International Journal of Infectious Diseases 38 (2015) 101–107

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Viruses and Gram-negative bacilli dominate the etiology of community-acquired pneumonia in Indonesia, a cohort study

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ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 20 July 2015
Accepted 25 July 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Etiology
Community-aquired pneumonia
Asia
Virus
Gram-negative bacilli
Tuberculosis

ABSTRACT

Objective: Knowledge about the etiology of community-acquired pneumonia (CAP) is essential for adequate management. Presently, few studies about CAP are available from Southeast Asia. This study aimed to investigate the etiology, severity, and outcome of CAP in the most populous Southeast Asia country, Indonesia.

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

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

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 have a higher mortality risk.

1. 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. Many countries in Southeast Asia have no published or peer reviewed studies on CAP, including Indonesia, Brunei, Myanmar, Cambodia, East Timor, Laos, Vietnam, and most published studies used limited or unstandardized microbiological methods.[2–5] The etiology of CAP has been reported to differ in different geography and demography settings, thus, the management should not directly be adopted from other countries. Local data should be provided using a systematic and standardized method. This study aimed to describe the current etiology of CAP using a full range of diagnostic methods. Antimicrobial susceptibility of bacterial pathogens, underlying diseases, severity and outcome of these CAP patients were analyzed.

2. Materials and Methods

2.1. 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).

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http://dx.doi.org/10.1016/j.ijid.2015.07.023

2.2. 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 ≥2 of 6 criteria (cough, purulent sputum, temperature >38.5^{0}C, abnormal chest auscultation, white blood cell count >10 or <4 $\times 10^9$/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/uL, steroid treatment >20 mg/day for more than two weeks), had terminal stage of malignancy, or evidence of other causes of abnormalities on the X-ray.

2.3. Data collection

All patients were assessed upon admission, followed up 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 left to the discretion of the physicians in charge.

2.4. The microbiological evaluation

Sputum, throat swab, blood, paired sera with a 4-week interval, and urine were sent to the 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. Blood was cultured by inoculating four BACTEC bottles [Becton-Dickinson, Rochester, UK] per patient, and sputum samples were inoculated on blood agar, chocolate agar, chocolate agar with gentamicin 5 mg/L, and MacConkey agar (Oxoid, Basingstoke, UK). Crystal violet colistin 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 -80ºC, 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 ($bla_{\text{OXA-51-like}}$).[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 confirmed by 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 β-lactamase (ESBL)-production was suspected, isolates were further analyzed 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 syndrome compatible with leptospirosis. ELISA for respiratory viruses (Virion\Serion) was performed in the Laboratory of Viroscience, Erasmus MC. Real-time PCRs (RT-PCRs) were done on sputum and on 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[11,12] and PCR for Leptospira and for *Chlamydia psittaci*[13,14] were done as described elsewhere. 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.

2.5. 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 >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 ≥1 bottle for established pathogens or ≥2 bottles for other species.

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 immunogloblin for Leptospira.

RT-PCR were considered positive if the CT value was <50 for *M. pneumoniae*, ≤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 visible reaction of the urinary antigen tests was considered positive as instructed by the manufacturer.

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

2.6. Statistical analysis

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.

2.7. 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.