoost-Azië van januari 2000 tot december 2011 met focus op de epidemiologie, klinische presentatie, etiologie, risicofactoren, behandeling en uitkomst. De algemene conclusie die getrokken kon worden was dat er zeer beperkt informatie over het onderwerp is. Slechts uit drie landen zijn onderzoeksgegevens gepubliceerd waarbij de onderzoeksmethoden varieerden tussen de verschillende landen en niet optimaal waren gestandaardiseerd.

In deel II worden de karakteristieken van CAP patiënten, de verwekkers van CAP en de uitkomsten van de ziekenhuisopnamen onderzocht in twee ziekenhuizen in de stad Semarang, Midden-Java, Indonesië, met behulp van de meest recente diagnostische tests. **Hoofdstuk 3** presenteert de resultaten van de prospectieve studie van 148 patiënten met CAP die in het ziekenhuis werden opgenomen. Het etiologische patroon van CAP bleek sterk te verschillen van dat in de economisch ontwikkelde landen in Europa, Noord-Amerika en Australië. De respiratoire virussen waren het meest frequent aangetoond, namelijk bij 45% van de patiënten, met influenza A en B als meest voorkomende (18%). Gram-negatieve bacteriën (GNB), waaronder *Klebsiella pneumoniae* (14%) en andere GNB (5%) waren de meest voorkomende bacteriële oorzaak van CAP bij in het ziekenhuis opgenomen patiënten, gevolgd door *Streptococcus pneumoniae* (13%). Gemengde infecties kwamen veel voor. De mortaliteit onder de 148 patiënten was hoog (30%) en was geassocieerd met ernst van de ziekte en met het ontbreken van een etiologische diagnose. **Hoofdstuk 4** rapporteert vijf patiënten met CAP bij wie de diagnose leptospirose vastgesteld werd met verscheidene onderzoeken: sneltest voor leptospirose, serologie met een “enzyme-linked immunosorbent assay”-techniek (ELISA), microscopische agglutinatietest (MAT) en een polymerase kettingreactie (PCR) op serum uit de acute fase en de herstelfase, urine, sputum en keeluitstrijkjes. De bevinding dat het resultaat van de PCR op het keeluitstrijkje consistent was met de PCR op serum uit de acute fase is belangwekkend, omdat het de mogelijkheid biedt om keeluitstrijkjes te gebruiken als een niet-invasief alternatief voor de diagnose van leptospirose.

Het is algemeen bekend dat longontsteking vaak wordt voorafgegaan door nasofaryngeaal dragerschap van respiratoire pathogenen. Deel III verkent het dragerschap van *S. pneumoniae*, *K. pneumoniae* en andere GNB bij gezonde individuen en vergelijkt de

kenmerken van deze bacteriestammen met de stammen die geïsoleerd zijn van patiënten met longontsteking. **Hoofdstuk 5** en **hoofdstuk 6** beschrijven de resultaten van onze studie naar het dragerschap van *S. pneumoniae*, *K. pneumoniae* en andere GNB bij gezonde personen en de analyse van risicofactoren voor dat dragerschap. Nasofaryngeaal dragerschap van *S. pneumoniae* was 43% bij kinderen van 6-60 maanden en 11% bij volwassenen van 45-70 jaar, hetgeen vergelijkbaar is met het dragerschappercentage in economisch ontwikkelde landen zoals Nederland en Japan. Nasofaryngeaal dragerschap van *K. pneumoniae* was 7% bij kinderen en 15% bij volwassenen, terwijl dat voor andere GNB 19% in beide groepen was. Deze percentages zijn veel hoger dan die gerapporteerd in economisch ontwikkelde landen. Dragerschap met GNB was geassocieerd met bepaalde socio-economische omstandigheden, met name blootstelling aan slechte water- en voedselhygiëne.

**Hoofdstuk 7** beschrijft in hoeverre *K. pneumoniae* stammen geïsoleerd van gezonde individuen en van CAP patiënten verschillen met betrekking tot hypermucoviscositeit, het antimicrobiële gevoeligheidsprofiel, kapseltypen en de aanwezigheid van de virulentiefactoren *rmpA* en *wcaG*. Bacteriestammen van gezonde volwassenen en volwassenen met longontsteking waren virulenter dan die van kinderen en leken afkomstig te zijn van nauw verwante klonen gelet op de overeenkomsten betreffende hypermucoviscositeit, kapseltypen en de aanwezigheid van *rmpA* en *wcaG*-genen. De bacteriestammen van gezonde kinderen leken minder virulent te zijn en van andere klonen te komen, want zij verschilden met die van gezonde volwassenen wat betreft hypermucoviscositeit en de aanwezigheid van *wcaG*-genen. Het nasofaryngeaal dragerschap van *K. pneumoniae* lijkt dus met name van belang bij volwassenen, omdat zij door deze blootstelling luchtweginfecties zouden kunnen krijgen met deze bacteriën.

Deel IV, hoofdstuk 8, beschrijft de ontwikkeling van kwaliteitsindicatoren (QI) voor de beoordeling van de kwaliteit van de verleende zorg, inzake de behandeling van CAP patiënten met antibiotica. Met behulp van de Delphi-methode, werden slechts 6 van de 15 voorgestelde QI's uiteindelijk uitgekozen. De meeste indicatoren werden afgewezen vanwege de slechte uitvoerbaarheid. Ter verbetering van de ontwikkeling en de toepassing van de QI's, is het raadzaam om een gemodificeerde Delphi-methode toe te passen, waarbij het mogelijk is om tijdens een bijeenkomst van deskundigen opinies uit te wisselen en aan elkaar te toetsen, om een zo goed mogelijke medische documentatie in het ziekenhuis op te zetten en om de dienstverlening van het microbiologisch laboratorium te verbeteren.

In deel V, hoofdstuk 9 en 10, worden de belangrijkste resultaten samengevat en besproken. Tevens worden er aanbevelingen gedaan voor de verbetering van de gezondheidszorg en voor verder onderzoek.



# Ringkasan

## Indonesian summary

Pneumonia komunitas (PK) merupakan salah satu penyakit infeksi utama di seluruh dunia. Di negara-negara berpenghasilan rendah-menengah, penyakit ini merupakan salah satu penyebab kesakitan dan kematian terpenting. Tujuan thesis ini adalah untuk meningkatkan pengetahuan tentang PK di Indonesia, khususnya dalam aspek mikrobiologi dan aspek klinis. Informasi tersebut diperlukan agar dapat disusun suatu pedoman manajemen pasien PK yang berdasarkan bukti yang spesifik dan sesuai untuk Indonesia.

Bagian I membahas latar belakang informasi mengenai PK di Asia Tenggara. **Bab 2** menyajikan ikhtisar dari literatur-literatur ilmiah mengenai PK di Asia Tenggara dari Januari 2000-Desember 2011 dengan fokus pada epidemiologi, penampilan klinis, etiologi, faktor risiko, pengelolaan, dan luaran pasien-pasien PK. Sebagai kesimpulan umum, dapat dinyatakan bahwa informasi mengenai PK di Asia Tenggara masih jarang karena hanya tiga Negara (Malaysia, Thailand, Singapura) yang pernah mempublikasikan hasil penelitian tentang PK. Selain itu, kebanyakan penelitian tidak menggunakan metode penelitian yang baku.

Pada bagian II, kami mengeksplorasi karakteristik pasien PK yang dirawat di dua rumah sakit di kota Semarang, Jawa Tengah, pathogen-patogen penyebab PK dengan menggunakan pemeriksaan mikrobiologi mutakhir, serta luaran dari perawatan di rumah sakit. **Bab 3** menyajikan hasil penelitian prospektif berbasis rumah sakit yang mencakup 148 pasien. Pola etiologi PK terbukti cukup berbeda dengan yang didapatkan di negara-negara maju di Eropa, Amerika Utara, atau Australia. Penyebab PK terbanyak pada penelitian ini adalah virus-virus respiratori, yang ditemukan pada 45% dari total pasien, dengan virus influenza A dan B sebagai etiologi yang paling sering (18%).

Kuman bentuk batang Gram negatif (KBBGN), terdiri dari *Klebsiella pneumoniae* (14%) dan KBBGN-lain (5%) merupakan penyebab bakterial paling sering pada pasien PK yang dirawat inap, diikuti oleh *Streptococcus pneumoniae* (13%). Infeksi polimikrobal juga sering didapatkan. Angka kematian-30-hari dari 148 pasien tersebut tinggi (30%) dan berhubungan secara signifikan dengan derajat berat penyakit, dan kegagalan dalam menegakkan diagnosis etiologi.

**Bab 4** berisi laporan kasus leptospirosis dengan manifestasi klinik PK, yang didiagnosis dengan berbagai pemeriksaan mikrobiologi seperti tes cepat (*rapid test*), serologi dengan *enzyme-linked immunosorbent assay* (ELISA) dan *microscopic agglutination test* (MAT), and polymerase chain reaction (PCR) pada specimen serum akut-konvalesens, urin, sputum, dan swab tenggorok. Fakta bahwa hasil PCR pada swab tenggorok bersesuaian dengan hasil PCR pada sampel serum akut merupakan temuan yang menarik, menimbulkan harapan untuk menggunakan swab tenggorok sebagai alternative sampel non invasif untuk diagnosis leptospirosis.

Sebagaimana telah banyak diketahui, pneumonia didahului oleh kolonisasi atau bawaan (*carriage*) patogen respiratori pada nasofaring, maka bagian III membahas

tentang bawaan *S. pneumoniae*, *K. pneumoniae* pada individu sehat dan membandingkan karakteristik dari strain yang diperoleh dari individu sehat tersebut dengan strain yang berasal dari pasien PK. **Bab 5** dan **bab 6** memaparkan hasil penelitian kami tentang bawaan *S. pneumoniae*, *K. pneumoniae*, dan KBBGN lainnya pada nasofaring individu sehat, serta faktor risiko bawaan patogen tersebut. Angka bawaan *S. pneumoniae* pada nasofaring didapatkan sebesar 43% pada anak sehat usia 6-60 bulan dan 11% pada orang dewasa sehat usia 45-70 tahun. Angka ini hampir sebanding dengan yang didapatkan di Belanda dan Jepang. Angka bawaan untuk *K. pneumoniae* sebesar 7% pada anak dan 15% pada orang dewasa, sedangkan untuk KBBGN lainnya sebesar 19% baik pada anak maupun dewasa. Angka bawaan KBBGN ini sangat tinggi dibandingkan dengan yang dilaporkan di negara maju. Bawaan KBBGN pada tenggorok atau nasofaring berkaitan dengan lingkungan sosioekonomi, khususnya paparan terhadap air dan makanan dengan higienitas rendah. **Bab 7** membandingkan perbedaan karakteristik strain *K. pneumoniae* yang diisolasi dari orang sehat dan dari pasien PK, khususnya dalam hal sifat hipermukoviskositas, profil kepekaan terhadap antibiotik, serta kepemilikan faktor virulensi *rmpA* dan *wcaG*. Strain dari individu dewasa yang sehat maupun yang sakit PK lebih virulen dari pada strain dari anak, dan tampaknya berasal dari klon yang berhubungan erat karena kemiripan karakteristik-karakteristik tersebut di atas. Strain dari anak sehat tampaknya kurang virulen dan berasal dari klon yang berbeda dari strain yang didapat dari orang dewasa sehat. Jadi, paparan dan bawan KBBGN, khususnya *K. pneumoniae*, mungkin penting pada populasi dewasa, dan merupakan predisposisi terjadinya infeksi traktus respiratorius oleh KBBGN.

Bagian IV, bab 8 membahas tentang pengembangan indikator kualitas (IK) perawatan, khususnya yang terkait dengan pengobatan antibiotik, pada pasien PK. Dengan menggunakan metode Delphi, hanya 6 dari 15 indikator kualitas perawatan yang diusulkan oleh panel pakar terkait yang dapat digunakan. Sebagian besar indikator tertolak karena kelayakannya terlalu rendah untuk digunakan pada sistem dan situasi perawatan kesehatan klinis di Indonesia. Agar dapat dihasilkan IK perawatan yang lebih baik, direkomendasikan untuk menggunakan metode Delphi yang dimodifikasi dengan menambahkan diskusi di antara putaran pembentukan konsensus, menggunakan sistem dokumentasi medis yang lebih baik, dan memperbaiki pelayanan laboratorium mikrobiologi.

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# Curriculum vitae

## Helmia Farida

Helmia Farida was born on December 13, 1966 in Jepara, Central Java, Indonesia. She graduated from elementary school (SD Islam Badan Wakaf III - Semarang) in 1979, junior high school (SMP Negeri 3 Semarang) in 1982, and senior high school (SMA Negeri 1 Semarang) in 1985. After completing her education from Medical Faculty, Diponegoro University, Semarang, as a medical doctor in 1991, she worked at a private hospital in Jakarta. She worked as a head of a Primary Health Centre in Bawan, Kapuas, Central Kalimantan from 1992-1993, and then in Genuk, Semarang, Central Java from 1993-1995. She joined a master program of Medical Microbiology in Airlangga University from 1995-1997, followed by a fellowship in Pediatrics from 1998-2005. She has been working as a lecturer in the Department of Microbiology, Faculty of Medicine, Diponegoro University from 2001 until presently. From 2006-2013 she also a member of the Clinical Microbiology and Infectious Disease team in the Laboratory of Clinical Microbiology of Dr. Kariadi Hospital. She was involved in the "Antimicrobial Resistance in Indonesia, Prevalence and Prevention (AMRIN)" Indonesian-Dutch Collaborative Study in 2002-2004. Thank to her participation in the study, she was also then involved in the development of the Indonesia National Antimicrobial Resistance Control Program (PPRA) in 2005-2013. She has been involved in the Community-Acquired Pneumonia in Indonesia (CAPSIN) Indonesian-Dutch Collaborative Study in 2007-2013. During the CAPSIN Study she was trained at the Department of Microbiology and Infectious Diseases of the Erasmus University Medical Centre, Rotterdam (Prof. H.A. Verbrugh, MD, PhD). Helmia is married to Anang Achmadi and they have three children, Faris (1994), Hana (1997), and Azky (2001).



# List of publications

Duerink DO, **Farida H**, Nagelkerke NJ, Wahyono H, Keuter M, Lestari ES, Hadi U, van den Broek PJ. Preventing nosocomial infections: improving compliance with standard precautions in an Indonesian teaching hospital. *J Hosp Infect.* 2006;64(1):36-43.

Kuntaman K, Santoso S, Wahjono H, Mertaniasih NM, Lestari ES, **Farida H**, Hapsari R, Firmanti SC, Noorhamdani AS, Santosaningsih D, Purwono PB, Kusumaningrum D. The sensitivity pattern of extended spectrum beta-lactamase-producing bacteria against six antibiotics routinely used in clinical setting. *J Indon Med Assoc.* 2011;61(12):482-6.

**Farida H**, Severin JA, Gasem MH, Keuter M, van den Broek P, Hermans PW, Wahyono H, Verbrugh HA. Nasopharyngeal carriage of *Klebsiella pneumoniae* and other Gram-negative bacilli in pneumonia-prone age groups in Semarang, Indonesia. *J Clin Microbiol.* 2013;51(5):1614-6.

**Farida H**, Severin JA, Gasem MH, Keuter M, Wahyono H, van den Broek P, Hermans PW, Wahyono H, Verbrugh HA. Nasopharyngeal carriage of *Streptococcus pneumoniae* in pneumonia-prone age groups in Semarang, Java Island, Indonesia. *PLoS one.* 2014;9(1).

Santosaningsih D, Santoso S, Budayanti NS, Kuntaman K, Lestari ES, **Farida H**, Hapsari R, Hadi P, Winarto W, Milheirico C, Maquelin K, Willemsse-Erix D, van Belkum A, Severin JA, Verbrugh HA. Epidemiology of *Staphylococcus aureus* harboring the *mecA* or Panton-Valentine leukocidin genes in hospitals in Java and Bali, Indonesia. *Am J Trop Med Hyg.* 2014;90(4):728-34.

**Farida H**, Rondags A, Gasem MH, Leong K, Adityana A, van den Broek P, Keuter M, Natsch S. Development of quality indicators to evaluate antibiotic treatment of patients with CAP in Indonesia. *In printing process at Trop Med Intl Health.* doi:10.1111/tmi.12452 (Epub ahead of print]

**Farida H**, Gasem MH, Suryanto A, Keuter M, Zulkarnain N, Satoto B, van der Eijk AA, Djokomoeljanto R, Wahyono H, Verbrugh HA, Severin JA, van den Broek PJ. . Viruses and Gram-negative bacilli dominate the etiology of community-acquired pneumonia in Indonesia. *Submitted for publication.*

Gasem MH, **Farida H**, Ahmed A, Verbrugh HA, Hartskeerl RA, van den Broek PJ. Is *Leptospira sp.* a causative pathogen of community-acquired pneumonia in Indonesia? A case series. *Submitted for publication*

**Farida H**, Suryanto A, Severin JA, Verbrugh HA, van den Broek PJ. Community acquired pneumonia in adults in Southeast Asia . A review. *Submitted for publication.*

# PhD Portfolio

## Helmia Farida

<b>Name PhD student</b> : drs. Helmia Farida	<b>PhD period</b> : 2008-2015
<b>Erasmus MC Departement:</b> Medical Microbiology and Infectious Diseases	<b>Promotor</b> : Prof. dr. Henri A. Verbrugh Prof. dr. Peterhans J. van den Broek
<b>Research School</b> : Postgraduate School Molecular Medicine	<b>Co-promotor</b> : dr. Juliëtte A. Severin dr. M. Hussein Gasem

1. PhD training	Year	Workload	
		Hours	ECTS
<b>General courses</b>			
Program of Preparation for Doctorate in Medical Sciences/ Program Persiapan Pendidikan Doktor Ilmu Kedokteran (P3DIK), Diponegoro University, Semarang, Indonesia	2008	120	4
Writing successful grant proposal, Erasmus MC, Rotterdam, the Netherlands	2011	8	0.25
How to finish your PhD in 4 years	2011	8	0.25
<b>Specific course</b>			
International Summer School in Infectious Diseases, Gottingen University – Indonesian Association of Clinical Microbiologist Aceh Chapter, Banda Aceh, Indonesia	2008	54	2
Course in Virology, Erasmus MC, Rotterdam, the Netherlands	2010	45	2
Microscopic Image Analysis, Erasmus MC, Rotterdam, the Netherlands	2012	28	1
Training for tutors in the Indonesian Antimicrobial Resistance Control Program	2009	32	1
Workshop on Clinical Microbiology and Infectious Disease, Diponegoro University, Semarang, Indonesia	2009	28	1
Workshop on Interpretative Reading of Antimicrobial Susceptibility Test : Why and How ? – Indonesian Association of Clinical Microbiologist, Jakarta, Indonesia	2011	8	0.25
Workshop on Expertise in Antimicrobial Susceptibility Test, Indonesian Association of Clinical Microbiologists, Jakarta, Indonesia	2013	8	0.25
Workshop on The National Antimicrobial Resistance Control for Indonesian Hospitals in Jakarta, Bandung, Denpasar	2011	28	1
	2012	28	1
	2013	28	1
Workshop on Improving quality of analytic phase: From colony selection to ASTand Optimizing Clinical Microbiology Laboratory: Focus on Gram Negative Bacteria	2014	16	0,5
<b>Congress, Seminars and Presentation</b>			
Participant in the National Congress on the Indonesian Antimicrobial Resistance Control Program organized by the Ministry of Health of Republic Indonesia	2008, 2010, 2012		3
Participant in the National Congress of the Indonesian Association of Microbiology (PERMI)	2008		1
Participant in the International Congress on Biomedical Sciences, Bandung, Indonesia	2011		1
Participant at The 8 <sup>th</sup> National Congress of Indonesian Clinical Microbiologist, Denpasar, Indonesia	2012		1
Presentation in the International Congress on Biomedical Sciences, Bandung, Indonesia	2011		1
Two presentations at The 8 <sup>th</sup> National Congress of Indonesian Clinical Microbiologist, Denpasar, Indonesia	2012		1

Participant in the Annual Scientific Meeting of the Indonesian Association of Clinical Microbiologist, Semarang, Indonesia	2013	1
Participant in the Indonesian Antimicrobial Resistance Watch IX, Jakarta, Indonesia	2014	1
Presentation in the In-house training on Hospital Associated Infection Surveillance and Control, Tugu Hospital, Semarang	2008	1
Two presentations at inner seminars (on Infection Control and on Antimicrobial Resistance Control) in Dr. Kariadi Hospital	2010, 2013	1
Presentation in the Regional Scientific Meeting of Indonesian Internal Medicine Specialists, Semarang, Indonesia	2011	1
<b>Subtotal</b>		<b>28.5</b>

<b>2. Teaching</b>		<b>Hours</b>	<b>ECTS</b>
Teaching, practical supervising for medical students	2008 - 2011 2013 - 2014	56	2
Supervising Internal Medicine resident theses (2 residents)	2008 - 2012	56	2
Supervising medical doctor student theses (24 students @ 14 hours)	2008 - 2014	336	12
<b>Subtotal</b>			<b>16</b>

<b>TOTAL</b>	<b>44.5</b>
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