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# Epidemiology of community-acquired pneumonia among hospitalized children in Indonesia: a multicenter, prospective study

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Epidemiology of community-acquired pneumonia among hospitalized children in Indonesia: a multicenter, prospective study

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ABSTRACT:

Objective: To identify etiologies of childhood community-acquired pneumonia (CAP) based on a comprehensive diagnostic approach.

Design: “Partnerships for Enhanced Engagement in Research - Pneumonia in Pediatrics (PEER-PePPeS)” study was an observational prospective-cohort study conducted from July 2017 through September 2019.

Setting: Government referral teaching hospitals and satellite sites in three cities in Indonesia: Semarang, Yogyakarta, and Tangerang.

Participants: Hospitalized children aged 2–59-months who met the criteria for pneumonia were eligible. Children were excluded if they had been hospitalized for >24 hours; had malignancy or history of malignancy;
35 a history of long-term (>2 months) steroid therapy, or conditions that might interfere with compliance
36 with the study procedures.
37
38 **Main outcome(s) measure(s):**
39 Causative bacterial, viral, or mixed pathogen for the pneumonia was determined using
40 microbiological, molecular, and serologic test from routinely collected specimens (blood, sputum, and
41 nasopharyngeal swabs). We applied a previously published algorithm (PEER-PePPeS rules) to
determine the causative pathogen(s).
42
43 **Results:**
44 188 subjects were enrolled. Based on our algorithm, 48 (25.5%) had a bacterial infection, 31 (16.5%)
45 had a viral infection, 76 (40.4%) had mixed bacterial and viral infections, and 33 (17.6%) were unable
to be classified. The five most common causative pathogens identified were *Haemophilus influenzae*
46 non-type B (N=73), respiratory syncytial virus (RSV) (N=51), *Klebsiella pneumoniae* (N=43),
47 *Streptococcus pneumoniae* (N=29), and Influenza virus (N=25). RSV and Influenza virus diagnoses were
48 highly associated with Indonesia's rainy season (November-March). The polymerase chain reaction
49 (PCR) assays on IS specimens captured most of the pathogens identified in this study.
50
51 **Conclusions:**
52 CAP in hospitalized Indonesian children is most commonly associated with mixed infections, with *H.
53 influenzae* non-type B and RSV being the most frequently identified pathogens. Our study highlights
54 the importance of PCR for diagnosis and by extension, appropriate use of antimicrobials.
55
56 **Keywords:** Pneumonia; Children; Indonesia; Etiology; Epidemiology
57
58 **STRENGTHS AND LIMITATIONS OF THIS STUDY**
59
60 • Prospective multisite study conducted over 27-months
61 • Used a comprehensive approach (culture, molecular, and paired serology assays) to identify
62 causative pathogens from routinely collected specimens (blood, sputum, and nasopharyngeal
63 swabs)
64 • Did not include healthy control children, limiting ability to estimate the adjusted population
65 attributable fraction (aPaF) for each pathogen.
66 • Did not collect lung aspirates or pleural fluid specimens, which are preferred for determination
67 of pneumonia etiology
Several cases of pneumonia attributed to unknown etiology, which could be due to administration of antibiotics before culture, poor sputum quality, limited bacterial and viral panels used, lack of fungal testing or another factor

INTRODUCTION

Pneumonia is the leading infectious cause of child mortality. It accounts for approximately one million deaths annually among children under 5 years old, with a greater burden in low- and middle-income countries (LMICs).[1] In 2017, pneumonia contributed to 15% of childhood deaths and was the second highest cause of death amongst Indonesian children under five years.[2] One strategy to reduce child mortality due to pneumonia in Indonesia is through implementation of the Integrated Management of Childhood Illness (IMCI) guidelines which support early detection and management of possible pneumonia in young children with the goal of reducing deaths.[3,4]

IMCI is adapted from the World Health Organization (WHO) guideline, which was based on data from the 1970s through early 1990s when bacteria such as *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* caused the majority of fatal pneumonias in children.[3,5,6] WHO and other practice guidelines, including the 2011 guidelines from the British Thoracic Society (BTS) and the Pediatric Infectious Diseases Society/Infectious Diseases Society of America (IDSA/PIDS), recommend empiric antibiotics as the first-line treatment to ensure that all potential pneumonia cases receive effective antibiotic therapy in an effort to decrease mortality.[7–9]

Several recent studies of community-acquired pneumonia (CAP) in children have highlighted the role of viral etiologies. Increased recognition of viral etiologies of CAP is likely due to both enhanced molecular diagnostic capacity and wide deployment of Hib and pneumococcal conjugate vaccines [PCV].[10,11] Treatment of non-bacterial pneumonia with antibiotics may engender avoidable antimicrobial resistance. Thus, current data on the etiologies of childhood pneumonia is needed and should be regularly evaluated to inform vaccination policies, empiric management decisions, and targeted treatment.[12]

From a diagnostic standpoint, direct demonstration of organisms by culture (or staining) of lung aspirates was the standard for determining the microbial etiology of CAP.[13] In the current era, many use less-invasive biological specimens (e.g. blood, naso/oropharyngeal secretions, bronchoalveolar lavage, or induced sputum) and employ diverse methods (e.g. culture, PCR, antigen detection, or paired serology) to identify organisms.[14] However, such comprehensive methods are costly and often require specialized equipment and human resources, limiting feasibility in low-resource settings.[15,16]
Prospective community-based cohort studies that define pathogen(s) causing CAP in Indonesian children are scarce. We conducted a “Partnerships for Enhanced Engagement in Research - Pneumonia in Pediatrics (PEER-PePPeS)” study, which aimed to identify etiologies of childhood CAP using comprehensive diagnostic methods.

**METHODS**

**Study design and study sites**

PEER-PePPeS was a multi-site observational cohort study seeking to determine etiologies of CAP amongst children aged 2–59 months in Indonesia. The study was conducted by the Indonesia Research Partnership on Infectious Disease (INA-RESPOND) and enrolled participants initially at three government referral teaching hospitals in three provinces: Kariadi Hospital (Central Java), Sardjito Hospital (Yogyakarta), and Tangerang District Hospital (Banten), as shown in Supplementary Fig. 1. Satellite sites located near the primary sites were added during the study to facilitate subject recruitment. An-Nisa Hospital was Tangerang District Hospital’s satellite; Adhyatma Hospital and Bhakti Wira Tamtama Hospital served as satellites sites to Kariadi Hospital; Sardjito Hospital did not have a satellite. The Sardjito and Kariadi Hospitals are tertiary health care facilities equipped with 850+ beds each and Tangerang District Hospital with 437 beds. They have specialty physicians/departments and diagnostic laboratories that can perform routine hematological, biochemical, microbiological, molecular, and serological testing.

**Study Definitions**

In this study, pneumonia in children was defined as cough or fever with at least one of the following: shortness of breath (indicated by at least one of the following signs: head bobbing; nasal flaring; chest indrawing or intercostal retracting), tachypnea, grunting, crackles, rhonchi, decreased vesicular breath sounds, bronchial breath sounds or chest x-ray findings consistent with pneumonia. Tachypnea was defined as respiratory rate >50/min for infants 2–12 months and >40/min for children >12–60 months.[17] Abnormal chest x-ray findings consistent with pneumonia were defined as presence of either focal or diffuse infiltrates, a silhouette sign, pleural effusion, or air bronchogram.[18] Chest x-rays were read by the pediatrician.

Based on WHO classification and treatment of childhood pneumonia at health facilities (2014 version), for children 2–59 months of age, severe pneumonia was defined as pneumonia (tachypnea and/or chest indrawing) accompanied by presence of any danger signs, which including the inability...
to drink, persistent vomiting, convulsions, lethargy or loss of consciousness, stridor in a calm child, or severe malnutrition.[17]

Study Participants

PEER-PePPeS study enrolled children aged 2-59 months, who were hospitalized between July 18th, 2017 until September 25th, 2019, and met the definition for pneumonia. Eligible subjects were enrolled within 24 hours of admission. Children were excluded if they had been hospitalized for >24 hours; had a malignancy or history of malignancy; a history of long term (>2 months) steroid therapy; or conditions that might interfere with compliance with the study procedures (e.g., very ill patients for whom specimens could not be obtained or living outside the area for which follow-up was practical).

Study Procedures

Demographic and anthropometric data, current signs and symptoms, pregnancy history, vaccination status, breastfeeding history, antibiotic and steroid exposure, family history, medical history, risk factors, hematology profiles, chemistry results, and chest x-ray (per standard of care) were collected at enrollment. Clinical examination (vital signs, general examination, lung auscultation, SpO2); nasopharyngeal (NP) swab for molecular tests; induced sputum (IS) for culture and molecular tests; collection of blood specimens for routine blood count, cultures, molecular tests, serologic tests, C-reactive protein (CRP), and procalcitonin (PCT) were also performed. We prospectively followed subjects daily until hospital discharge; data on vital signs, respiratory signs, intensive care admission, intubation, complications and treatment were collected. On Day 14, we performed clinical examinations and collected convalescent sera for serology tests; subjects discharged before day 14 returned to clinic for their evaluation. We conducted a telephone interview on Day 30 (±4 days) to assess clinical outcome.

This study used several widely available bacterial and viral respiratory molecular pathogen panels and serologic assays.[19–22] NP and IS specimens were tested with a PCR panel that included twelve-viruses (Influenza A, Influenza B, Adenovirus, Enterovirus, Bocavirus, Respiratory Syncytial Virus (RSV) A, RSV B, Human Metapneumovirus, Rhinovirus, Parainfluenza Virus 1-4, Coronavirus OC43, and Coronavirus NL63). NP specimens were evaluated by PCR for five bacteria (Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, and Klebsiella pneumoniae), while IS specimens were tested for nine (Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Bordetella pertussis, Moraxella catarrhalis, Staphylococcus aureus, Klebsiella pneumoniae, and Legionella pneumoniae). Good quality
(<10 squamous epithelial cells per low power field[12]) IS specimens underwent culture and gram stain.[23] For whole blood, qPCR was performed for three bacteria (Haemophilus influenzae, Streptococcus pneumoniae, and Staphylococcus aureus). Serologic testing for seven viruses (Influenza A, Influenza B, Adenovirus, Parvovirus B19, Echovirus/Enterovirus, RSV, Parainfluenza Virus) and four bacteria (Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumoniae, and Bordetella pertussis) was performed using paired acute-convalescent sera.

Blood culture, IS culture and Gram stain, routine blood count, CRP, PCT, and chest x-ray were performed by the laboratory/radiology department at the hospital site. qPCR and serology assays were performed at the INA-RESPOND Reference Laboratory located in Tangerang District Hospital. Details of blood culture, sputum culture, molecular and serology test techniques are shown in Supplementary Table 1.

Pathogen Identification

Causative bacterial, viral, or mixed pathogen for the pneumonia was determined based on an algorithm (PEER-PePPeS rules) for interpretation of microbiological, molecular, and serologic test results previously published.[12] In brief, we considered all organisms detected by blood culture, detected by whole blood PCR, or that grew from good quality IS specimen in high quantities with a compatible primary Gram stain as potential causative bacterial pathogens. Bacteria commonly considered contaminants were excluded. For the nasopharynx, potential colonizing bacteria (e.g. H. influenzae, S. pneumoniae, and S. aureus) and potential innocent bystander viruses (e.g. Bocavirus, Adenovirus, non-SARS human Coronavirus (hCoVs), Enterovirus, and Rhinovirus) were determined to be causative based on a PCR density cut-off and/or serodiagnosis criteria for paired acute and convalescent sera (seroconversion or a two to four-fold increase in antibody titers in the convalescent specimen).[12]

Data collection and statistical analysis

Data were recorded on paper case report forms and entered in duplicate into OpenClinica (OpenClinica, LLC, MA, USA) by research staff. Categorical variables were summarized using absolute values and percentages, and continuous variables as medians and interquartile ranges (IQRs). Differences in categorical variables and continuous variables were compared using the Pearson χ2 test and Student’s t-test, respectively. Statistical analyses were performed using Statistical Package for Social Science (SPSS) software version 23 (IBM Corporation, Armonk, NY, USA). All p-values were two-sided. Level of significance was set at P < 0.05.
Patient and public involvement statement

Patients or the public were not involved in study design or study conduct at any stage from inception to completion and dissemination of this project. Patients who met the eligibility criteria as described above were recruited to this study.

RESULTS

Study Population

Of 444 children who were hospitalized with CAP, 188 (42.3%) were eligible and enrolled in the study. Of 256 screening failures, 31.8% were due to hospitalization >24 hours at the time of screening and 22.1% to circumstances that might interfere with the study procedures. Of the 188 enrolled children, 184 (97.9%) had radiologic evidence of pneumonia. 179 (95.1%) subjects completed the study, including 19 (10.1%) who died. Eight subjects (4.3%) were lost to follow up, and one subject (0.5%) withdrew from the study. The study flow is shown in Figure 1.

Demographic and clinical characteristics are presented in Table 1. Overall, subject characteristics were similar across the three study sites. Median age was 9 months (IQR, 5 to 20), and 54.7% of subjects were male. The most common comorbid conditions were developmental delay (27.7%), congenital heart disease (26.1%), and severe malnutrition (18.6%). The percentage of subjects who had been vaccinated (age-adjusted) against pneumococcus, influenza, Hib-DPT, and measles vaccines were 4.8%, 6.4%, 56.4%, and 76.6%, respectively.

Table 1. Baseline Characteristics of Subjects.

| Demographic Characteristics | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) |
|----------------------------|------------|----------------|------------------|------------------|
| Age, median (IQR) months   | 9 (5 – 20) | 9 (5.5 – 21)  | 8 (4 – 13.3)     | 11 (5-20)        |
| Gender, Male (%)           | 103 (54.7) | 29 (61.7)     | 26 (50)          | 48 (53.9)        |
| Household Characteristics, (%): |          |                |                  |                  |
| Low Education of Parents*  | 163 (86.7) | 37 (78.7)     | 42 (80.8)        | 84 (94.4)        |
| Living in a dense neighborhood* | 121 (64.4) | 19 (40.4)     | 42 (80.8)        | 60 (67.4)        |
| Sick household contact <14 days | 109 (58.0) | 22 (46.8)     | 43 (82.7)        | 44 (49.4)        |
| Exposure to cigarette smoke | 120 (63.8) | 24 (51.1)     | 27 (51.9)        | 69 (77.5)        |
| Attending daycare          | 4 (2.1)    | 2 (4.3)       | 1 (1.9)          | 1 (1.1)          |
| Medical history (%)        |            |                |                  |                  |
| Premature baby             | 34 (18.1)  | 4 (8.5)       | 16 (30.8)        | 14 (15.7)        |
| Low birth weight           | 46 (24.4)  | 12 (25.5)     | 20 (38.5)        | 14 (15.7)        |
| Developmental delay        | 52 (27.7)  | 16 (34.0)     | 21 (40.4)        | 15 (16.8)        |
• Congenital heart disease 49 (26.1)
• Severe malnutrition\(^\dagger\) 35 (18.6)
• Neurological disorder 25 (13.3)
• Asthma 9 (4.8)
• HIV disease\(^\dagger\) 2 (1.1)
• Tuberculosis (recent/cured) 10 (5.3)

49 (26.1) 16 (34.0) 24 (46.2) 9 (10.1)
35 (18.6) 10 (21.3) 13 (25.0) 12 (13.5)
25 (13.3) 5 (10.6) 17 (32.7) 3 (3.4)
9 (4.8) 3 (6.4) 1 (1.9) 5 (5.6)
2 (1.1) 1 (2.1) 1 (1.9) 0 (0)
10 (5.3) 4 (8.5) 2 (3.8) 4 (4.5)

Immunization history, fully vaccinated for age\(^\dagger\) (%):

- DPT-Hib 106 (56.4)
- Influenza 12 (6.4)
- Pneumococcus 9 (4.8)
- Measles 144 (76.6)

106 (56.4) 31 (66.0) 25 (48.1) 50 (56.2)
12 (6.4) 3 (6.4) 6 (11.5) 3 (3.5)
9 (4.8) 3 (6.4) 5 (9.6) 1 (1.1)
144 (76.6) 40 (85.1) 41 (78.8) 63 (70.8)

SpO\(_2\) <90% and/or Cyanosis, (%):

- Severe pneumonia (WHO Classification 2014 version), (%):
  - Pleural effusion 5 (2.7)
  - Interstitial infiltrate 131 (69.7)
  - Alveolar infiltrate 125 (66.5)

43 (22.9) 7 (14.9) 17 (32.7) 19 (21.3)
5 (2.7) 1 (2.1) 2 (3.8) 2 (2.2)
131 (69.7) 26 (55.3) 30 (57.7) 75 (84.3)
125 (66.5) 41 (87.2) 44 (84.6) 40 (44.9)

Antibiotic administration prior to blood collection for blood culture, (%)

- Antibiotic administration prior to blood culture, (%):
  - Pleural effusion 150 (79.8)
  - Interstitial infiltrate 39 (83.0)
  - Alveolar infiltrate 49 (94.2)

150 (79.8) 39 (83.0) 49 (94.2) 62 (69.7)

\(\dagger\) Low education of parents was defined by highest level of parents’ formal education being high school diploma or less; \(\dagger\) A densely populated neighborhood was defined as >200 people/km\(^2\) or <8 m\(^2\)/person in the subject’s home; \(\dagger\) Severe malnutrition was defined as weight for height below -3 standard deviations from the median of the WHO Child Growth Standards; \(\dagger\) Subjects were tested for HIV infection if a parent / guardian provided consent and a specimen was available (n=160); \# Full vaccination was defined as being up to date for age per vaccination schedule at study enrollment.

The most common symptoms were cough (91.0%), shortness of breath (90.6%), and fever (80.9%). Signs noted during the initial examination included intercostal retraction (91.0%), rhonchi (89.4%), and chest indrawing (66.5%). Of 188 subjects, 172 (91.4%) and 167 (88.8%) had CRP and PCT measured with median values of 9.0 (IQR, 3.6 – 28.0; Ref range \(\leq 5\)) mg/L and 0.2 (IQR, 0.1 – 1.7; Ref range \(\leq 0.15\)) ng/mL, respectively. Interstitial infiltrate (69.7%) was the most common radiographic finding. 47.3% of cases were classified as severe pneumonia according to the WHO classification system. All 188 enrolled cases were treated with antibiotics. The combination of ampicillin and gentamicin (37.8%), cefotaxime (26.1%), and ceftriaxone (19.1%) were the three most frequent regimens used during hospitalization (data not shown).

**Detection of Pathogens**

Blood and sputum cultures were performed on specimens from 184 (97.9%) and 183 (97.3%) subjects, respectively. A total of 150 (79.8%) children received antibiotics prior to collection of blood
for culture. Seventy-five (41.0%) sputum culture isolates were analyzed from specimens meeting the required quality criteria. A NP or OP swab was obtained from 187 (99.5%) subjects, IS for PCR from 176 (93.6%), whole blood for PCR from 163 (86.7%), and paired acute-convalescent serum specimens for serology from 116 (61.7%) (Fig. 1).

The PEER-PePPeS algorithm was used to determine the causative pathogen(s) from those identified by culture, molecular, and serologic assay. Amongst the 188 study participants, 48 (25.5%) had bacterial infection, 31 (16.5%) had viral infection, 76 (40.4%) were of mixed bacterial and viral etiology, and 33 (17.6%) were of unknown etiology (Fig. 2, Panel A). Mixed infection, the most common overall etiology, was seen in 38.7% of 2-11 month-olds and in 42.7% of 12-59 month-olds (Fig 2. Panel B). Mixed infection was also the predominant etiology across all study sites (Supplementary Fig. 2).

H. influenzae non-type B (N=73), RSV (N=51), K. pneumoniae (N=43), S. pneumoniae (N=29), Influenza virus (N=25), S. aureus (N=20), PIV (N=17), hMPV (N=11), Rhinovirus (N=10), and B. pertussis (N=7) were the top ten pathogens identified, more commonly appearing in mixed infection as opposed to as a sole pathogen (Fig 2. Panel C). Influenza virus was significantly higher in the age group 12-59 mo vs 2-11 mo (N=16, 64%, P=0.027), while S. aureus was significantly more common in 2-11 mo vs 12-59 mo (N=16, 80%, P=0.024). Though not statistically significant, other pathogens trended toward more frequent detection in age group 2-11 mo (except B. pertussis) (Fig 2. Panel D). Amongst 76 mixed infection cases, RSV + H. influenzae non-type B was the most common co-infection (N=22, 28.9%), followed by RSV + S. pneumoniae (N=10, 13.2%), Influenza virus + H. influenzae non-type B (N=10, 13.2%), RSV + K. pneumoniae (N=9, 11.8%), and Parainfluenza virus + H. influenzae non-type B (N=9, 11.8%) (Data not shown).

We observed no difference in pathogen distribution by pneumonia severity based on WHO classification system (Supplementary Table 2 and Supplementary Fig. 3). By pathogen, there was no significant difference in distribution between pneumonia severity status or mortality, except for S. pneumoniae which was found in significantly more severe cases using the WHO system (P=0.033) (Supplementary Table 2).

A comparison of positivity rates for each causative pathogen by detection method is shown in Table 2. Overall, PCR captured more bacterial pathogens than culture and more viral pathogens than acute-convalescent paired serology. Paired serology was generally helpful in identifying atypical bacteria, such as C. pneumoniae and L. pneumophila, and upper respiratory tract viruses, such as Rhinovirus and Enterovirus. When comparing blood and IS culture, IS yielded more positive bacterial pathogen results. Similarly, IS PCR captured more pathogens than NP/OP PCR.

Table 2. Causative Pathogens per PEER-PePPeS Rules by Detection Method
### Pathogen

| Pathogen       | N  | Blood culture (% N) | IS culture (% N) | Whole blood PCR (% N) | NP / OP PCR (% N) | IS PCR (% N) | Serology Test (% N) |
|----------------|----|---------------------|------------------|-----------------------|-------------------|--------------|----------------------|
| **Gram-positive cocci bacteria** |    |                     |                  |                       |                   |              |                      |
| *S. pneumoniae* | 29 | 1 (3.4%)            | 3 (10.3%)        | 0 (0.0%)              | 21 (72.4%)        | 28 (96.6%)   |                      |
| *S. aureus*     | 20 | 0 (0.0%)            | 7 (35%)          | 0 (0.0%)              | 11 (55%)          | 19 (95%)     |                      |
| *S. mitis*      | 4  | 0 (0.0%)            | 4 (100%)         | 0 (0.0%)              |                   |              |                      |
| *S. pyogenes*   | 1  | 0 (0.0%)            | 1 (100%)         | 0 (0.0%)              |                   |              |                      |
| **Gram-negative cocci bacteria** |    |                     |                  |                       |                   |              |                      |
| *M. catarrhalis*| 2  | 0 (0.0%)            | 2 (100%)         | 2 (100%)              | 2 (100%)          |              |                      |
| **Gram-negative rods bacteria** |    |                     |                  |                       |                   |              |                      |
| *H. inf non-type b* | 73 | 0 (0.0%)            | 0 (0.0%)         | 8 (10.9%)             | 60 (82.2%)        | 71 (98.6%)   |                      |
| *K. pneumoniae* | 43 | 0 (0.0%)            | 17 (39.5%)       | 2 (4.7%)              | 34 (79.1%)        |              |                      |
| *B. pertussis*  | 7  | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              | 7 (100%)          |              |                      |
| *E. coli*       | 5  | 1 (20%)             | 4 (80%)          | 0 (0.0%)              |                   |              |                      |
| *P. aeruginosa* | 4  | 0 (0.0%)            | 4 (100%)         | 0 (0.0%)              |                   |              |                      |
| *A. baumannii*  | 3  | 0 (0.0%)            | 3 (100%)         | 0 (0.0%)              |                   |              |                      |
| *H. inf type b* | 2  | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              | 0 (0.0%)          | 2 (100%)     |                      |
| *N. meningitidis* | 1 | 1 (100%)            | 1 (100%)         | 0 (0.0%)              |                   |              |                      |
| **Atypical-bacteria** |    |                     |                  |                       |                   |              |                      |
| *C. pneumoniae* | 5  | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              |                   |              |                      |
| *M. pneumoniae* | 5  | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              |                   |              |                      |
| *L. pneumophila* | 1 | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              |                   |              |                      |
| **Virus**       |    |                     |                  |                       |                   |              |                      |
| RSV            | 51 |                     |                  | 36 (70.6%)            | 45 (88.2%)        | 10 (19.6%)   |                      |
| RSV A          | 15 |                     |                  | 10 (66.7%)            | 13 (86.7%)        |              |                      |
| RSV B          | 36 |                     |                  | 26 (72.2%)            | 32 (88.8%)        |              |                      |
| Influenza Virus | 25 |                     |                  | 16 (64%)              | 22 (88%)          | 9 (36%)      |                      |
| Inf A (H1N1)   | 7  |                     |                  | 7 (100%)              | 7 (100%)          | 7 (70%)      |                      |
| Inf A (H3N2)   | 3  |                     |                  | 3 (100%)              | 3 (100%)          |              |                      |
| Inf B          | 14 |                     |                  | 6 (42.9%)             | 12 (85.7%)        | 2 (14.3%)    |                      |
| PIV            | 17 |                     |                  | 16 (94.1%)            | 15 (88.2%)        | 3 (17.6%)    |                      |
| PIV 1          | 5  | 5 (100%)            | 4 (80%)          | 0 (0.0%)              |                   |              |                      |
| PIV 2          | 0  |                     |                  | 0 (0.0%)              |                   |              |                      |
| PIV 3          | 11 | 10 (90.9%)          | 10 (90.9%)       | 3 (17.6%)             |                   |              |                      |
| PIV 4          | 1  | 1 (100%)            | 1 (100%)         | 0 (0.0%)              |                   |              |                      |
| hMPV           | 11 |                     |                  | 5 (45.5%)             | 10 (90.9%)        |              |                      |
| Rhinovirus     | 10 |                     |                  | 10 (100%)             | 6 (60%)           | 4 (40%)      |                      |
| Enterovirus    | 5  | 3 (60%)             | 3 (60%)          | 3 (60%)               |                   |              |                      |
| Bocavirus      | 3  | 2 (66.7%)           | 3 (100%)         | 0 (0.0%)              |                   |              |                      |
| CorNL63        | 2  | 2 (100%)            | 2 (100%)         | 0 (0.0%)              |                   |              |                      |

Grey-box indicates the assay was not performed

### Mortality

Nineteen (10.1%) subjects died during the 30-day study period. Seven were male, and most (17 subjects) were less than 1 year old. Among deceased subjects, median study duration was 12 (IQR, 4 – 17.5) days; 8 were admitted to ICU, and 6 received mechanical ventilation. Twelve died due to
respiratory failure, three due to sepsis, and three for unknown reasons after discharge (data not shown). Most deaths occurred in the 2-11 mo age group compared with the 12-59 mo age group (78.9% vs. 21.1%, \( p = 0.036 \)). Infection of deceased subjects was bacterial-only in 7, viral-only in 2, mixed in 5, and unknown in 5 subjects, with no significant differences between alive and deceased subjects. \( H. \text{ influenzae} \) non-type B was the most common pathogen identified in deceased subjects (N=8), followed by \( K. \text{ pneumooniae} \) (N=6), influenza virus (N=3), \( B. \text{ pertussis} \) (N=2), and RSV (N=2) (Supplementary Table 2). Some deceased subjects had pre-existing health conditions, most common were congenital heart disease (10 subjects), severe malnutrition (7 subjects), and developmental delay (7 subjects) (data not shown).

**Seasonality**

During the 27-month study period, infections caused by RSV and influenza were seen year-round with peak activity occurring during the wet season (November to March) in Indonesia (66.7%, \( p < 0.001 \); and 64.0%, \( p = 0.012 \), respectively). However, there was little variation in detection of the most common respiratory bacterial infections by month and season. \( H. \text{ influenzae} \) non-type B shows peaks in August (N=12, 16.4%) and March (N=11, 15.1%), while \( K. \text{ pneumooniae} \) and \( S. \text{ pneumooniae} \) fluctuate at lower levels throughout the year (Figure 3).

**DISCUSSION:**

PEER-PePPeS, a prospective multisite study, addresses a critical knowledge gap about the current epidemiology of pathogens causing CAP in children 2-59 months old in Indonesia. The study found: (1) mixed bacterial and viral infection is the predominant cause of childhood CAP, irrespective of age group and pneumonia severity; (2) bacterial infections were common (66% of cases) with \( H. \text{ influenzae} \) non-b type, \( K. \text{ pneumooniae} \), and \( S. \text{ pneumooniae} \) as the three most common bacterial etiologies; (3) viral pathogens were also common (57% of PEER-PePPeS subjects), with 16.5% of cases attributed to virus only and RSV and Influenza Virus being the most common viruses identified; and (4) PCR on IS specimens was the most sensitive assay for pathogen identification in this study.

A mixed bacterial and viral infection was the most commonly identified etiology in our study. While this is consistent with findings from other studies the clinical significance of the mixed infection remains controversial and it is not clear whether or not both agents are acting as true pathogens.[20,24] PEER-PePPeS did not demonstrate a correlation of mixed infection with pneumonia severity and 30-day mortality. Many deceased cases occurred at a younger age (less than 1 year old), and in the presence of comorbidities, such as congenital heart disease and severe malnutrition, as also
shown by previous reports.[25,26] Such factors may need to be considered in the prevention and management of childhood pneumonia to reduce mortality rate.

In recent years, there has been an increased focus on the role of respiratory viruses in childhood pneumonia, partly due to the reduction in bacterial disease associated with the use of conjugate pneumococcal and Hib vaccines and the increased capacity to detect viruses through PCR methods.[19,20,27,28] In PEER-PePPeS, viruses were found in 57% of subjects (virus only + mixed infection), with RSV and influenza virus being the viruses most commonly detected. RSV and influenza infection may be associated with Indonesia's wet/rainy season, consistent with other reports from tropical regions.[29–31] A high prevalence of RSV was also observed in the GABRIEL and PERCH international case-control studies of childhood pneumonia etiology.[20,28] In terms of mixed infections, we found that RSV + H. influenzae non-type B and RSV + S. pneumoniae were most common. Since respiratory viruses such as RSV can predispose to secondary bacterial infections, particularly S. pneumoniae and H. influenzae[32], and conversely bacteria can increase RSV susceptibility[32,33], these co-infections highlight the need for optimizing RSV surveillance, prevention and treatment.

Though Influenza virus also increases risk for secondary bacterial infections and is a major cause of childhood morbidity and mortality worldwide, data from developing countries is scarce.[34] In a previous Indonesian study of hospitalized patients with a severe acute respiratory infection (SARI), the prevalence of the Influenza virus was 10.6% in children less than 5 years old, and was never diagnosed during hospitalization.[35] PEER-PePPeS confirms the need for improved diagnostic strategies, management optimization, and influenza vaccination in children. Of note, our study was conducted before identification of COVID-19 in Indonesia[36], so did not address the role of COVID-19 in childhood pneumonia.

We also found that 66% of cases were caused by bacterial infection (bacteria only + mixed infection). Overall, H. influenzae non-type B was the most common bacteria implicated, followed by K. pneumoniae and S. pneumoniae. H. influenzae non-type B predominance was also observed in a Malaysian study, where 90% of enrolled children were vaccinated against Hib as part of the national immunization program.[22] With our moderate (56.4%) Hib vaccine coverage, high incidence of H. influenzae non-type B may represent true prevalence or strains not covered by Hib vaccine.[37] This finding agrees with current data that non-typeable H. influenzae (NTHi) can cause significant illness, and argues for strengthening the diagnostic laboratory capacity for pediatric specimens.

Identification of K. pneumoniae as the second most common bacterial etiology of childhood CAP is consistent with previously reported high carriage rates (~7%) in healthy Indonesian children. Carriage has been related to poor water and food hygiene and may give rise to pneumonia, especially
in children with malnutrition.[38] Given \textit{K. pneumoniae}’s potential for antibiotic resistance and high virulence of some strains, proactive detection and management strategies should be prioritized.[39]

The relatively low prevalence (15.4%) of \textit{S. pneumoniae} in PEER-PePPes was surprising since carriage rates are high and PCV coverage low in Indonesia.[40] A similar relatively low prevalence of \textit{S. pneumoniae} in childhood CAP was also reported from Malaysia, where PCV coverage is 8.7%.[22] and in the PERCH study, reflecting temporal shifts in childhood pneumonia etiologies.[20] As only 4.8% of PEER-PePpeS subjects had received PCV, vaccination alone cannot account for the low \textit{S. pneumoniae} prevalence. It is possible that antibiotic exposure prior to obtaining specimens may reduce median colonization density and lower positive findings yield of \textit{S. pneumoniae} by both culture and PCR.[41] Moreover, our panel did not include \textit{S. pneumoniae} paired serology, which may be useful to increase pneumococcal diagnosis in young children.[42] Nonetheless, \textit{S. pneumoniae} remains an important etiological agent of severe/complicated CAP globally.[43] Our finding that \textit{S. pneumoniae} was significantly associated with severe cases by the WHO classification system supports the need for ongoing surveillance, vaccination and prevention of transmission between adults and children.

Inclusion of several pathogen identification strategies in PEER-PePPes demonstrates the differential utility of assays and specimen types. Our findings highlight the value of molecular assays, especially in culture-negative cases where microorganisms may be nonrecoverable in culture due to prior antibiotics or presence of otherwise hard to culture bacteria.[44,45] PCR is also less laborious and boasts a shorter turn-around-time than conventional culture. PCR can additionally identify genes associated with antibiotic resistance, though conventional culture methods will still be required to confirm the phenotypic resistance.[46,47] Even with the limited PCR panels used in our study, molecular assays had greater sensitivity for identification of bacterial pathogens than blood or sputum culture when using the PEER-PePpeS rules. Regardless of the method of detection, targeted treatment can follow empiric treatment once an organism is identified. This facilitates optimization of management while minimizing risk of adverse events and development of antimicrobial resistance due to unnecessary, prolonged use of antibiotics.

Although sensitive for detection, PCR does not provide information regarding infectiousness or viability. Genome fragments from dead organisms may be detected, often at a low level, even after clinical resolution.[45] Furthermore, negative results may occur due to differential viral kinetics along the respiratory tract. Thus lower respiratory tract specimens, such as IS, should be sought as they originate from the actual site of infection.[10,12] Accordingly, we observed a higher yield from PCR on IS than NP specimens. This finding may be confounded as IS and NP detection panels varied slightly. We also found that the use of paired serologies increased the diagnostic yield and was useful for
384 pathogen confirmation, particularly in the setting of innocent bystander viruses and atypical bacteria.[12]

386 PEER-PePPeS used a comprehensive approach for pathogen detection to increase diagnostic yield. It also enrolled patients over a 27-month study period, facilitating assessment of seasonality.

388 However, our study has several limitations. The relatively small sample size and geographic limitation to the island of Java may limit generalizability. Second, we did not enroll healthy control children, limiting the ability to estimate the adjusted population attributable fraction (aPaF) of each pathogen.[27,28] A healthy control group would have revealed the baseline carriage rate, minimizing over-attribution of disease to non-pathogenic organisms.[19,20,27,28] Third, we did not collect lung aspirates or pleural fluid specimens, which are superior for determination of pneumonia etiology. [13]

394 Fourth, several subjects had pneumonia of unknown etiology; this may have been due to administration of antibiotics before culture, poor IS quality, the limited panel of bacterial and viral pathogens tested, lack of fungal testing, or currently unrecognized causes of pediatric pneumonia.

In conclusion, the epidemiology of childhood CAP is constantly evolving in step with social and environmental factors and thus, should be regularly assessed. Our study found that H. influenzae non-type B and RSV were the most common pathogens causing hospitalized CAP among Indonesian children aged 2-59 months old, suggesting a changing pathogen profile from the 1970-1990s etiology studies that mainly detected S. pneumoniae and H. influenzae type B as the most important causes of childhood pneumonia in LMICs[3,5,6]. PCR on IS had the best sensitivity for pathogen identification, highlighting the need for accessible multiplexed point-of-care molecular assays to guide management.

397 Optimization of pathogen detection to understand changing childhood CAP epidemiology will inform public policy on prevention and management.

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CONTRIBUTORS:

DL, HF, RT, YM, HK, AMN, ATA, CYL, HCL designed and conceptualized the study. DL, HF, RT, AB, C, MSA, DW, M, SD, AS performed clinical assessments and were responsible for data entry. DL, HF, RT, YM, HK, AMN performed data analysis, interpretation and drafted the first manuscript. YM, HK, AMN, NL, AK, CYL designed the methodology for pathogen identification. YM, HK, AMN assisted with manuscript writing, analysis, and interpretation of data. All authors contributed to manuscript development, edited for critical content, and have approved the final version.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICS APPROVAL:

This study was approved by the Ethical Clearance Committee of Faculty of Medicine, Universitas Indonesia (No. 567/UN2.F1/ETIK/2017). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or guardians before enrollment.

DATA AVAILABILITY STATEMENT:

Data are available upon reasonable request. The anonymized data set will be shared following the signing of a data-sharing agreement, with permission of the ethical clearance committee, study authors, and all project partners, exclusively for non-commercial purposes. Please contact the corresponding author with any queries.
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**FIGURE CAPTIONS:**

**Figure 1. Subject screening, enrolment, and monitoring flowchart.** CAP, community-acquired pneumonia; RR, respiratory rate; CXR, chest X-Ray; CRP, C-reactive protein; PCT, procalcitonin; NP, nasopharyngeal; IS, induced sputum; PCR, polymerase chain reaction.

**Figure 2. Pathogen Distribution.** (A) Overall proportion of identified viral/bacterial/mixed pathogen, (B) Viral/bacterial/mixed pathogens by age group, (C) Pattern of detection of the ten most identified pathogens, (D) Distribution of ten most identified pathogens by age group. *P*<0.05

**Figure 3. Distribution of the (A) monthly count and (B) seasonal pattern of infection caused by *H. influenzae* non-type B, RSV, *K. pneumoniae*, *S. pneumoniae*, and Influenza virus during a 27-month study period.

**SUPPLEMENTARY INFORMATION:**

Details of Microbiological, Molecular and Serologic Methods, *Supplementary Table 1*

Pathogen distribution by WHO severity classification status and mortality, *Supplementary Table 2*

PEER-PePPeS Study sites, *Supplementary Figure 1*

Proportion of Identified Pathogen in each Sites, *Supplementary Figure 2*

Proportion of Identified Pathogen between WHO Severity Status, *Supplementary Figure 3*
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml
A. Proportion of identified Pathogens

- Bacterial (N=48)
- Viral (N=31)
- Mixed (N=76)
- Unknown (N=33)

Total=108

B. 12-18 mo

- Bacterial (N=17)
- Viral (N=16)
- Mixed (N=32)
- Unknown (N=15)

Total=82

C. Single vs. Co-infection Pathogen Detection

- Detected as single pathogen
- In coordination with other(s) pathogens

D. Age Groups Distribution of Detected Pathogens

- H. influenzae
- RSV
- Rhinovirus
- Parainfluenza Virus
- R. pneumoniae
- E. pneumoniae

381x381mm (300 x 300 DPI)
A. Detection of Pathogens by Enrollment Months (Number of Cases)

B. Detection of Pathogens by Season (Percentage of Cases)

381x177mm (300 x 300 DPI)
Supplementary Table 1. Microbiological, Molecular and Serologic Methods

| No. | Assays | Procedures |
|-----|--------|------------|
| 1.  | Gram stain | Gram-stained smears were obtained from the most purulent portion of each induced sputum specimen. The good quality specimen was defined as <10 squamous epithelium per low-power field (magnification, 100×) 1. The procedure of the Gram stain required four basic steps that include applied a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram’s Iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstained with safranin 2. The Gram-stained smears interpreted as follows: Gram-positive lancet-shaped diplococci (GPDC) suggest *Streptococcus pneumoniae*; Gram-positive diplococci (GPDC) or cocci in chains suggest *Streptococcus pyogenes*; Gram-positive cocci in clusters (GPC-cluster) suggest *Staphylococcus aureus*; Gram-negative coccobacilli (GNCB) suggest *Haemophilus influenzae*, *Bordetella pertussis* or *Acinetobacter baumannii*; Gram-negative diplococci (GNDC) suggest *Moraxella catarrhalis*; large Gram-negative rods (GNR-large) suggest *Klebsiella pneumoniae* or *Escherichia coli*; and small Gram-negative rods (GNR-small) suggest *Pseudomonas aeruginosa* 3. |
| 2.  | Induced Sputum Culture | The most purulent portion of induced sputum was inoculated onto sheep blood, chocolate, and MacConkey agars, streaked out using a standard 4-quadrant streaking method, and incubated at 35°C for 48 hours. Cultures were examined at 24 hours and 48 hours, and predominant bacteria were identified and quantified according to the farthest quadrant with visible colonies (first quadrant, scanty; second quadrant, 1+; third quadrant, 2+; fourth quadrant, 3+) 4. Then, the predominant bacteria isolates were inoculated into the appropriate VITEK identification strip using the VITEK® 2 COMPACT (BioMérieux, Germany). Briefly, a bacterial suspension was adjusted to a McFarland standard of 0.50 in a solution of 0.45 % sodium chloride using DensiLameter. The time between preparation of the solution and filling of the card was always less than 1 h. Analysis was done using the identification card and automatically read every 15 min. Bacteria identification and antibiotic susceptibility testing results were analyzed using the VITEK 2 software according to the manufacturer’s instructions 5. |
| 3.  | Blood Culture | Up to 2 mL of blood samples (2 bottle sets) were collected and sent to the site laboratory with standardized procedures. Blood cultures were incubated for at least 5 days, unless positive, using automated systems (BacT/ALERT in Tangerang Hospital; BACTEC at other sites) 6. Organisms were identified according to standard microbiological methods as described in induced sputum culture section. The following organisms were considered to be contaminants when identified in blood cultures: Coagulase-negative *staphylococci*, *Micrococcus* spp., *Propionibacterium* spp., Alpha-hemolytic streptococci (except... |
|   |   |
|---|---|
| 4. Viral RNA Extraction | *Viral RNA was extracted from viral transport media (VTM) containing respiratory swab as well as sputum, using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Briefly, 140 µl of VTM or sputum coat was lysed in 560 of carrier RNA-containing AVL buffer, followed by the binding of viral RNA to the QIAamp membrane. Contaminants were removed from viral RNA in two separate washing steps using two different wash buffers, AW1 and AW2. Viral RNA was eluted in 60 µl of AVE buffer and kept in -80°C if not directly used.*  

5. Bacterial DNA Extraction | *Bacterial DNA was extracted from viral transport media (VTM) containing respiratory swab as well as sputum, using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Briefly, 20 µl of QIAGEN Protease and 200 µl of VTM or sputum coat was lysed in 200 of AL buffer, followed binding of DNA to the QIAamp membrane. Contaminants were removed from DNA in two separate washing steps using two different wash buffers, AW1 and AW2. Bacterial DNA was eluted in 200 µl of AE buffer and kept in -80°C if not directly used.*  

6. qPCR for Respiratory Viruses | *The realtime PCR for respiratory virus detection was done followed the protocol of Beld et al., 2004 and Jansen et al., 2011. Positive control is a synthetic plasmid carrying the nucleotide sequence of the detection target. Primers, probes, and positive controls were synthesized and purified by an outside vendor (Integrated DNA Technologies, Iowa, US). Realtime PCR was done using the TaqManTM Fast Virus 1-Step Master Mix (Thermo Fisher Scientific; Cat#: 4444432) in an Applied Biosystems 7500 Fast Realtime PCR System (Thermo Fisher Scientific, MA, US). The reaction mixture composition was 1X TaqManTM Fast Virus 1-Step Master Mix, 0.5 µM of each primer, 0.25 µM probe, and 4 µl RNA, in a total 20 µl volume. The cycle condition was 50°C reverse transcription for 5 minutes, 95°C initial denaturation for 20 seconds, followed by 45 cycles of denaturation (95°C, 3 seconds) and annealing/elongation (55°C, 30 seconds). Realtime PCR works correctly when the positive control demonstrates the amplification curve and the template-free (negative) control demonstrates no amplification curve (no Ct values).*  

7. qPCR for Respiratory Bacteria | *In real-time PCR (qPCR) a portion of bacterial DNA genome specific to the pathogen(s) of interest is amplified using a specific pair of primers and probes for each bacteria, that were selected from the available literature 10–14. A detector (TaqMan® probe) is used in the reaction. Mastermix is prepared in a 1.5-ml tube for total reaction. qPCR assays were carried out in a total volume of 20 µL, comprising 10 µL of TaqMan® Fast Universal PCR Master Mix, 1.4 µL of nuclease-free water (Promega), 3.6 µL of oligonucleotide mixtures, and 4 µL of DNA extract. The cycle condition was 95°C initial denaturation for 20 seconds, followed by
45 cycles of denaturation (95°C, 3 seconds) and annealing/elongation (58°C, 30 seconds). Realtime PCR works correctly when the positive control demonstrates the amplification curve and the template-free (negative) control demonstrates no amplification curve (no Ct values).

8. Serology Test

Assays were obtained from SERION ELISA classic kit (Institut Virion/Serion Laboratories, Germany) and used according to the insert of SERION kit. SERION ELISA classic is a qualitative and quantitative immunoassay for detecting human antibodies in serum or plasma with their corresponding antigen. The indirect enzyme immunosorbent assay in this kit was coated with specific antigens of the pathogen of interest. Patient sera are diluted in a rheumatoid factor and then diluted in Sample Diluent (containing phosphate with tween 20 and Bromphenol blue) and incubated in the coated microwells to bind serum antibody to the solid-phase antigen. The microwells are then washed to remove unreacted serum proteins, and enzyme conjugate (anti-human IgA, IgG, or IgM APC_Alkaline phosphatase) is added to label the bound antibody. After further incubation, the microwells are washed to remove unbound APC Conjugate. The pNPP (para-nitrophenyl phosphate) substrate is then added to quantitate the Conjugate-bound p-nitrophenyl phosphate portion. The colorless substrate p-nitrophenyl phosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the serum antibody. This timed reaction is interrupted with a Stop Solution (sodium hydroxide). Color intensity (Absorbance) is measured at a wavelength of 405nm on a microtiter plate reader or spectrophotometer within 15 minutes of adding the stop solution. Antibody activities are calculated by the SERION evaluation software.15.
37 **Supplementary Table 2.** Pathogen distribution by WHO severity classification status and mortality.

| Pathogens          | WHO Classification System | Mortality Outcome | p-value |
|--------------------|---------------------------|-------------------|---------|
|                    | Severe (N=89)             | Non-severe (N=99) |         |
|                    | Died(N=19)                | Alive (N=169)     |         |
| Causative Pathogen |                           |                   |         |
| *H. influenzae* non-type b | 31 (34.8%) | 42 (42.4%) | 0.286 | 8 (42.1%) | 65 (38.5%) | 0.757 |
| RSV                | 25 (28.1%) | 26 (26.3%) | 0.778 | 2 (10.5%) | 49 (29.0%) | 0.086 |
| *K. pneumoniae*    | 15 (16.9%) | 28 (28.3%) | 0.062 | 6 (31.6%) | 37 (21.9%) | 0.388 |
| *S. pneumoniae*    | 19 (21.3%) | 10 (10.1%) | 0.033 | 1 (5.2%) | 28 (16.6%) | 0.317 |
| Influenza virus    | 9 (10.1%) | 16 (16.2%) | 0.223 | 3 (15.8%) | 22 (13.0%) | 0.723 |
| *S. aureus*        | 8 (9.0%) | 12 (12.1%) | 0.487 | 0 (0.0%) | 20 (11.8%) | 0.230 |
| PIV                | 8 (9.0%) | 9 (9.1%) | 0.981 | 1 (5.3%) | 16 (9.5%) | 1.000 |
| hMPV               | 6 (6.7%) | 5 (5.1%) | 0.622 | 1 (5.3%) | 10 (5.9%) | 1.000 |
| Rhinovirus         | 7 (7.9%) | 3 (3.0%) | 0.196 | 1 (5.3%) | 9 (5.3%) | 1.000 |
| *B. pertussis*     | 4 (4.5%) | 3 (3.0%) | 0.709 | 2 (10.5%) | 5 (3.0%) | 0.150 |
| Infection Type     |                           |                   |         |
| Bacterial pathogen | 17 (19.1%) | 31 (31.3%) | 0.055 | 7 (36.8%) | 41 (24.3%) | 0.268 |
| Viral pathogen     | 16 (18.0%) | 15 (15.2%) | 0.602 | 2 (10.5%) | 29 (17.2%) | 0.744 |
| Mixed pathogen     | 38 (42.7%) | 38 (38.4%) | 0.547 | 5 (26.3%) | 71 (42.0%) | 0.186 |
| Unknown pathogen   | 18 (20.2%) | 15 (15.2%) | 0.361 | 5 (26.3%) | 28 (16.6%) | 0.337 |

For comparison, Chi-square test for categorical variables was done.
Supplementary Figure 1. PEER-PePPeS Study sites:

1. Kariadi Hospital, Semarang
Satellite sites: Adhyatma Hospital and Bhakti Wira Tamtama Hospital
2. Sardjito Hospital, Yogyakarta
3. Tangerang District Hospital, Tangerang
Satellite site: An-Nisa Hospital

Supplementary Figure 2. Proportion of Identified Pathogen in each Sites. (A) Semarang, (B) Yogyakarta, and (C) Tangerang
Supplementary Figure 3. Proportion of Identified Pathogen between WHO Severity Status. (A) Non-severe Pneumonia, (B) Severe Pneumonia.
STROBE Statement—Checklist of items that should be included in reports of cohort studies

| Paragraph/Line number | Recommendation |
|------------------------|----------------|
| **Title and abstract** | (a) Indicate the study's design with a commonly used term in the title or the abstract |
|                        | (b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction**       |                |
| Background/rationale   | Explain the scientific background and rationale for the investigation being reported |
| Objectives             | State specific objectives, including any prespecified hypotheses |
| **Methods**            |                |
| Study design           | Present key elements of study design early in the paper |
| Setting                | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants           | (a) Give the eligibility criteria, and the sources and methods of selection of participants |
| Variables              | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/measurement | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias                   | Describe any efforts to address potential sources of bias |
| Study size             | Explain how the study size was arrived at |
| Quantitative variables | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods    | (a) Describe all statistical methods, including those used to control for confounding |
|                        | (b) Describe any methods used to examine subgroups and interactions |
|                        | (c) Explain how missing data were addressed |
|                        | (d) If applicable, describe analytical methods taking account of sampling strategy |
|                        | (e) Describe any sensitivity analyses |
| **Results**            | (a) Report numbers of individuals at each stage of study—e.g numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed |
| Participants           | (b) Give reasons for non-participation at each stage |
|                        | (c) Consider use of a flow diagram |
Descriptive data
Results, paragraph 2 and 3. Table 1. (Page 7-8)
(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
(b) Indicate number of participants with missing data for each variable of interest

Outcome data
Results, paragraph 4 and 5 (Page 8-9)
Report numbers of outcome events or summary measures

Main results
Results, paragraph 5, 7, and 9. Table 2. (Page 9-11). Fig. 2 and Fig. 3
(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
(b) Report category boundaries when continuous variables were categorized
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses
Results, paragraph 6 (Page 9).
Results, paragraph 8. (Page 10-11).
Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion
Key results
Discussion, paragraph 1 (Page 11)
Summarise key results with reference to study objectives

Limitations
Discussion, paragraph 10 (Page 14)
Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias

Interpretation
Discussion, paragraph 2-9 (Page 11-14)
Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence

Generalisability
Discussion, paragraph 3-5 (Page 12)
Discussion, paragraph 7 (Page 13)
Discussion, paragraph 10-11 (Page 14)
Discuss the generalisability (external validity) of the study results

Other information
Funding
Funding statement (Page 15)
Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.
# Epidemiology of community-acquired pneumonia among hospitalized children in Indonesia: a multicenter, prospective study

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Epidemiology of Community-acquired pneumonia among hospitalized children in Indonesia: a multicenter, prospective study

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ABSTRACT:

Objective:

To identify etiologies of childhood community-acquired pneumonia (CAP) based on a comprehensive diagnostic approach.

Design:

“Partnerships for Enhanced Engagement in Research - Pneumonia in Pediatrics (PEER-PePPeS)” study was an observational prospective-cohort study conducted from July 2017 through September 2019.

Setting:
Government referral teaching hospitals and satellite sites in three cities in Indonesia: Semarang, Yogyakarta, and Tangerang.

Participants:
Hospitalized children aged 2–59-months who met the criteria for pneumonia were eligible. Children were excluded if they had been hospitalized for >24 hours; had malignancy or history of malignancy; a history of long-term (>2 months) steroid therapy, or conditions that might interfere with compliance with study procedures.

Main outcome(s) measure(s):
Causative bacterial, viral, or mixed pathogen(s) for the pneumonia were determined using microbiological, molecular, and serologic tests from routinely collected specimens (blood, sputum, and nasopharyngeal swabs). We applied a previously published algorithm (PEER-PePPeS rules) to determine the causative pathogen(s).

Results:
188 subjects were enrolled. Based on our algorithm, 48 (25.5%) had a bacterial infection, 31 (16.5%) had a viral infection, 76 (40.4%) had mixed bacterial and viral infections, and 33 (17.6%) were unable to be classified. The five most common causative pathogens identified were *Haemophilus influenzae* non-type B (N=73, 38.8%), respiratory syncytial virus (RSV) (N=51, 27.1%), *Klebsiella pneumoniae* (N=43, 22.9%), *Streptococcus pneumoniae* (N=29, 15.4%), and Influenza virus (N=25, 13.3%). RSV and Influenza virus diagnoses were highly associated with Indonesia’s rainy season (November-March). The polymerase chain reaction (PCR) assays on induced sputum (IS) specimens captured most of the pathogens identified in this study.

Conclusions:
Our study found that *H. influenzae* non-type B and RSV were the most frequently identified pathogens causing hospitalized CAP among Indonesian children aged 2-59 months old. Our study also highlights the importance of PCR for diagnosis and by extension, appropriate use of antimicrobials.

Keywords: Pneumonia; Children; Indonesia; Etiology; Epidemiology

STRENGTHS AND LIMITATIONS OF THIS STUDY
- Prospective multisite study conducted over 27-months
- Used a comprehensive approach (culture, molecular, and paired serology assays) to identify causative pathogens from routinely collected specimens (blood, sputum, and nasopharyngeal swabs)
The relatively small sample size, geographic limitation to the island of Java and observational design may limit generalizability and causal inference.

We did not collect lung aspirates or pleural fluid specimens, which are preferred for the determination of pneumonia etiology, and did not include healthy control children, limiting ability to estimate the adjusted population attributable fraction (aPaF) for each pathogen.

Several cases of pneumonia attributed to unknown etiology, which could be due to administration of antibiotics before culture, poor sputum quality, limited bacterial and viral panels used, lack of fungal testing or another factor.

INTRODUCTION

Pneumonia is the leading infectious cause of child mortality, with a greater burden in low- and middle-income countries (LMICs).[1] In Indonesia, pneumonia contributed to 15% of childhood deaths and was the second leading cause of death amongst children under five years in 2017.[2] Indonesian practice guidelines are adapted from the World Health Organization (WHO) guidelines, which are based on 1970’s – 1990’s data showing bacteria such as *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* caused the majority of fatal pneumonias in children.[3–5] Therefore empiric antibiotics are considered first-line treatment for children with community-acquired pneumonia (CAP).[6–8] Despite evidence that appropriate antibiotics are lifesaving, rational selection of antibiotics for pneumonia is hampered by low adherence to guidelines and scarcity of point-of-care diagnostics.[9–11] Consequently, healthcare providers, particularly those in LMIC, are likely to overtreat non-bacterial pneumonia with antibiotics.[11,12]

Several recent studies of community-acquired pneumonia (CAP) in children have highlighted the role of viral etiologies. Increased recognition of viral etiologies of CAP is likely due to both enhanced molecular diagnostic capacity and wide deployment of Hib and pneumococcal conjugate vaccines [PCV].[13,14] Treatment of non-bacterial pneumonia with antibiotics may engender avoidable antimicrobial resistance. Thus, current data on the etiologies of childhood pneumonia is needed and should be regularly evaluated to inform vaccination policies, empiric management decisions, and targeted treatment.[12]

From a diagnostic standpoint, direct demonstration of organisms by culture (or staining) of lung aspirates has been the standard for determining microbial etiology of CAP.[15] In the current era, many use less-invasive biological specimens (e.g. blood, naso/oropharyngeal secretions, bronchoalveolar lavage, or induced sputum) and employ diverse methods (e.g. culture, PCR, antigen detection, or paired serology) to identify organisms.[16] However, such comprehensive methods are...
costly and often require specialized equipment and human resources, limiting feasibility in low-resource settings.[17,18]

Prospective community-based cohort studies that define pathogen(s) causing CAP in Indonesian children are scarce. We conducted a “Partnerships for Enhanced Engagement in Research - Pneumonia in Pediatrics (PEER-PePPeS)” study, which aimed to identify etiologies of childhood CAP using comprehensive diagnostic methods.

METHODS

Study design and study sites

PEER-PePPeS was a multi-site observational cohort study seeking to determine etiologies of CAP amongst children aged 2–59 months in Indonesia. The study was conducted by the Indonesia Research Partnership on Infectious Disease (INA-RESPOND) and enrolled participants initially at three government referral teaching hospitals in three provinces: Kariadi Hospital (Central Java), Sardjito Hospital (Yogyakarta), and Tangerang District Hospital (Banten), as shown in Supplementary Fig. 1. Satellite sites located near the primary sites were added during the study to facilitate subject recruitment.

Study Definitions

In this study, pneumonia in children was defined as cough or fever with at least one of the following: shortness of breath (indicated by at least one of the following signs: head bobbing; nasal flaring; chest indrawing or intercostal retracting), tachypnea, grunting, crackles, rhonchi, decreased vesicular breath sounds, bronchial breath sounds or chest x-ray findings consistent with pneumonia. Tachypnea was defined as respiratory rate >50/min for infants 2–12 months and >40/min for children >12–60 months.[19] Abnormal chest x-ray findings consistent with pneumonia were defined as presence of either focal or diffuse infiltrates, a silhouette sign, pleural effusion, or air bronchogram.[20] Chest x-rays were read by the pediatrician.

Based on WHO classification and treatment of childhood pneumonia at health facilities (2014 version), for children 2–59 months of age, severe pneumonia was defined as pneumonia (tachypnea and/or chest indrawing) accompanied by presence of any danger signs, which included the inability to drink, persistent vomiting, convulsions, lethargy or loss of consciousness, stridor in a calm child, or severe malnutrition.[19]

Study Participants
PEER-PePPeS study enrolled children aged 2-59 months, who were hospitalized between July 18th, 2017 until September 25th, 2019, and met the definition for pneumonia. Eligible subjects were enrolled within 24 hours of admission. Children were excluded if they had been hospitalized for >24 hours; had a malignancy or history of malignancy; a history of long term (>2 months) steroid therapy; or conditions that might interfere with compliance with study procedures (e.g., very ill patients for whom specimens could not be obtained or living outside the area for which follow-up was not practical).

**Study Procedures**

Demographic and anthropometric data, current signs and symptoms, pregnancy history, vaccination status, breastfeeding history, antibiotic and steroid exposure, family history, medical history, risk factors, hematologic profiles, chemistry results, and chest x-ray (per standard of care) were collected at enrollment. Clinical examination (vital signs, general examination, lung auscultation, SpO2); nasopharyngeal (NP) swab for molecular tests; induced sputum (IS) for culture and molecular tests; collection of blood specimens for routine blood count, cultures, molecular tests, serologic tests, C-reactive protein (CRP), and procalcitonin (PCT) were also performed. We prospectively followed subjects daily until hospital discharge; data on vital signs, respiratory signs, intensive care admission, intubation, complications, and treatment were collected. On Day 14, we performed clinical examinations and collected convalescent sera for serology tests; subjects discharged before day 14 returned to clinic for their evaluation. We conducted a telephone interview on Day 30 (±4 days) to assess clinical outcome.

This study used several widely available bacterial and viral respiratory molecular pathogen panels and serologic assays.[21–24] NP and IS specimens were tested with a PCR panel that included twelve-viruses (influenza A, influenza B, adenovirus, enterovirus, bocavirus, respiratory syncytial virus (RSV) A, RSV B, human metapneumovirus (hMPV), rhinovirus, parainfluenza virus (PIV) 1-4, coronavirus OC43, and coronavirus NL63). NP specimens were evaluated by PCR for five bacteria (**Haemophilus influenzae**, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, and Klebsiella pneumoniae), while IS specimens were tested for nine (**Haemophilus influenzae**, Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Bordetella pertussis, Moraxella catarrhalis, Staphylococcus aureus, Klebsiella pneumoniae, and Legionella pneumophila). Good quality (<10 squamous epithelial cells per low power field[12]) IS specimens underwent culture and gram stain.[25] For whole blood, qPCR was performed for three bacteria (**Haemophilus influenzae**, Streptococcus pneumoniae, and Staphylococcus aureus). Serologic testing for seven viruses (influenza A, influenza B, adenovirus, parvovirus B19, echovirus/enterovirus, RSV, parainfluenza virus) and four
bacteria (*Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumoniae, and Bordetella pertussis*) was performed using paired acute-convalescent sera.

Blood culture, IS culture and Gram stain, routine blood count, CRP, PCT, and chest x-ray were performed by the laboratory/radiology department at the hospital site. qPCR and serology assays were performed retrospectively at the INA-RESPOND Reference Laboratory located in Tangerang District Hospital. Details of blood culture, sputum culture, molecular and serology test techniques are shown in **Supplementary Table 1**.

**Pathogen Identification**

Causative bacterial, viral, or mixed pathogen for the pneumonia was determined based on an algorithm (PEER-PePPeS rules) for interpretation of microbiological, molecular, and serologic test results previously published.[12] In brief, we considered all organisms detected by blood culture, detected by whole blood PCR, or that grew from good quality IS specimen in high quantities with a compatible primary Gram stain as potential causative bacterial pathogens. Bacteria commonly considered contaminants were excluded. For the nasopharynx, potential colonizing bacteria (e.g. *H. influenzae*, *S. pneumoniae*, and *S. aureus*) and potential innocent bystander viruses (e.g. bocavirus, adenovirus, non-SARS human Coronavirus (hCoVs), enterovirus, and rhinovirus) were determined to be causative based on a PCR density cut-off and/or serodiagnosis criteria for paired acute and convalescent sera (seroconversion or a two to four-fold increase in antibody titers in the convalescent specimen).[12]

**Data collection and statistical analysis**

Data were recorded on paper case report forms and entered in duplicate into OpenClinica (OpenClinica, LLC, MA, USA) by research staff. Categorical variables were summarized using absolute values and percentages, and continuous variables as medians and interquartile ranges (IQRs). Differences in categorical variables were compared using Pearson χ² or Fisher’s exact test when the expected values in any of the contingency table cells were below 5. Differences in continuous variables were compared using One-way ANOVA or Kruskal-Wallis H-test for data which did not follow the normal distribution based on Levene’s test. Statistical analyses were performed using Statistical Package for Social Science (SPSS) software version 23 (IBM Corporation, Armonk, NY, USA). All p-values were two-sided. Level of significance was set at *P* < 0.05.

**Patient and public involvement statement**
Patients or the public were not involved in study design or study conduct at any stage from inception to completion and dissemination of this project. Patients who met the eligibility criteria as described above were recruited to this study.

RESULTS

Study Population

Of 444 children who were hospitalized with CAP, 188 (42.3%) were eligible and enrolled in the study. Of 256 screening failures, 31.8% were due to hospitalization >24 hours at the time of screening and 22.1% to circumstances that might interfere with the study procedures. Of the 188 enrolled children, 184 (97.9%) had radiologic evidence of pneumonia. 179 (95.1%) subjects completed the study, including 19 (10.1%) who died. Eight subjects (4.3%) were lost to follow up, and one subject (0.5%) withdrew from the study. The study flow is shown in Figure 1.

Demographic and clinical characteristics are presented in Table 1. Age, gender, laboratory values, and pneumonia severity by WHO classification were similar across the three study sites. The median age was nine months (IQR, 5 to 20), and 54.7% of subjects were male. The most common comorbid conditions were developmental delay (27.7%), congenital heart disease (26.1%), and severe malnutrition (18.6%), with subjects from Yogyakarta site having the greatest proportion of those comorbidities. The percentage of subjects who had been vaccinated (age-adjusted) against pneumococcus, influenza, Hib-DPT, and measles vaccines were 2.1%, 1.1%, 55.9%, and 75.0%, respectively.

Table 1. Baseline Characteristics of Subjects.

| Demographic Characteristics | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|-----------------------------|-------------|-----------------|-------------------|------------------|---------|
| Age, median (IQR) months    | 9 (5 – 20)  | 9 (5.5 – 21)    | 8 (4 – 13.3)      | 11 (5-20)        | 0.442   |
| Gender, Male, (%)           | 103 (54.7)  | 29 (61.7)       | 26 (50)           | 48 (53.9)        | 0.493   |
| Household Characteristics, (%):
  - Low Education of Parents* | 163 (86.7)  | 37 (78.7)       | 43 (82.7)         | 84 (94.3)        | 0.019   |
  - Living in a dense neighborhood† | 121 (64.4) | 19 (40.4)       | 42 (80.8)         | 60 (67.4)        | <0.001  |
  - Living near waste disposal | 70 (37.2)   | 12 (25.5)       | 29 (55.8)         | 29 (32.6)        | 0.004   |
  - Sick household contact <14 days | 109 (58.0) | 22 (46.8)       | 43 (82.7)         | 44 (49.4)        | <0.001  |
  - Exposure to cigarette smoke | 120 (63.8) | 24 (51.1)       | 27 (51.9)         | 69 (77.5)        | 0.001   |
| Demographic Characteristics | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|-----------------------------|-------------|----------------|------------------|-----------------|--------|
| Attending daycare           | 4 (2.1)     | 2 (4.3)        | 1 (1.9)          | 1 (1.1)         | 0.374  |

Medical history (%):  
- Premature baby 34 (18.1) 4 (8.5) 16 (30.8) 14 (15.7) 0.012  
- Low birth weight 46 (24.4) 12 (25.5) 20 (38.5) 14 (15.7) 0.011  
- Developmental delay 52 (27.7) 16 (34.0) 21 (40.4) 15 (16.8) 0.003  
- Congenital heart disease 49 (26.1) 16 (34.0) 24 (46.2) 9 (10.1) <0.001  
- Severe malnutrition<sup>1</sup> 35 (18.6) 10 (21.3) 13 (25.0) 12 (13.5) 0.205  
- Neurological disorder 25 (13.3) 5 (10.6) 17 (32.7) 3 (3.4) <0.001  
- Asthma 9 (4.8) 3 (6.4) 1 (1.9) 5 (5.6) 0.563  
- HIV disease<sup>3</sup> 2 (1.1) 1 (2.1) 1 (1.9) 0 (0) 0.315  
- Tuberculosis 10 (5.3) 4 (8.5) 2 (3.8) 4 (4.5) 0.588  

Immunization history, fully vaccinated for age<sup>11</sup> (%):  
- DPT-Hib 105 (55.9) 30 (63.8) 25 (48.1) 50 (56.2) 0.233  
- Influenza 2 (1.1) 0 (0) 2 (3.8) 0 (0) 0.132  
- Pneumococcus 4 (2.1) 0 (0) 4 (7.7) 0 (0) 0.009  
- Measles 141 (75.0) 38 (80.9) 41 (78.8) 62 (69.7) 0.175  

Symptoms and signs (%):  
- Cough 171 (91.0) 40 (85.1) 42 (80.8) 89 (100) <0.001  
- Shortness of breath 174 (92.6) 41 (87.2) 48 (92.3) 85 (95.5) 0.214  
- Fever 152 (80.9) 34 (72.3) 35 (67.3) 83 (93.3) <0.001  
- Decreased Consciousness 7 (3.7) 1 (2.1) 1 (1.9) 5 (5.6) 0.612  
- Inability to drink 13 (6.9) 4 (8.5) 5 (9.6) 4 (4.5) 0.425  
- Diarrhea 36 (19.1) 6 (12.8) 4 (7.7) 26 (29.2) 0.003  
- Vomiting 14 (7.4) 4 (8.5) 5 (9.6) 5 (5.6) 0.595  
- Seizure 6 (3.2) 1 (2.1) 0 (0) 5 (5.6) 0.203  
- Fast breathing 80 (42.6) 15 (31.9) 43 (82.7) 22 (24.7) <0.001  
- Intercostal retraction 171 (91.0) 43 (91.5) 52 (100) 76 (85.4) 0.005  
- Rhonchi 168 (89.4) 42 (89.4) 39 (75.0) 87 (97.8) <0.001  
- Wheezing 35 (18.6) 9 (19.1) 10 (19.2) 16 (18.0) 1.000  
- Chest indrawing 125 (66.5) 36 (76.6) 43 (82.7) 46 (51.7) <0.001  
- SpO₂ <90% and/or Cyanosis 43 (22.9) 7 (14.9) 17 (32.7) 19 (21.3) 0.098  

Leukocyte count, median (IQR) x 10<sup>9</sup>/μL  
- 14.0 (10.4 – 18.9) 14.9 (11.1 – 18.8) 12.1 (9.8 – 17.8) 14.0 (10.4 – 19.0) 0.356  

Neutrophil-lymphocyte ratio (NLR), median (IQR)  
- 1.4 (0.9 – 2.8) 1.3 (0.9 – 2.6) 1.0 (0.6 – 2.0) 1.9 (1.1 – 3.2) 0.367
The most common symptoms were shortness of breath (92.6%), cough (91.0%), and fever (80.9%). Signs noted during the initial examination included intercostal retraction (91.0%), rhonchi (89.4%), and chest indrawing (66.5%). Of 188 subjects, 172 (91.4%) and 167 (88.8%) had CRP and PCT measured with median values of 9.0 (IQR, 3.6 – 28.0; Ref range ≤5) mg/L and 0.2 (IQR, 0.1 – 1.7; Ref range ≤0.15) ng/mL, respectively. Interstitial infiltrate (69.7%) was the most common radiographic finding. 47.3% of cases were classified as severe pneumonia according to the WHO classification system. All 188 enrolled cases were treated with antibiotic, and 150 of them (79.8%) had received antibiotic prior to blood collection for blood culture, with the combination of ampicillin and gentamicin (34.0%), cefotaxime (17.5%), and ceftriaxone (13.8%) were the three most frequent regimens used.

Detection of Pathogens

Blood and sputum cultures were performed on specimens from 184 (97.9%) and 183 (97.3%) subjects, respectively. A total of 150 (79.8%) children received antibiotics prior to collection of blood for culture. Seventy-five (41.0%) sputum culture isolates were analyzed from specimens meeting the...
required quality criteria. A NP or OP swab was obtained from 187 (99.5%) subjects, IS for PCR from 176 (93.6%), whole blood for PCR from 163 (86.7%), and paired acute-convalescent serum specimens for serology from 116 (61.7%) (Fig. 1).

The PEER-PePPeS algorithm was used to determine the causative pathogen(s) from those identified by culture, molecular, and serologic assay. Amongst the 188 study participants, 48 (25.5%) had bacterial infection, 31 (16.5%) had viral infection, 76 (40.4%) were of mixed bacterial and viral etiology, and 33 (17.6%) were of unknown etiology (Fig. 2, Panel A). Mixed infection, the most common overall etiology, was seen in 38.7% of 2-11 month-olds and in 42.7% of 12-59 month-olds (Fig 2. Panel B). Mixed infection was also the predominant etiology across all study sites (Supplementary Fig. 2). H. influenzae non-type B (N=73), RSV (N=51), K. pneumoniae (N=43), S. pneumoniae (N=29), Influenza virus (N=25), S. aureus (N=20), PIV (N=17), hMPV (N=11), Rhinovirus (N=10), and B. pertussis (N=7) were the top ten pathogens identified, more commonly appearing in mixed infection as opposed to a sole pathogen (Fig 2. Panel C). Influenza virus was significantly higher in the age group 12-59 mo vs 2-11 mo (N=16, 64%, P=0.027), while S. aureus was significantly more common in 2-11 mo vs 12-59 mo (N=16, 80%, P=0.024). Though not statistically significant, other pathogens trended toward more frequent detection in age group 2-11 mo (except B. pertussis) (Fig 2. Panel D). Amongst 76 mixed infection cases, RSV + H. influenzae non-type B was the most common co-infection (N=22, 28.9%), followed by RSV + S. pneumoniae (N=10, 13.2%), Influenza virus + H. influenzae non-type B (N=10, 13.2%), RSV + K. pneumoniae (N=9, 11.8%), and Parainfluenza virus + H. influenzae non-type B (N=9, 11.8%) (Data not shown).

We observed no difference in pathogen distribution by pneumonia severity based on WHO classification system (Supplementary Table 2 and Supplementary Fig. 3). By pathogen, there was no significant difference in distribution between pneumonia severity status or mortality, except for S. pneumoniae which was found in significantly more severe cases using the WHO system (P=0.033) (Supplementary Table 2).

A comparison of positivity rates for each causative pathogen by detection method is shown in Table 2. Overall, PCR captured more bacterial pathogens than culture and more viral pathogens than acute-convalescent paired serology. Paired serology was generally helpful in identifying atypical bacteria, such as C. pneumoniae and L. pneumophila, and upper respiratory tract viruses, such as Rhinovirus and Enterovirus. When comparing blood and IS culture, IS yielded more positive bacterial pathogen results. Similarly, IS PCR captured more pathogens than NP/OP PCR.

**Table 2. Causative Pathogens per PEER-PePPeS Rules by Detection Method**
### Pathogen Results

| Pathogen          | N  | Blood culture (% N) | IS culture (% N) | Whole blood PCR (% N) | NP / OP PCR (% N) | IS PCR (% N) | Serology Test (% N) |
|-------------------|----|---------------------|------------------|-----------------------|-------------------|-------------|---------------------|
| **Gram-positive cocci bacteria** |    |                     |                  |                       |                   |             |                     |
| *S. pneumoniae*   | 29 | 1 (3.4%)            | 3 (10.3%)        | 0 (0.0%)              | 21 (72.4%)        | 28 (96.6%)  |                     |
| *S. aureus*       | 20 | 0 (0.0%)            | 7 (35%)          | 0 (0.0%)              | 11 (55%)          | 19 (95%)    |                     |
| *S. mitis*        | 4  | 0 (0.0%)            | 4 (100%)         | 0 (0.0%)              |                   |             |                     |
| *S. pyogenes*     | 1  | 0 (0.0%)            | 1 (100%)         | 0 (0.0%)              |                   |             |                     |
| **Gram-negative cocci bacteria** |    |                     |                  |                       |                   |             |                     |
| *M. catarrhalis*  | 2  | 0 (0.0%)            | 2 (100%)         | 2 (100%)              | 2 (100%)          |             |                     |
| **Gram-negative rods bacteria** |    |                     |                  |                       |                   |             |                     |
| *H. inf non-type b* | 73 | 0 (0.0%)            | 0 (0.0%)         | 8 (10.9%)             | 60 (82.2%)        | 71 (98.6%)  |                     |
| *K. pneumoniae*   | 43 | 0 (0.0%)            | 17 (39.5%)       | 2 (4.7%)              | 34 (79.1%)        |             |                     |
| *B. pertussis*    | 7  | 0 (0.0%)            | 0 (0.0%)         |                        |                   | 7 (100%)    |                     |
| *E. coli*         | 5  | 1 (20%)             | 4 (80%)          | 0 (0.0%)              |                   |             |                     |
| *P. aeruginosa*   | 4  | 0 (0.0%)            | 4 (100%)         | 0 (0.0%)              |                   |             |                     |
| *A. baumannii*    | 3  | 0 (0.0%)            | 3 (100%)         | 0 (0.0%)              |                   |             |                     |
| *H. inf type b*   | 2  | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              | 0 (0.0%)          | 2 (100%)    |                     |
| *N. meningitidis* | 1  | 1 (100%)            | 1 (100%)         | 0 (0.0%)              | 0 (0.0%)          |             |                     |
| **Atypical bacteria** |    |                     |                  |                       |                   |             |                     |
| *C. pneumoniae*   | 5  | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              | 5 (100%)          |             |                     |
| *M. pneumoniae*   | 5  | 0 (0.0%)            | 0 (0.0%)         | 5 (100%)              | 1 (20%)           |             |                     |
| *L. pneumophila*  | 1  | 0 (0.0%)            | 0 (0.0%)         | 0 (0.0%)              | 1 (100%)          |             |                     |
| **Virus**         |    |                     |                  |                       |                   |             |                     |
| RSV               | 51 |                     |                  |                       | 36 (70.6%)        | 45 (88.2%)  | 10 (19.6%)          |
| RSV A             | 15 |                     |                  |                       | 10 (66.7%)        | 13 (86.7%)  |                     |
| RSV B             | 36 |                     |                  |                       | 26 (72.2%)        | 32 (88.8%)  |                     |
| Influenza virus   | 25 |                     |                  |                       | 16 (64%)          | 22 (88%)    | 9 (36%)             |
| inf A (H1N1)     | 7  |                     |                  |                       | 7 (100%)          | 7 (100%)    | 7 (70%)             |
| inf A (H3N2)     | 3  |                     |                  |                       | 3 (100%)          | 3 (100%)    |                     |
| inf B             | 14 |                     |                  |                       | 6 (42.9%)         | 12 (85.7%)  | 2 (14.3%)           |
| PIV 1            | 17 |                     |                  |                       | 16 (94.1%)        | 15 (88.2%)  | 3 (17.6%)           |
| PIV 1            | 17 |                     |                  |                       | 16 (94.1%)        | 15 (88.2%)  | 3 (17.6%)           |
| PIV 2            | 17 |                     |                  |                       | 16 (94.1%)        | 15 (88.2%)  | 3 (17.6%)           |
| PIV 3            | 17 |                     |                  |                       | 16 (94.1%)        | 15 (88.2%)  | 3 (17.6%)           |
| PIV 4            | 17 |                     |                  |                       | 16 (94.1%)        | 15 (88.2%)  | 3 (17.6%)           |
| hMPV             | 11 |                     |                  |                       | 5 (45.5%)         | 10 (90.9%)  |                     |
| Rhinovirus       | 10 |                     |                  |                       | 10 (100%)         | 6 (60%)     | 4 (40%)             |
| Enterovirus      | 5  |                     |                  |                       | 3 (60%)           | 3 (60%)     | 3 (60%)             |
| Bocavirus        | 3  |                     |                  |                       | 2 (66.7%)         | 3 (100%)    |                     |
| hCoV-NL63        | 2  |                     |                  |                       | 2 (100%)          | 2 (100%)    |                     |

Grey-box indicates the assay was not performed

### Mortality

Nineteen (10.1%) subjects died during the 30-day study period. Seven were male, and most (17 subjects) were less than 1 year old. Among deceased subjects, median study duration was 12 (IQR, 4 – 17.5) days; 8 were admitted to ICU, and 6 received mechanical ventilation. Twelve died due to...
respiratory failure, three due to sepsis, and three for unknown reasons after discharge (data not shown). Most deaths occurred in the 2-11 mo age group compared with the 12-59 mo age group (78.9% vs. 21.1%, $p=0.036$). Infection of deceased subjects was bacterial-only in 7, viral-only in 2, mixed in 5, and unknown in 5 subjects, with no significant differences between alive and deceased subjects. *H. influenzae* non-type B was the most common pathogen identified in deceased subjects (N=8, with the case fatality rate [CFR] in this study of 11.0%), followed by *K. pneumoniae* (N=6, CFR of 13.9%), Influenza virus (N=3, CFR of 12.0%), *B. pertussis* (N=2, CFR of 28.6%), and RSV (N=2, CFR of 3.9%) (Supplementary Table 2). Some deceased subjects had pre-existing health conditions or comorbidities, most common were congenital heart disease (10 subjects), severe malnutrition (7 subjects), and developmental delay (7 subjects). A clinical summary of the fatal cases is shown in Supplementary Table 3.

**Seasonality**

During the 27-month study period, infections caused by RSV and influenza were seen year-round with peak activity occurring during the wet season (November to March) in Indonesia (66.7%, $p<0.001$; and 64.0%, $p=0.012$, respectively). However, there was little variation in detection of the most common respiratory bacterial infections by month and season. *H. influenzae* non-type B shows peaks in August (N=12, 16.4%) and March (N=11, 15.1%), while *K. pneumoniae* and *S. pneumoniae* fluctuate at lower levels throughout the year (Figure 3).

**DISCUSSION:**

PEER-PePPeS, a prospective multisite study, addresses a critical knowledge gap about the current epidemiology of pathogens causing CAP in children 2-59 months old in Indonesia. There are no recent prospective Indonesian studies on this topic. Our study found: (1) mixed bacterial and viral infection is the most frequent (N=76, 40.4%) cause of childhood CAP, irrespective of age group and pneumonia severity; (2) bacterial infections were common (66% of cases) with *H. influenzae* non-b type, *K. pneumoniae*, and *S. pneumoniae* as the three most common bacterial etiologies; (3) viral pathogens were also common (57% of PEER-PePPeS subjects), with 16.5% of cases attributed to virus only and RSV and Influenza Virus being the most common viruses identified; and (4) PCR on IS specimens was the most sensitive assay for pathogen identification in this study.

While the finding that mixed bacterial and viral infection is the most identified etiology of childhood CAP is consistent with other studies, clinical significance of the mixed infection remains controversial. It is unclear if both agents act as true pathogens.[22,26] PEER-PePPeS did not
demonstrate a correlation of mixed infection with pneumonia severity and 30-day mortality. Many deceased cases occurred at a younger age (less than 1 year old), and in the presence of comorbidities, such as congenital heart disease and severe malnutrition, as also shown by previous reports.[27,28] Such factors may need to be considered in the prevention and management of childhood pneumonia to reduce mortality rate.

In recent years, there has been an increased focus on the role of respiratory viruses in childhood pneumonia, partly due to the reduction in bacterial disease associated with the use of conjugate pneumococcal and Hib vaccines and the increased capacity to detect viruses through PCR methods.[21,22,29,30] In PEER-PePPeS, viruses were found in 57% of subjects (virus only + mixed infection), with 16.5% of cases attributed to virus only. Thus, many patients probably received unnecessary antibiotics when they were covered empirically according to current Indonesia guidelines. Improving ability to discriminate between viral and bacterial infections would facilitate optimization of antibiotic administration and counter antimicrobial resistance, a major global health challenge.[31]

RSV and influenza virus are the most commonly detected viruses in this study and may be associated with Indonesia’s wet/rainy season, consistent with other reports from tropical regions.[32–34] A high prevalence of RSV was also observed in the GABRIEL and PERCH international case-control studies of childhood pneumonia etiology.[22,30] In terms of mixed infections, we found that RSV + H. influenzae non-type B and RSV + S. pneumoniae were most common. Since respiratory viruses such as RSV can predispose to secondary bacterial infections, particularly S. pneumoniae and H. influenzae[35], and conversely bacteria can increase RSV susceptibility[35,36], these co-infections highlight the need for optimizing RSV surveillance, prevention and treatment.

Though influenza virus also increases risk for secondary bacterial infections and is a major cause of childhood morbidity and mortality worldwide, data from developing countries is scarce.[37] In a previous Indonesian study of hospitalized patients with a severe acute respiratory infection (SARI), the prevalence of the influenza virus was 10.6% in children less than 5 years old, and was never diagnosed during hospitalization.[38] PEER-PePPeS confirms the need for improved diagnostic strategies, management optimization, and influenza vaccination in children. Of note, our study was conducted before identification of COVID-19 in Indonesia[39], so did not address the role of COVID-19 in childhood pneumonia.

We also found that 66% of cases were caused by bacterial infection (bacteria only + mixed infection). Overall, H. influenzae non-type B was the most common bacteria implicated, followed by K. pneumoniae and S. pneumoniae. H. influenzae non-type B predominance was also observed in a Malaysian study, where 90% of enrolled children were vaccinated against Hib as part of the national
immunization program.[24] With our moderate (56.4%) Hib vaccine coverage, high incidence of *H. influenzae* non-type B may represent true prevalence or strains not covered by Hib vaccine.[40] This finding agrees with current data that non-typeable *H. influenzae* (NTHi) can cause significant illness, and argues for strengthening the diagnostic laboratory capacity for pediatric specimens.

Identification of *K. pneumoniae* as the second most common bacterial etiology of childhood CAP is consistent with previously reported high carriage rates (~7%) in healthy Indonesian children. Carriage has been related to poor water and food hygiene and may give rise to pneumonia, especially in children with malnutrition.[41] Given *K. pneumoniae*’s potential for antibiotic resistance and high virulence of some strains, proactive detection and management strategies should be prioritized.[42]

The relatively low prevalence (15.4%) of *S. pneumoniae* in PEER-PePPes was surprising since carriage rates are high and PCV coverage low in Indonesia.[43] A similar relatively low prevalence of *S. pneumoniae* in childhood CAP was also reported from Malaysia, where PCV coverage is 8.7%[24] and in the PERCH study, reflecting temporal shifts in childhood pneumonia etiologies.[22] As only 4.8% of PEER-PePPeS subjects had received PCV, vaccination alone cannot account for the low *S. pneumoniae* prevalence. It is possible that antibiotic exposure prior to obtaining specimens may reduce median colonization density and lower positive findings yield of *S. pneumoniae* by both culture and PCR.[44] Moreover, our panel did not include *S. pneumoniae* paired serology, which may be useful to increase pneumococcal diagnosis in young children.[45] Nonetheless, *S. pneumoniae* remains an important etiological agent of severe/complicated CAP globally.[46] Our finding that *S. pneumoniae* was significantly associated with severe cases by the WHO classification system supports the need for ongoing surveillance, vaccination and prevention of transmission between adults and children.

Inclusion of several pathogen identification strategies in PEER-PePPes demonstrates the differential utility of assays and specimen types. Our findings highlight the value of molecular assays, especially in culture-negative cases where microorganisms may be nonrecoverable in culture due to prior antibiotics or presence of otherwise hard to culture bacteria.[47,48] PCR is also less laborious and boasts a shorter turn-around-time than conventional culture. PCR can additionally identify genes associated with antibiotic resistance, though conventional culture methods will still be required to confirm phenotypic resistance.[49,50] Even with the limited PCR panels used in our study, molecular assays had greater sensitivity for identification of bacterial pathogens than blood or sputum culture when using the PEER-PePPeS rules. Regardless of the method of detection, targeted treatment can follow empiric treatment once an organism is identified. This facilitates optimization of management while minimizing risk of adverse events and development of antimicrobial resistance due to unnecessary, prolonged use of antibiotics.
Although sensitive for detection, PCR does not provide information regarding infectiousness or viability. Genome fragments from dead organisms may be detected, often at a low level, even after clinical resolution.[48] Furthermore, negative results may occur due to differential viral kinetics along the respiratory tract. Thus lower respiratory tract specimens, such as IS, should be sought as they originate from the actual site of infection.[12,13] Accordingly, we observed a higher yield from PCR on IS than NP specimens. This finding may be confounded as IS and NP detection panels varied slightly.

We also found that the use of paired serologies increased the diagnostic yield and was useful for pathogen confirmation, particularly in the setting of innocent bystander viruses and atypical bacteria.[12]

PEER-PePPeS used a comprehensive approach for pathogen detection to increase diagnostic yield. It also enrolled patients over a 27-month study period, facilitating assessment of seasonality. However, our study has several limitations. The relatively small sample size, geographic limitation to the island of Java and observational design may limit generalizability and causal inference. Second, most subjects (79.8%) received antibiotics before specimens' collection, which is an inherent limitation of this observational study due to early antibiotics administration as per national guideline. To overcome this, our inclusion criteria were to enroll subjects within 24 hours of admission, and specimens were collected as soon as possible to hopefully minimize the effects of antibiotics on culture results. Third, we did not enroll healthy control children, limiting the ability to estimate the adjusted population attributable fraction (aPaF) of each pathogen.[29,30] A healthy control group could have revealed baseline carriage rates, minimizing over-attribution of disease to non-pathogenic organisms.[21,22,29,30] Fourth, we did not collect lung aspirates or pleural fluid specimens, which are superior for determination of pneumonia etiology. [15] Fifth, several subjects had pneumonia of unknown etiology; this may have been due to administration of antibiotics before culture which could reduce sensitivity, poor IS quality, the limited panel of bacterial and viral pathogens tested, lack of fungal testing, or currently unrecognized causes of pediatric pneumonia.

In conclusion, the epidemiology of childhood CAP is constantly evolving in step with social and environmental factors and thus, should be regularly assessed. Our study found that H. influenzae non-type B and RSV were the most common pathogens causing hospitalized CAP among Indonesian children aged 2-59 months old, suggesting a changing pathogen profile from the 1970-1990s etiology studies that mainly detected S. pneumoniae and H. influenzae type B as the most important causes of childhood pneumonia in LMICs.[3–5] PCR on IS demonstrated the best sensitivity for pathogen identification. We recommend incorporating molecular assays for pathogen detection, preferably multiplexed point-of-care assays, into practice guidelines. Improvements in Indonesia’s lab infrastructure during the COVID-19 pandemic can be leveraged to facilitate use of molecular assays.
for evaluation of childhood CAP. Optimization of pathogen detection to understand changing
childhood CAP epidemiology will also inform public policy on prevention and management.

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DL, HF, RT, YM, HK, AMN, ATA, CYL, HCL designed and conceptualized the study. DL, HF, RT, AB, C,
MSA, DW, M, SD, AS performed clinical assessments and were responsible for data entry. DL, HF, RT,
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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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This study was approved by the Ethical Clearance Committee of Faculty of Medicine, Universitas Indonesia (No. 567/UN2.F1/ETIK/2017). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or guardians before enrollment.

DATA AVAILABILITY STATEMENT:

Data are available upon reasonable request. The anonymized data set will be shared following the signing of a data-sharing agreement, with permission of the ethical clearance committee, study authors, and all project partners, exclusively for non-commercial purposes. Please contact the corresponding author with any queries.

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**FIGURE CAPTIONS:**

*Figure 1. Subject screening, enrolment, and monitoring flowchart.* CAP, community-acquired pneumonia; RR, respiratory rate; CXR, chest X-Ray; CRP, C-reactive protein; PCT, procalcitonin; NP, nasopharyngeal; IS, induced sputum; PCR, polymerase chain reaction.

*Figure 2. Pathogen Distribution.* (A) Overall proportion of identified viral/bacterial/mixed pathogen, (B) Viral/bacterial/mixed pathogens by age group, (C) Pattern of detection of the ten most identified pathogens, (D) Distribution of ten most identified pathogens by age group. *P<0.05*
Figure 3. Distribution of the (A) monthly count and (B) seasonal pattern of infection caused by *H. influenzae* non-type B, RSV, *K. pneumoniae*, *S. pneumoniae*, and Influenza virus during a 27-month study period.

**SUPPLEMENTARY INFORMATION:**

Details of Microbiological, Molecular and Serologic Methods, **Supplementary Table 1**

Pathogen distribution by WHO severity classification status and mortality, **Supplementary Table 2**

Summary of fatal cases, **Supplementary Table 3**.

PEER-PePPeS Study sites, **Supplementary Figure 1**

Proportion of Identified Pathogen in each Sites, **Supplementary Figure 2**

Proportion of Identified Pathogen between WHO Severity Status, **Supplementary Figure 3**
Figure 1. Subject screening, enrolment, and monitoring flowchart. CAP, community-acquired pneumonia; RR, respiratory rate; CXR, chest X-Ray; CRP, C-reactive protein; PCT, procalcitonin; NP, nasopharyngeal; IS, induced sputum; PCR, polymerase chain reaction.
Figure 2. Pathogen Distribution. (A) Overall proportion of identified viral/bacterial/mixed pathogen, (B) Viral/bacterial/mixed pathogens by age group, (C) Pattern of detection of the ten most identified pathogens, (D) Distribution of ten most identified pathogens by age group. *P<0.05

381x381mm (300 x 300 DPI)
Figure 3. Distribution of the (A) monthly count and (B) seasonal pattern of infection caused by H. influenzae non-type B, RSV, K. pneumoniae, S. pneumoniae, and Influenza virus during a 27-month study period.

381x177mm (300 x 300 DPI)
Supplementary Table 1. Microbiological, Molecular and Serologic Methods

| No. | Assays               | Procedures                                                                                                                                                                                                 |
|-----|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.  | Gram stain           | Gram-stained smears were obtained from the most purulent portion of each induced sputum specimen. The good quality specimen was defined as <10 squamous epithelium per low-power field (magnification, 100×) 1. The procedure of the Gram stain required four basic steps that include applied a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram’s Iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstained with safranin 2. The Gram-stained smears interpreted as follows: Gram-positive lancet-shaped diplococci (GPDC) suggest *Streptococcus pneumoniae*; Gram-positive diplococci (GPDC) or cocci in chains suggest *Streptococcus pyogenes*; Gram-positive cocci in clusters (GPC-cluster) suggest *Staphylococcus aureus*; Gram-negative coccobacilli (GNCB) suggest *Hemophilus influenzae*, *Bordetella pertussis* or *Acinetobacter baumannii*; Gram-negative diplococci (GNDC) suggest *Moraxella catarrhalis*; large Gram-negative rods (GNR-large) suggest *Klebsiella pneumoniae* or *Escherichia coli*; and small Gram-negative rods (GNR-small) suggest *Pseudomonas aeruginosa* 3. |
| 2.  | Induced Sputum       | The most purulent portion of induced sputum was inoculated onto sheep blood, chocolate, and MacConkey agars, streaked out using a standard 4-quadrant streaking method, and incubated at 35°C for 48 hours. Cultures were examined at 24 hours and 48 hours, and predominant bacteria were identified and quantified according to the farthest quadrant with visible colonies (first quadrant, scanty; second quadrant, 1+; third quadrant, 2+; fourth quadrant, 3+) 4. Then, the predominant bacteria isolates were inoculated into the appropriate VITEK identification strip using the VITEK® 2 COMPACT (BioMérieux, Germany). Briefly, a bacterial suspension was adjusted to a McFarland standard of 0.50 in a solution of 0.45 % sodium chloride using DensiLameter. The time between preparation of the solution and filling of the card was always less than 1 h. Analysis was done using the identification card and automatically read every 15 min. Bacteria identification and antibiotic susceptibility testing results were analyzed using the VITEK 2 software according to the manufacturer’s instructions 5. |
| 3.  | Blood Culture        | Up to 2 mL of blood samples (2 bottle sets) were collected and sent to the site laboratory with standardized procedures. Blood cultures were incubated for at least 5 days, unless positive, using automated systems (BacT/ALERT in Tangerang Hospital; BACTEC at other sites) 6. Organisms were identified according to standard microbiological methods as described in induced sputum culture section. The following organisms were considered to be contaminants when identified in blood cultures: Coagulase-negative *staphylococci*, *Micrococcus* spp., *Propionibacterium* spp., Alpha-hemolytic streptococci (except
| No. | Assays                                                                 | Procedures                                                                                                                                 |
|-----|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
|     | pneumococcus, Streptococcus anginosus, and Streptococcus mitis, Enterococcus spp., Corynebacterium spp. (diphtheroids), Bacillus spp. (except Bacillus anthracis), Pseudomonas spp. (except Pseudomonas aeruginosa), Stomatococcus, Aeroccocus, Neiserria subflava, Veillonella spp., other environmental non-fermenting Gram negative rods, and Candida spp. |                                                                                                                                              |
| 4.  | Viral RNA Extraction                                                   | Viral RNA was extracted from viral transport media (VTM) containing respiratory swab as well as sputum, using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Briefly, 140 µl of VTM or sputum coat was lysed in 560 of carrier RNA-containing AVL buffer, followed by the binding of viral RNA to the QIAamp membrane. Contaminants were removed from viral RNA in two separate washing steps using two different wash buffers, AW1 and AW2. Viral RNA was eluted in 60 µl of AVE buffer and kept in -80°C if not directly used 8,9. |
| 5.  | Bacterial DNA Extraction                                               | Bacterial DNA was extracted from viral transport media (VTM) containing respiratory swab as well as sputum, using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Briefly, 20 µl of QIAGEN Protease and 200 µl of VTM or sputum coat was lysed in 200 of AL buffer, followed binding of DNA to the QIAamp membrane. Contaminants were removed from DNA in two separate washing steps using two different wash buffers, AW1 and AW2. Bacterial DNA was eluted in 200 µl of AE buffer and kept in -80°C if not directly used |
| 6.  | qPCR for Respiratory Viruses                                           | The realtime PCR for respiratory virus detection was done followed the protocol of Beld et al., 2004 and Jansen et al., 2011. Positive control is a synthetic plasmid carrying the nucleotide sequence of the detection target. Primers, probes, and positive controls were synthesized and purified by an outside vendor (Integrated DNA Technologies, Iowa, US). Realtime PCR was done using the TaqManTM Fast Virus 1-Step Master Mix (Thermo Fisher Scientific; Cat#: 4444432) in an Applied Biosystems 7500 Fast Realtime PCR System (Thermo Fisher Scientific, MA, US). The reaction mixture composition was 1X TaqManTM Fast Virus 1-Step Master Mix, 0.5 µM of each primer, 0.25 µM probe, and 4 µl RNA, in a total 20 µl volume. The cycle condition was 50°C reverse transcription for 5 minutes, 95°C initial denaturation for 20 seconds, followed by 45 cycles of denaturation (95°C, 3 seconds) and annealing/elongation (55°C, 30 seconds). Realtime PCR works correctly when the positive control demonstrates the amplification curve and the template-free (negative) control demonstrates no amplification curve (no Ct values) 8,9. |
| 7.  | qPCR for Respiratory Bacteria                                          | In real-time PCR (qPCR) a portion of bacterial DNA genome specific to the pathogen(s) of interest is amplified using a specific pair of primers and probes for each bacteria, that were selected from the available literature 10–14. A detector (TaqMan® probe) is used in the reaction. Mastermix is prepared in a 1.5-ml tube for total reaction. qPCR assays were carried out in a total volume of 20 µl, comprising 10 µl of TaqMan® Fast Universal PCR Master Mix, 1.4 µl of nuclease-free water (Promega), 3.6 µl of oligonucleotide mixtures, and 4 µl of |
DNA extract. The cycle condition was 95°C initial denaturation for 20 seconds, followed by 45 cycles of denaturation (95°C, 3 seconds) and annealing/elongation (58°C, 30 seconds).

Realtime PCR works correctly when the positive control demonstrates the amplification curve and the template-free (negative) control demonstrates no amplification curve (no Ct values).

Serology Test

Assays were obtained from SERION ELISA classic kit (Institut Virion/Serion Laboratories, Germany) and used according to the insert of SERION kit. SERION ELISA classic is a qualitative and quantitative immunoassay for detecting human antibodies in serum or plasma with their corresponding antigen. The indirect enzyme immunoassorbent assay in this kit was coated with specific antigens of the pathogen of interest. Patient sera are diluted in a rheumatoid factor and then diluted in Sample Diluent (containing phosphate with tween 20 and Bromphenol blue) and incubated in the coated microwells to bind serum antibody to the solid-phase antigen. The microwells are then washed to remove unreacted serum proteins, and enzyme conjugate (anti-human IgA, IgG, or IgM APC-Alkaline phosphatase) is added to label the bound antibody. After further incubation, the microwells are washed to remove unbound APC Conjugate. The pNPP (para-nitrophenyl phosphate) substrate is then added to quantify the Conjugate-bound p-nitrophenyl phosphate portion. The colorless substrate p-nitrophenyl phosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the serum antibody. This timed reaction is interrupted with a Stop Solution (sodium hydroxide). Color intensity (Absorbance) is measured at a wavelength of 405nm on a microtiter plate reader or spectrophotometer within 15 minutes of adding the stop solution. Antibody activities are calculated by the SERION evaluation software.

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Footnote References:
Supplementary Table 2. Pathogen distribution by WHO severity classification status and mortality.

| Pathogens | WHO Classification System | p-value | Mortality Outcome | p-value |
|-----------|---------------------------|---------|------------------|---------|
|           | Severe (N=89) | Non-severe (N=99) | Died (N=19) | Alive (N=169) |
| H. influenzae non-type b | 31 (34.8%) | 42 (42.4%) | 0.286 | 8 (42.1%) | 65 (38.5%) | 0.757 |
| RSV | 25 (28.1%) | 26 (26.3%) | 0.778 | 2 (10.5%) | 49 (29.0%) | 0.086 |
| K. pneumoniae | 15 (16.9%) | 28 (28.3%) | 0.062 | 6 (31.6%) | 37 (21.9%) | 0.388 |
| S. pneumoniae | 19 (21.3%) | 10 (10.1%) | **0.033** | 1 (5.2%) | 28 (16.6%) | 0.317 |
| Influenza virus | 9 (10.1%) | 16 (16.2%) | 0.223 | 3 (15.8%) | 22 (13.0%) | 0.723 |
| S. aureus | 8 (9.0%) | 12 (12.1%) | 0.487 | 0 (0.0%) | 20 (11.8%) | 0.230 |
| PIV | 8 (9.0%) | 9 (9.1%) | 0.981 | 1 (5.3%) | 16 (9.5%) | 1.000 |
| hMPV | 6 (6.7%) | 5 (5.1%) | **0.622** | 1 (5.3%) | 10 (5.9%) | 1.000 |
| Rhinovirus | 7 (7.9%) | 3 (3.0%) | 0.196 | 1 (5.3%) | 9 (5.3%) | 1.000 |
| B. pertussis | 4 (4.5%) | 3 (3.0%) | 0.709 | 2 (10.5%) | 5 (3.0%) | 0.150 |

**Infection Type**

| Pathogens | p-value | Mortality Outcome | p-value |
|-----------|---------|------------------|---------|
| Bacterial pathogen | 17 (19.1%) | 31 (31.3%) | 0.055 | 7 (36.8%) | 41 (24.3%) | 0.268 |
| Viral pathogen | 16 (18.0%) | 15 (15.2%) | 0.602 | 2 (10.5%) | 29 (17.2%) | 0.744 |
| Mixed pathogen | 38 (42.7%) | 38 (38.4%) | 0.547 | 5 (26.3%) | 71 (42.0%) | 0.186 |
| Unknown pathogen | 18 (20.2%) | 15 (15.2%) | 0.361 | 5 (26.3%) | 28 (16.6%) | 0.337 |

Differences in categorical variables were compared using Pearson χ² or Fisher’s exact test when the expected values in any of the contingency table cells were below 5.

Supplementary Table 3. Summary of fatal cases.

| Case, Site, Gender (Age, mo) | Medical History | Signs and Symptoms (SS), Vital Signs (VS), Laboratory parameter (Lab) at admission | CXR | Causative Pathogen | ABX during Hospitalization | Hospitalization status | Cause of Death |
|-------------------------------|----------------|---------------------------------------------------------------------------------|-----|-------------------|------------------------|----------------------|---------------|
| RL1, SMG, Male (4) | Recurrent pneumonia, congenital heart disease, severe malnutrition | • SS: Cough, fever, dyspnea, chest indrawing, intercostal retraction, rhonchi • VS: 38°C, RR 44/min, SpO₂ 97% • Lab: Hb 9.6 g/dL, WBC 24.1 ×10⁹/L, Platelets ×10³/L, NLR 4.63, CRP 25.70 mg/L, PCT 2.41 ng/mL | Alveolar infiltrate | Rhinovirus, H. influenzae non-type b | Ampicillin, Gentamicin, Ceftriaxon, Cefoperazone Sulbactam | On mechanical ventilator, ICU admission (25 days) | Died on Day-26 | Cardiopulmonary failure | Sepsis |
| Case, Site, Gender (Age, mo) | Medical History | Signs and Symptoms (SS), Vital Signs (VS), Laboratory parameter (Lab) at admission | CXR | Causative Pathogen | ABX during Hospitalization | Hospitalization status | Cause of Death |
|-----------------------------|----------------|--------------------------------------------------------------------------------|-----|-------------------|---------------------------|-----------------------|-----------------|
| #02, SMG, Female (23)       | Recurrent pneumonia, congenital heart disease, incomplete NIP (DPT-Hib), malnutrition, developmental delay | $SS$: Cough, fever, dyspnea, chest indrawing, intercostal retraction, rhonchhi | Alveolar and interstitial infiltrates | Influenza A (H1N1) | Ampicillin, Gentamicin, Metronidazole, Ceftriaxone, Meropenem | On mechanical ventilator ICU admission (9 days) | Died on Day 21  |
| #03, SMG, Female (11)       | Low birth weight, low birth weight disease, incomplete NIP (Measles), severe malnutrition, developmental delay | $SS$: Cough, fever, dyspnea, diarrhea, nasal flaring, chest indrawing, intercostal retraction, rhonchhi | Alveolar and interstitial infiltrates | Influenza A (H3N2), B. pertussis, H. influenzae non-type b, K. pneumoniae | Ampicillin, Gentamicin, Azithromycin | On nasal cannula Died on Day 19 | Cardiopulmonary failure |
| #04, SMG, Male (45)         | Recurrent pneumonia, frontonasal dysplasia syndrome, epilepsy, developmental delay | $SS$: Cough, dyspnea, nasal flaring, intercostal retraction, | Alveolar infiltrate | Unknown | Ampicillin, Gentamicin | On nasal cannula Died on Day 2 | Respiratory failure |
| #05, SMG, Male (5)          | Premature birth, low birth weight, recurrent pneumonia, congenital heart disease, incomplete NIP (DPT-Hib) | $SS$: Cough, dyspnea, nasal flaring, chest indrawing, intercostal retraction, | Alveolar infiltrate | K. pneumoniae | Ampicillin, Gentamicin | On simple mask ICU admission (1 day) | Died on Day 6  |
| #06, SMG, Female (3)        | Recurrent pneumonia, incomplete NIP (DPT-Hib), malnutrition | $SS$: Cough, dyspnea, chest indrawing, intercostal retraction, rhonchhi | Alveolar infiltrate | Unknown | Ampicillin, Gentamicin, Ceftriaxone, Metronidazole, Meropenem | On mechanical ventilator ICU admission (7 days) | Died on Day 18 |
| #07, YGV, Female (10)       | Congenital heart disease, incomplete NIP (DPT-Hib, and Measles), severe malnutrition, developmental delay | $SS$: Cough, fever, dyspnea, head bobbing, chest indrawing, intercostal retraction, rhonchhi | Alveolar infiltrate | Unknown | Amoxicillin, Gentamicin, Ceftriaxone, Metronidazole, Cefoperazone | On mechanical ventilator ICU admission (12 days) | Died on Day 17 |
| #08, YGV, Female (3)        | Low birth weight, congenital heart disease, incomplete NIP (DPT-Hib), severe malnutrition | $SS$: Cough, fever, dyspnea, chest indrawing, intercostal retraction, rhonchhi | Alveolar and interstitial infiltrates | Unknown | Amoxicillin, Ceftriaxone | On nasal cannula Hospital discharge on day 10 | Died on Day 29 (outside hospitalization) | Acute Respiratory Distress Syndrome |
| #09, YGV, Female (5)        | Congenital heart disease, incomplete NIP (DPT-Hib), severe malnutrition | $SS$: Cough, dyspnea, inability to drink, nasal flaring, chest indrawing, intercostal retraction, rhonchhi | Alveolar and interstitial infiltrates | N. influenzae non-type b, K. pneumoniae | Ampicillin, Gentamicin | On nasal cannula Died on day 15 | Aspiration, mucous hypersecretion |
| #10, YGV, Male (6)          | Recurrent pneumonia, congenital heart disease, tuberculosis, incomplete NIP (DPT-Hib) | $SS$: Cough, fever, dyspnea, nasal flaring, chest indrawing, intercostal retraction, rhonchhi, wheezing | Alveolar and interstitial infiltrates, pleural effusion | K. pneumoniae | Ampicillin, Gentamicin, Ceftriaxone | On no-rebreather mask | Died on day 4 | Sepsis |
| #11, TRG, Female (5)        | Premature birth, developmental delay | $SS$: Cough, fever, dyspnea, nasal flaring, rhonchhi, wheezing | Alveolar infiltrate | A. baumannii (MDR) | Cefotaxime | On Nasal cannula Hospital discharge on day 7 | Unknown death |
| Case, Site, Gender (Age, mo) | Medical History | Signs and Symptoms (SS), Vital Signs (VS), Laboratory parameter (Lab) at admission | CXR | Causative Pathogen | ABX during Hospitalization | Hospitalization status | Cause of Death |
|----------------------------|-----------------|---------------------------------------------------------------------------------|-----|-------------------|--------------------------|-----------------------|-----------------|
|                            |                 | VS: 37.5°C, RR 48x/min, SpO₂ 91% Lab: Hb 8.5 g/dL, WBC 12.1 \(\times10^9/\text{L}\), PT 208 \(\times10^9/\text{L}\), ANC 8.6, NLR 3.2, CRP 0.91 mg/L, PCT 0.74 ng/mL |     | Alveolar and interstitial infiltrates | Ceftriaxone, Ceftazidime, Azithromycin | Died on day 17 (outside hospitalization) | Sepsis |
| #12, TRG, Female (2)       | Incomplete NIP (DPT-Hib) | SS: Cough, fever, dyspnea, diarrhea, skin rash, intercostal retraction, rhonchi, wheezing |     | Unknown | | | |
|                            |                 | VS: 37.6°C, RR 63x/min, SpO₂ 93% Lab: Hb 10.5 g/dL, WBC 13.6 \(\times10^9/\text{L}\), PT 289 \(\times10^9/\text{L}\), ANC 10.2, NLR 3.9, CRP 175.30 mg/L, PCT 0.74 ng/mL |     | Alveolar and interstitial infiltrates, pleural effusion | | | |
| #13, TRG, Female (2)       | Incomplete NIP (DPT-Hib) | SS: Cough, fever, dyspnea, nasal flaring, chest in-drawing, intercostal retraction, rhonchi, wheezing |     | Unknown | Cefotaxime | Died on day 8 | Respiratory Failure |
| #14, TRG, Female (2)       | Congenital heart disease, incomplete NIP (DPT-Hib), severe malnutrition | SS: Cough, fever, dyspnea, nasal flaring, chest in-drawing, intercostal retraction, rhonchi, wheezing |     | Alveolar and interstitial infiltrates | | | Respiratory Failure |
| #15, TRG, Male (9)         | Incomplete NIP (Measles) | SS: Cough, fever, dyspnea, nasal flaring, chest in-drawing, intercostal retraction, rhonchi |     | Interstitial infiltrate | | | Meningoencephalitis, Respiratory Failure |
| #16, TRG, Female (4)       | Premature birth, low birth weight, congenital heart disease, incomplete NIP (DPT-Hib) | SS: Cough, fever, dyspnea, diarrhea, chest in-drawing, intercostal retraction, rhonchi |     | H. influenzae non-type b | | | Unknown death |
| #17, TRG, Female (20)      | Developmental delay, incomplete NIP (DPT-Hib) | SS: Cough, fever, dyspnea, chest in-drawing, intercostal retraction, rhonchi |     | H. influenzae non-type b, S. pneumoniae | Cefotaxime, Gentamicin, Ceftiraxone | On mechanical ventilator ICU admission (8 days) Died on day 12 | Sepsis shock, Cardiopulmonary failure |
| #18, TRG, Male (4)         | Low birth weight, developmental delay, recurrent pneumonia, incomplete NIP (DPT-Hib), severe malnutrition | SS: Cough, fever, dyspnea, nasal flaring, chest in-drawing, intercostal retraction, rhonchi |     | Alveolar and interstitial infiltrates, pleural effusion | | | Respiratory failure |
| #19, TRG, Male (15)        | Incomplete NIP (DPT-Hib and Measles) | SS: Cough, fever, dyspnea, rhonchi |     | Interstitial infiltrate | RSV B, B. pertussis, H. influenzae non-type b | | Unknown death |

Abbreviations: SMG: Semarang site; YGY: Yogyakarta site; TGR: Tangerang site; NIP: mandatory National Immunization Program; DPT-Hib: a combined vaccine of acellular pertussis, tetanus toxoids, and diphtheria and of Haemophilus influenzae type b conjugate vaccines; CXR: chest X-ray; ABX: Antibiotics; RSV: Respiratory Syncytial Virus; hMPV: Human Metapneumovirus; PIV: Parainfluenza Virus; MDR: Multiple drug resistance.

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Supplementary Figure 1. PEER-PePPeS Study sites

1. Kariadi Hospital, Semarang
   Satellite sites: Adhyatma Hospital and Bhakti Wira Tamtama Hospital
2. Sardjito Hospital, Yogyakarta
3. Tangerang District Hospital, Tangerang
   Satellite site: An-Nisa Hospital

Supplementary Figure 2. Proportion of Identified Pathogen in each Sites. (A) Semarang, (B) Yogyakarta, and (C) Tangerang
Supplementary Figure 3. Proportion of Identified Pathogen between WHO Severity Status. (A) Non-severe Pneumonia, (B) Severe Pneumonia.
# STROBE Statement—Checklist of items that should be included in reports of cohort studies

| Paragraph/ Line number | Recommendation |
|------------------------|----------------|
| **Title and abstract**  |  |
| Page 1-2               | (a) Indicate the study’s design with a commonly used term in the title or the abstract  |
|                        | (b) Provide in the abstract an informative and balanced summary of what was done and what was found  |
| **Introduction**       |  |
| Background/rationale   | Introduction, paragraph 1-3 (Page 3-4) Explain the scientific background and rationale for the investigation being reported |
| Objectives            | Introduction, paragraph 4 (Page 4) State specific objectives, including any prespecified hypotheses  |
| **Methods**           |  |
| Study design          | Methods, paragraph 1 (Page 4) Present key elements of study design early in the paper  |
| Setting               | Methods, paragraph 1 and 4 (Page 4-5) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection  |
| Participants          | Methods, paragraph 4 (Page 5) (a) Give the eligibility criteria, and the sources and methods of selection of participants  |
| Variables             | Methods, paragraph 2, 3, and 5 (Page 4-5) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable  |
| Data sources/measurement | Methods, paragraph 5, 6, 7, and 8 (Page 5-6) For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group  |
| Bias                  | Methods, paragraph 9 and 10 (Page 6-7) Describe any efforts to address potential sources of bias  |
| Study size            | Methods, paragraph 1 and 4 (Page 4-5) Explain how the study size was arrived at  |
| Quantitative variables | Methods, paragraph 9 (Page 6) Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why  |
| Statistical methods   | Methods, paragraph 9 (Page 6) (a) Describe all statistical methods, including those used to control for confounding  |
|                        | (b) Describe any methods used to examine subgroups and interactions  |
|                        | (c) Explain how missing data were addressed  |
|                        | (d) If applicable, describe analytical methods taking account of sampling strategy  |
|                        | (e) Describe any sensitivity analyses  |
| **Results**           |  |
| Participants          | Results, paragraph 1 (Page 7) and Flow diagram/Fig. 1 (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed  |
|                        | (b) Give reasons for non-participation at each stage  |
|                        | (c) Consider use of a flow diagram  |
| Descriptive data      | Results, paragraph 2 and 3. Table 1. (Page 7-9) (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders  |
|                        | (b) Indicate number of participants with missing data for each variable of interest  |
| Outcome data          | Results, paragraph 4 and 5 (Page 9-10) Report numbers of outcome events or summary measures  |
| Main results          |  |
|                        | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval)  |
Results, paragraph 5, 7, and 9. Table 2. (Page 10-12). Fig. 2 and Fig. 3 interval). Make clear which confounders were adjusted for and why they were included.

(b) Report category boundaries when continuous variables were categorized.

(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.

Other analyses

Results, paragraph 6 (Page 10). Results, paragraph 8. (Page 11-12). Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses.

Discussion

Key results

Discussion, paragraph 1 (Page 12) Summarise key results with reference to study objectives.

Limitations

Discussion, paragraph 11 (Page 15) Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.

Interpretation

Discussion, paragraph 2-10 (Page 12-15) Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

Generalisability

Discussion, paragraph 3-8 (Page 13-14) Discuss the generalisability (external validity) of the study results.

Other information

Funding

Funding statement (Page 16) Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based.

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.
**Epidemiology of community-acquired pneumonia among hospitalized children in Indonesia: a multicenter, prospective study**

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| Keywords | Epidemiology < TROPICAL MEDICINE, INFECTIOUS DISEASES, PAEDIATRICS |
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Epidemiology of Community-acquired pneumonia among hospitalized children in Indonesia: a multicenter, prospective study

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ABSTRACT:

Objective: To identify etiologies of childhood community-acquired pneumonia (CAP) based on a comprehensive diagnostic approach.

Design: “Partnerships for Enhanced Engagement in Research - Pneumonia in Pediatrics (PEER-PePPeS)” study was an observational prospective-cohort study conducted from July 2017 through September 2019.

Setting:
Participants:
Hospitalized children aged 2–59-months who met the criteria for pneumonia were eligible. Children were excluded if they had been hospitalized for >24 hours; had malignancy or history of malignancy; a history of long-term (>2 months) steroid therapy, or conditions that might interfere with compliance with study procedures.

Main outcome(s) measure(s):
Causative bacterial, viral, or mixed pathogen(s) for pneumonia were determined using microbiological, molecular, and serologic tests from routinely collected specimens (blood, sputum, and nasopharyngeal swabs). We applied a previously published algorithm (PEER-PePPeS rules) to determine the causative pathogen(s).

Results:
188 subjects were enrolled. Based on our algorithm, 48 (25.5%) had a bacterial infection, 31 (16.5%) had a viral infection, 76 (40.4%) had mixed bacterial and viral infections, and 33 (17.6%) were unable to be classified. The five most common causative pathogens identified were *Haemophilus influenzae* non-type B (N=73, 38.8%), respiratory syncytial virus (RSV) (N=51, 27.1%), *Klebsiella pneumoniae* (N=43, 22.9%), *Streptococcus pneumoniae* (N=29, 15.4%), and Influenza virus (N=25, 13.3%). RSV and Influenza virus diagnoses were highly associated with Indonesia’s rainy season (November-March). The polymerase chain reaction (PCR) assays on induced sputum (IS) specimens captured most of the pathogens identified in this study.

Conclusions:
Our study found that *H. influenzae* non-type B and RSV were the most frequently identified pathogens causing hospitalized CAP among Indonesian children aged 2-59 months old. Our study also highlights the importance of PCR for diagnosis and by extension, appropriate use of antimicrobials.

Keywords: Pneumonia; Children; Indonesia; Etiology; Epidemiology

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Prospective multisite study conducted over 27-months
- Used a comprehensive diagnostic approach (culture, molecular testing, and paired serologic assays) to identify causative pathogens from routinely collected specimens (blood, sputum, and nasopharyngeal swabs)
The relatively small sample size, geographic limitation to the island of Java and observational design limit generalizability and causal inference. We did not collect lung aspirates or pleural fluid specimens, which are preferred for determination of pneumonia etiology, and did not include healthy control children, limiting ability to estimate the adjusted population attributable fraction (aPaF) for each pathogen. Several cases of pneumonia were attributed to unknown etiology, which could be due to administration of antibiotics before culture, poor sputum quality, limited bacterial and viral panels, lack of fungal testing or another factor.

INTRODUCTION

Pneumonia is the leading infectious cause of child mortality, with a greater burden in low- and middle-income countries (LMICs). In Indonesia, pneumonia contributed to 15% of childhood deaths and was the second leading cause of death amongst children under five years in 2017. Indonesian practice guidelines are adapted from the World Health Organization (WHO) guidelines, which are based on 1970’s – 1990’s data showing bacteria such as Haemophilus influenzae type b (Hib) and Streptococcus pneumoniae caused the majority of fatal pneumonias in children. Therefore empiric antibiotics are considered first-line treatment for children with community-acquired pneumonia (CAP). Despite evidence that appropriate antibiotics are lifesaving, rational selection of antibiotics for pneumonia is hampered by low adherence to guidelines and scarcity of point-of-care diagnostics. Consequently, healthcare providers, particularly those in LMIC, are likely to overtreat non-bacterial pneumonia with antibiotics.

Several recent studies of community-acquired pneumonia (CAP) in children have highlighted the role of viral etiologies. Increased recognition of viral etiologies of CAP is likely due to both enhanced molecular diagnostic capacity and wide deployment of Hib and pneumococcal conjugate vaccines [PCV]. Treatment of non-bacterial pneumonia with antibiotics may engender avoidable antimicrobial resistance. Thus, current data on the etiologies of childhood pneumonia is needed and should be regularly evaluated to inform vaccination policies, empiric management decisions, and targeted treatment.

From a diagnostic standpoint, direct demonstration of organisms by culture (or staining) of lung aspirates has been the standard for determining microbial etiology of CAP. In the current era, many use less-invasive biological specimens (e.g. blood, naso/oropharyngeal secretions, bronchoalveolar lavage, or induced sputum) and employ diverse methods (e.g. culture, PCR, antigen detection, or paired serology) to identify organisms. However, such comprehensive methods are
costly and often require specialized equipment and human resources, limiting feasibility in low-resource settings.[17,18]

Prospective community-based cohort studies that define pathogen(s) causing CAP in Indonesian children are scarce. We conducted a “Partnerships for Enhanced Engagement in Research - Pneumonia in Pediatrics (PEER-PePPeS)” study, which aimed to identify etiologies of childhood CAP using comprehensive diagnostic methods.

METHODS

Study design and study sites

PEER-PePPeS was a multi-site observational cohort study seeking to determine etiologies of CAP amongst children aged 2–59 months in Indonesia. The study was conducted by the Indonesia Research Partnership on Infectious Disease (INA-RESPOND) and enrolled participants initially at three government referral teaching hospitals in three provinces: Kariadi Hospital (Central Java), Sardjito Hospital (Yogyakarta), and Tangerang District Hospital (Banten), as shown in Supplementary Fig. 1. Satellite sites located near the primary sites were added during the study to facilitate subject recruitment.

Study Definitions

In this study, pneumonia in children was defined as cough or fever with at least one of the following: shortness of breath (indicated by at least one of the following signs: head bobbing; nasal flaring; chest indrawing or intercostal retracting), tachypnea, grunting, crackles, rhonchi, decreased vesicular breath sounds, bronchial breath sounds or chest x-ray findings consistent with pneumonia. Tachypnea was defined as respiratory rate >50/min for infants 2–12 months and >40/min for children >12–60 months.[19] Abnormal chest x-ray findings consistent with pneumonia were defined as presence of either focal or diffuse infiltrates, a silhouette sign, pleural effusion, or air bronchogram.[20] Chest x-rays were read by the pediatrician.

Based on WHO classification and treatment of childhood pneumonia at health facilities (2014 version), for children 2–59 months of age, severe pneumonia is defined as pneumonia (tachypnea and/or chest indrawing) accompanied by presence of any danger signs, including inability to drink, persistent vomiting, convulsions, lethargy or loss of consciousness, stridor in a calm child, or severe malnutrition.[19]

Study Participants
PEER-PePPeS study enrolled children aged 2-59 months, who were hospitalized between July 18th, 2017 until September 25th, 2019, and met the definition for pneumonia. Eligible subjects were enrolled within 24 hours of admission. Children were excluded if they had been hospitalized for >24 hours; had a malignancy or history of malignancy; a history of long term (>2 months) steroid therapy; or conditions that might interfere with compliance with study procedures (e.g., very ill patients for whom specimens could not be obtained or living outside the area for which follow-up was practical).

**Study Procedures**

Demographic and anthropometric data, current signs and symptoms, pregnancy history, vaccination status, breastfeeding history, antibiotic and steroid exposure, family history, medical history, risk factors, hematology profiles, chemistry results, and chest x-ray (per standard of care) were collected at enrollment. Clinical examination (vital signs, general examination, lung auscultation, SpO2); nasopharyngeal (NP) swab for molecular tests; induced sputum (IS) for culture and molecular tests; collection of blood specimens for routine blood count, cultures, molecular tests, serologic tests, C-reactive protein (CRP), and procalcitonin (PCT) were also performed. We prospectively followed subjects daily until hospital discharge; data on vital signs, respiratory signs, intensive care admission, intubation, complications, and treatment were collected. On Day 14, we performed clinical examinations and collected convalescent sera for serology tests; subjects discharged before day 14 returned to clinic for their evaluation. We conducted a telephone interview on Day 30 (±4 days) to assess clinical outcome.

This study used several widely available bacterial and viral respiratory molecular pathogen panels and serologic assays.[21–24] NP and IS specimens were tested with a PCR panel that included twelve-viruses (influenza A, influenza B, adenovirus, enterovirus, bocavirus, respiratory syncytial virus (RSV) A, RSV B, human metapneumovirus (hMPV), rhinovirus, parainfluenza virus (PIV) 1-4, coronavirus OC43, and coronavirus NL63). NP specimens were evaluated by PCR for five bacteria (Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, and Klebsiella pneumoniae), while IS specimens were tested for nine (Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae, Bordetella pertussis, Moraxella catarrhalis, Staphylococcus aureus, Klebsiella pneumoniae, and Legionella pneumophila). Good quality (<10 squamous epithelial cells per low power field[12]) IS specimens underwent culture and gram stain.[25] For whole blood, qPCR was performed for three bacteria (Haemophilus influenzae, Streptococcus pneumoniae, and Staphylococcus aureus). Serologic testing for seven viruses (influenza A, influenza B, adenovirus, parvovirus B19, echovirus/enterovirus, RSV, parainfluenza virus) and four
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6

169 bacteria (*Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumoniae,* and *Bordetella pertussis*) was performed using paired acute-convalescent sera.

170 Blood culture, IS culture and Gram stain, routine blood count, CRP, PCT, and chest x-ray were performed by the laboratory/radiology department at the hospital site. qPCR and serology assays were performed retrospectively at the INA-RESPOND Reference Laboratory located in Tangerang District Hospital. Details of blood culture, sputum culture, molecular and serology test techniques are shown in *Supplementary Table 1.*

177 **Pathogen Identification**

178 Causative bacterial, viral, or mixed pathogen for the pneumonia was determined based on an algorithm (PEER-PePPeS rules) for interpretation of microbiological, molecular, and serologic test results published previously.\[12\] In brief, we considered all organisms detected by blood culture, detected by whole blood PCR, or that grew from good quality IS specimen in high quantities with a compatible primary Gram stain as potential causative bacterial pathogens. Bacteria commonly considered contaminants were excluded. For the nasopharynx, potential colonizing bacteria (e.g. *H. influenzae, S. pneumoniae,* and *S. aureus*) and potential innocent bystander viruses (e.g. bocavirus, adenovirus, non-SARS human Coronavirus (hCoVs), enterovirus, and rhinovirus) were determined to be causative based on a PCR density cut-off and/or serodiagnosis criteria for paired acute and convalescent sera (seroconversion or a two to four-fold increase in antibody titers in the convalescent specimen).\[12\]

190 **Data collection and statistical analysis**

191 Data were recorded on paper case report forms and entered in duplicate into OpenClinica (OpenClinica, LLC, MA, USA) by research staff. Categorical variables were summarized using absolute values and percentages, and continuous variables as medians and interquartile ranges (IQRs). Differences in categorical variables were compared using Pearson $\chi^2$ or Fisher’s exact test when the expected values in any of the contingency table cells were below 5. Differences in continuous variables were compared using One-way ANOVA or Kruskal-Wallis H-test for data which did not follow the normal distribution based on Levene’s test. Statistical analyses were performed using Statistical Package for Social Science (SPSS) software version 23 (IBM Corporation, Armonk, NY, USA). All p-values were two-sided. Level of significance was set at $P < 0.05$.

201 **Patient and public involvement statement**
Patients or the public were not involved in study design or study conduct at any stage from inception to completion and dissemination of this project. Patients who met the eligibility criteria as described above were recruited to this study.

RESULTS

Study Population

Of 444 children who were hospitalized with CAP, 188 (42.3%) were eligible and enrolled in the study. Of 256 screening failures, 31.8% were due to hospitalization >24 hours at the time of screening and 22.1% to circumstances that might interfere with the study procedures. Of the 188 enrolled children, 184 (97.9%) had radiologic evidence of pneumonia. 179 (95.1%) subjects completed the study, including 19 (10.1%) who died. Eight subjects (4.3%) were lost to follow up, and one subject (0.5%) withdrew from the study. The study flow is shown in Figure 1.

Demographic and clinical characteristics are presented in Table 1. Age, gender, laboratory values, and pneumonia severity by WHO classification were similar across the three study sites. The median age was nine months (IQR, 5 to 20), and 54.7% of subjects were male. The most common comorbid conditions / medical histories were developmental delay (27.7%), congenital heart disease (26.1%), low birth weight (24.4%), and severe malnutrition (18.6%), with subjects from Yogyakarta site having the greatest proportion of those comorbidities. The percentage of subjects who had been vaccinated (age-adjusted) against pneumococcus, influenza, Hib-DPT, and measles vaccines were 2.1%, 1.1%, 55.9%, and 75.0%, respectively.

Table 1. Baseline Characteristics of Subjects.

| Demographic Characteristics       | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|----------------------------------|-------------|-----------------|-------------------|-----------------|--------|
| Age, median (IQR) months         | 9 (5 – 20)  | 9 (5.5 – 21)    | 8 (4 – 13.3)      | 11 (5-20)       | 0.442  |
| Gender, Male, (%)                | 103 (54.7)  | 29 (61.7)       | 26 (50)           | 48 (53.9)       | 0.493  |
| Household Characteristics, (%)   |             |                 |                   |                 |        |
| Low Education of Parents         | 163 (86.7)  | 37 (78.7)       | 43 (82.7)         | 84 (94.3)       | 0.019  |
| Living in a dense neighborhood    | 121 (64.4)  | 19 (40.4)       | 42 (80.8)         | 60 (67.4)       | <0.001 |
| Exposure to cigarette smoke      | 120 (63.8)  | 24 (51.1)       | 27 (51.9)         | 69 (77.5)       | 0.001  |
| Sick household contact <14 days  | 109 (58.0)  | 22 (46.8)       | 43 (82.7)         | 44 (49.4)       | <0.001 |
| Living near waste disposal       | 70 (37.2)   | 12 (25.5)       | 29 (55.8)         | 29 (32.6)       | 0.004  |
| Attending daycare                | 4 (2.1)     | 2 (4.3)         | 1 (1.9)           | 1 (1.1)         | 0.374  |
| Medical history (%)              |             |                 |                   |                 |        |
| Demographic Characteristics                              | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------------------------------------|-------------|----------------|-------------------|------------------|---------|
| Developmental delay                                     | 52 (27.7)   | 16 (34.0)      | 21 (40.4)         | 15 (16.8)        | 0.003   |
| Congenital heart disease                               | 49 (26.1)   | 16 (34.0)      | 24 (46.2)         | 9 (10.1)         | <0.001  |
| Low birth weight                                       | 46 (24.4)   | 12 (25.5)      | 20 (38.5)         | 14 (15.7)        | 0.011   |
| Severe malnutrition[^1]                                 | 35 (18.6)   | 10 (21.3)      | 13 (25.0)         | 12 (13.5)        | 0.205   |
| Premature baby                                         | 34 (18.1)   | 4 (8.5)        | 16 (30.8)         | 14 (15.7)        | 0.012   |
| Neurological disorder                                  | 25 (13.3)   | 5 (10.6)       | 17 (32.7)         | 3 (3.4)          | <0.001  |
| Tuberculosis (recent/cured)                            | 10 (5.3)    | 4 (8.5)        | 2 (3.8)           | 4 (4.5)          | 0.588   |
| Asthma                                                 | 9 (4.8)     | 3 (6.4)        | 1 (1.9)           | 5 (5.6)          | 0.563   |
| HIV disease[^5]                                        | 2 (1.1)     | 1 (2.1)        | 1 (1.9)           | 0 (0)            | 0.315   |

Immunization history, fully vaccinated for age[^11] (%):

|                          | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------|-------------|----------------|-------------------|------------------|---------|
| Measles                  | 141 (75.0)  | 38 (80.9)      | 41 (78.8)         | 62 (69.7)        | 0.175   |
| DPT-Hib                  | 105 (55.9)  | 30 (63.8)      | 25 (48.1)         | 50 (56.2)        | 0.233   |
| Pneumococcus             | 4 (2.1)     | 0 (0)          | 4 (7.7)           | 0 (0)            | 0.009   |
| Influenza                | 2 (1.1)     | 0 (0)          | 2 (3.8)           | 0 (0)            | 0.132   |

Symptoms and signs (%):

|                          | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------|-------------|----------------|-------------------|------------------|---------|
| Shortness of breath      | 174 (92.6)  | 41 (87.2)      | 48 (92.3)         | 85 (95.5)        | 0.214   |
| Cough                    | 171 (91.0)  | 40 (85.1)      | 42 (80.8)         | 89 (100)         | <0.001  |
| Intercostal retraction   | 171 (91.0)  | 43 (91.5)      | 52 (100)          | 76 (85.4)        | 0.005   |
| Rhonchi                  | 168 (89.4)  | 42 (89.4)      | 39 (75.0)         | 87 (97.8)        | <0.001  |
| Fever                    | 152 (80.9)  | 34 (72.3)      | 35 (67.3)         | 83 (93.3)        | <0.001  |
| Chest indrawing          | 125 (66.5)  | 36 (76.6)      | 43 (82.7)         | 46 (51.7)        | <0.001  |
| Fast breathing           | 80 (42.6)   | 15 (31.9)      | 43 (82.7)         | 22 (24.7)        | <0.001  |
| SpO2 <90% and/or Cyanosis| 43 (22.9)   | 7 (14.9)       | 17 (32.7)         | 19 (21.3)        | 0.098   |
| Diarrhea                 | 36 (19.1)   | 6 (12.8)       | 4 (7.7)           | 26 (29.2)        | 0.003   |
| Wheezing                 | 35 (18.6)   | 9 (19.1)       | 10 (19.2)         | 16 (18.0)        | 1.000   |
| Vomiting                 | 14 (7.4)    | 4 (8.5)        | 5 (9.6)           | 5 (5.6)          | 0.595   |
| Inability to drink       | 13 (6.9)    | 4 (8.5)        | 5 (9.6)           | 4 (4.5)          | 0.425   |
| Decreased Consciousness  | 7 (3.7)     | 1 (2.1)        | 1 (1.9)           | 5 (5.6)          | 0.612   |
| Seizure                  | 6 (3.2)     | 1 (2.1)        | 0 (0)             | 5 (5.6)          | 0.203   |

Leukocyte count, median (IQR) x 10^9/L

|                          | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------|-------------|----------------|-------------------|------------------|---------|
|                          | 14.0 (10.4 – 18.9) | 14.9 (11.1 – 18.8) | 12.1 (9.8 – 17.8) | 14.0 (10.4 – 19.0) | 0.356   |

Neutrophil-lymphocyte ratio (NLR), median (IQR)

|                          | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------|-------------|----------------|-------------------|------------------|---------|
|                          | 1.4 (0.9 – 2.8) | 1.3 (0.9 – 2.6) | 1.0 (0.6 – 2.0) | 1.9 (1.1 – 3.2) | 0.367   |

CRP, median (IQR) mg/L

|                          | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------|-------------|----------------|-------------------|------------------|---------|
|                          | 9.0 (3.6 – 28.0) | 11.8 (1.6 – 23.3) | 9.0 (4.9 – 21.8) | 8.4 (1.5 – 34.1) | 0.665   |

PCT, median (IQR) ng/mL

|                          | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------|-------------|----------------|-------------------|------------------|---------|
|                          | 0.2 (0.1 – 1.7) | 0.2 (0.1 – 1.5) | 0.2 (0.1 – 1.0) | 0.2 (0.1 – 2.6) | 0.912   |
### Demographic Characteristics

|                          | All (N=188) | Semarang (N=47) | Yogyakarta (N=52) | Tangerang (N=89) | P-value |
|--------------------------|-------------|-----------------|-------------------|------------------|---------|
| Severe pneumonia (WHO Classification 2014 version) (%) |             |                 |                   |                  |         |
|                          | 89 (47.3)   | 26 (55.3)       | 26 (50.0)         | 37 (41.6)        | 0.281   |
| CXR Findings (%):        |             |                 |                   |                  |         |
| Interstitial infiltrate   | 131 (69.7)  | 26 (55.3)       | 30 (57.7)         | 75 (84.3)        | <0.001  |
| Alveolar infiltrate       | 125 (66.5)  | 41 (87.2)       | 44 (84.6)         | 40 (44.9)        | <0.001  |
| Pleural effusion          | 5 (2.7)     | 1 (2.1)         | 2 (3.8)           | 2 (2.2)          | 0.850   |
| Antibiotic administration prior to blood culture (%) | 150 (79.8)  | 39 (83.0)       | 49 (94.2)         | 62 (69.7)        | 0.002   |

*Low education of parents was defined by highest level of parents' formal education being high school diploma or less; † A densely populated neighborhood was defined as >200 people/km² or <8 m²/person in the subject's home; ‡ Severe malnutrition was defined as weight for height below -3 standard deviations from the median of the WHO Child Growth Standards; § Subjects were tested for HIV infection if a parent / guardian provided consent and a specimen was available (n=160); ‖ Full vaccination was defined as being up to date for age per vaccination schedule at study enrollment.

The most common symptoms were shortness of breath (92.6%), cough (91.0%), and fever (80.9%). Signs noted during the initial examination included intercostal retraction (91.0%), rhonchi (89.4%), and chest indrawing (66.5%). Of 188 subjects, 172 (91.4%) and 167 (88.8%) had CRP and PCT measured with median values of 9.0 (IQR, 3.6 – 28.0; Ref range ≤5) mg/L and 0.2 (IQR, 0.1 – 1.7; Ref range ≤0.15) ng/mL, respectively. Interstitial infiltrate (69.7%) was the most common radiographic finding. 47.3% of cases were classified as severe pneumonia according to the WHO classification system. All 188 enrolled cases were treated with antibiotics, and 150 of them (79.8%) had received 1 to 2 doses of antibiotics prior to collection of blood culture in the emergency unit, with the combination of ampicillin and gentamicin (34.6%), cefotaxime (17.0%), and ceftriaxone (14.4%) being the three most frequent regimens used. Details of antibiotic regimens administered before blood culture, including dosage and given frequency, are presented in Supplementary Table 2.

**Detection of Pathogens**

Blood and sputum cultures were performed on specimens from 184 (97.9%) and 183 (97.3%) subjects, respectively. A total of 150 (79.8%) children received antibiotics prior to collection of blood for culture. Seventy-five (41.0%) sputum culture isolates were analyzed from specimens meeting the required quality criteria. A NP or OP swab was obtained from 187 (99.5%) subjects, IS for PCR from 176 (93.6%), whole blood for PCR from 163 (86.7%), and paired acute-convalescent serum specimens for serology from 116 (61.7%) (Fig. 1).

The PEER-PePPeS algorithm was used to determine the causative pathogen(s) from those identified by culture, molecular, and serologic assay. Amongst the 188 study participants, 48 (25.5%)
had bacterial infection, 31 (16.5%) had viral infection, 76 (40.4%) were of mixed bacterial and viral etiology, and 33 (17.6%) were of unknown etiology (Fig. 2, Panel A). Mixed infection, the most common overall etiology, was seen in 38.7% of 2-11 month-olds and in 42.7% of 12-59 month-olds (Fig 2. Panel B). Mixed infection was also the predominant etiology across all study sites (Supplementary Fig. 2). *H. influenzae* non-type B (N=73, 38.8%), RSV (N=51, 27.1%), *K. pneumoniae* (N=43, 22.9%), *S. pneumoniae* (N=29, 15.4%), Influenza virus (N=25, 13.3%), *S. aureus* (N=20, 10.6%), PIV (N=17, 9.0%), hMPV (N=11, 5.8%), Rhinovirus (N=10, 5.3%), and *B. pertussis* (N=7, 3.7%) were the top ten pathogens identified, more commonly appearing in mixed infection as opposed to as a sole pathogen (Fig 2. Panel C). Influenza virus was significantly higher in the age group 12-59 mo vs 2-11 mo (N=16, 64%, P=0.027), while *S. aureus* was significantly more common in 2-11 mo vs 12-59 mo (N=16, 80%, P=0.024). Though not statistically significant, other pathogens trended toward more frequent detection in age group 2-11 mo (except *B. pertussis*) (Fig 2. Panel D). Amongst 76 mixed infection cases, RSV + *H. influenzae* non-type B was the most common co-infection (N=22, 28.9%), followed by RSV + *S. pneumoniae* (N=10, 13.2%), Influenza virus + *H. influenzae* non-type B (N=10, 13.2%), RSV + *K. pneumoniae* (N=9, 11.8%), and Parainfluenza virus + *H. influenzae* non-type B (N=9, 11.8%) (Data not shown).

We observed no difference in pathogen distribution by pneumonia severity based on WHO classification system (Supplementary Table 3 and Supplementary Fig. 3). By pathogen, there was no significant difference in distribution between pneumonia severity status or mortality, except for *S. pneumoniae* which was found in significantly more severe cases using the WHO system (P=0.033) (Supplementary Table 3).

A comparison of positivity rates for each causative pathogen by detection method is shown in Table 2. Overall, PCR captured more bacterial pathogens than culture and more viral pathogens than acute-convalescent paired serology. Paired serology was generally helpful in identifying atypical bacteria, such as *C. pneumoniae* and *L. pneumophila*, and upper respiratory tract viruses, such as Rhinovirus and Enterovirus. When comparing blood and IS culture, IS yielded more positive bacterial pathogen results. Similarly, IS PCR captured more pathogens than NP/OP PCR.

Table 2. Causative Pathogens per PEER-PePPEs Rules by Detection Method
| Pathogen               | N  | Blood culture N (%) | IS culture N (%) | Whole blood PCR N (%) | NP / OP PCR N (%) | IS PCR N (%) | Serology Test N (%) |
|------------------------|----|---------------------|------------------|-----------------------|-------------------|--------------|---------------------|
| **Gram-positive cocci bacteria** |    |                     |                  |                       |                   |              |                     |
| *S. pneumoniae*        | 29 | 1 (3.4%)            | 3 (10.3%)        | --                    | 21 (72.4%)        | 28 (96.6%)   |                     |
| *S. aureus*            | 20 | --                  | 7 (35%)          | --                    | 11 (55%)          | 19 (95%)     |                     |
| *S. mitis*             | 4  | --                  | 4 (100%)         | --                    | --                |              |                     |
| *S. pyogenes*          | 1  | --                  | 1 (100%)         | --                    | --                |              |                     |
| **Gram-negative cocci bacteria** |    |                     |                  |                       |                   |              |                     |
| *M. catarrhalis*       | 2  | --                  | 2 (100%)         | --                    | 2 (100%)          | 2 (100%)     |                     |
| **Gram-negative rods bacteria** |    |                     |                  |                       |                   |              |                     |
| *H. inf* non-type b    | 73 | --                  | --               | 8 (10.9%)             | 60 (82.2%)        | 71 (98.6%)   |                     |
| *K. pneumoniae*        | 43 | --                  | 17 (39.5%)       | --                    | 2 (4.7%)          | 34 (79.1%)   |                     |
| *B. pertussis*         | 7  | --                  | --               | --                    | --                | 7 (100%)     |                     |
| *E. coli*              | 5  | 1 (20%)             | 4 (80%)          | --                    | --                |              |                     |
| *P. aeruginosa*        | 4  | --                  | 4 (100%)         | --                    | --                |              |                     |
| *A. baumannii*         | 3  | --                  | 3 (100%)         | --                    | --                |              |                     |
| *H. inf* type b        | 2  | --                  | --               | --                    | --                | 2 (100%)     |                     |
| *N. meningitidis*      | 1  | 1 (100%)            | 1 (100%)         | --                    | --                |              |                     |
| **Atypical bacteria**  |    |                     |                  |                       |                   |              |                     |
| *C. pneumoniae*        | 5  | --                  | --               | --                    | --                | 5 (100%)     |                     |
| *M. pneumoniae*        | 5  | --                  | --               | --                    | --                | 5 (100%)     | 1 (20%)             |
| *L. pneumophila*       | 1  | --                  | --               | --                    | --                |              | 1 (100%)            |
| **Virus**              |    |                     |                  |                       |                   |              |                     |
| RSV                    | 51 | 36 (70.6%)          | 45 (88.2%)       | 10 (19.6%)            |                   |              |                     |
| RSV A                  | 15 | 10 (66.7%)          | 13 (86.7%)       | --                    | --                |              |                     |
| RSV B                  | 36 | 26 (72.2%)          | 32 (88.8%)       | --                    | --                |              |                     |
| Influenza virus        | 25 | 16 (64%)            | 22 (88%)         | 9 (36%)               |                   |              |                     |
| inf A (H1N1)           | 7  | 7 (100%)            | 7 (100%)         | 7 (70%)               |                   |              |                     |
| inf A (H3N2)           | 3  | 3 (100%)            | 3 (100%)         | --                    | --                |              |                     |
| inf B                  | 14 | 6 (42.9%)           | 12 (85.7%)       | 2 (14.3%)             |                   |              |                     |
| PIV                    | 17 | 16 (94.1%)          | 15 (88.2%)       | 3 (17.6%)             |                   |              |                     |
| PIV 1                  | 5  | 5 (100%)            | 4 (80%)          | 3 (17.6%)             |                   |              |                     |
| PIV 2                  | 0  | --                  | --               | --                    | --                |              |                     |
| PIV 3                  | 11 | 10 (90.9%)          | 10 (90.9%)       | --                    | --                |              |                     |
| PIV 4                  | 1  | 1 (100%)            | 1 (100%)         | --                    | --                |              |                     |
| hMPV                   | 11 | 5 (45.5%)           | 10 (90.9%)       | --                    | --                |              |                     |
| Rhinovirus             | 10 | 10 (100%)           | 6 (60%)          | 4 (40%)               |                   |              |                     |
| Enterovirus            | 5  | 3 (60%)             | 3 (60%)          | 3 (60%)               |                   |              |                     |
| Bocavirus              | 3  | 2 (66.7%)           | 3 (100%)         | --                    | --                |              |                     |
| hCoV-NL63              | 2  | 2 (100%)            | 2 (100%)         | --                    | --                |              |                     |

Grey-box indicates the assay was not performed

**Mortality**

Nineteen (10.1%) of the 188 subjects died during the 30-day study period. Seven (36.8%) of these 19 were male, and most (N=17, 89.5%) were less than 1 year old. Among the 19 deceased subjects, median study duration was 12 (IQR, 4 – 17.5) days; eight (42.1%) were admitted to ICU, and
six (31.6%) received mechanical ventilation. Twelve (63.2%) died due to respiratory failure, three (15.8%) due to sepsis, and three (15.8%) for unknown reasons after discharge (data not shown). Most deaths occurred in the 2-11 mo age group compared with the 12-59 mo age group (78.9% vs. 21.1%, \( p=0.036 \)). Causative pathogens for deceased subjects were bacterial-only in seven (36.8%), viral-only in two (10.5%), mixed in five (26.3%), and unknown in five subjects (26.3%). There were no significant differences in pathogen distribution between subjects that survived and died. *H. influenzae* non-type B was the most common pathogen identified in deceased subjects (N=8, with the case fatality rate [CFR] in this study of 11.0%), followed by *K. pneumoniae* (N=6, CFR of 13.9%), Influenza virus (N=3, CFR of 12.0%), *B. pertussis* (N=2, CFR of 28.6%), and RSV (N=2, CFR of 3.9%) (Supplementary Table 3). Pre-existing conditions amongst deceased subjects included congenital heart disease (N=10, 52.6%), severe malnutrition (N=7, 36.8%), and developmental delay (N=7, 36.8%). A clinical summary of the fatal cases is shown in Supplementary Table 4.

### Seasonality

During the 27-month study period, infections caused by RSV and influenza were seen year-round with peak activity occurring during the wet season (November to March) in Indonesia (66.7%, \( p<0.001 \); and 64.0%, \( p=0.012 \), respectively). However, there was little variation in detection of the most common respiratory bacterial infections by month and season. *H. influenzae* non-type B shows peaks in August (N=12, 16.4%) and March (N=11, 15.1%), while *K. pneumoniae* and *S. pneumoniae* fluctuate at lower levels throughout the year (Figure 3).

### DISCUSSION:

PEER-PePPeS, a prospective multisite study, characterized the current epidemiology of CAP in children 2-59 months old in Indonesia. No recent prospective Indonesian studies address this topic. Our study found: (1) mixed bacterial and viral infection is the most frequent (N=76, 40.4%) cause of childhood CAP, irrespective of age group and pneumonia severity; (2) bacterial infections were common (66% of cases) with *H. influenzae* non-b type, *K. pneumoniae*, and *S. pneumoniae* as the three most common bacterial etiologies; (3) viral pathogens were also common (57% of PEER-PePPeS subjects), with 16.5% of cases attributed to virus only and RSV and Influenza Virus being the most common viruses identified; and (4) PCR on IS specimens was the most sensitive assay for pathogen identification.

While our findings are consistent with other studies, clinical significance of mixed infection remains controversial. It is unclear if both agents act as true pathogens. [22,26] PEER-PePPeS did not
demonstrate a correlation of mixed infection with pneumonia severity and 30-day mortality. Many deaths occurred at a younger age (<1 year old) and with comorbidities, such as congenital heart disease and severe malnutrition, similar to previous reports. [27,28] Such factors should be considered in prevention and management of childhood pneumonia to reduce mortality rate.

In recent years, there has been an increased focus on the role of respiratory viruses in childhood pneumonia, partly attributable to use of conjugate pneumococcal and Hib vaccines and increased detection by PCR.[21,22,29,30] In PEER-PePPeS, viruses were found in 57% of subjects (virus only + mixed infection), with 16.5% of cases attributed to virus only. Thus, many patients probably received unnecessary antibiotics when covered empirically per current Indonesian guidelines. Improving ability to discriminate between viral and bacterial infections would facilitate optimization of antibiotic administration and counter antimicrobial resistance.[31]

RSV and influenza virus were the most commonly detected viruses in this study and may be associated with Indonesia’s wet/rainy season.[32–34] A high prevalence of RSV was also observed in the GABRIEL and PERCH international case-control studies of childhood pneumonia etiology.[22,30]

In terms of mixed infections, we found that RSV + *H. influenzae* non-type B and RSV + *S. pneumoniae* were most common. Since respiratory viruses such as RSV can predispose to secondary bacterial infections, particularly *S. pneumoniae* and *H. influenzae*[35], and conversely bacteria can increase RSV susceptibility[35,36], these co-infections highlight the need for optimizing RSV surveillance, prevention and treatment.

Though influenza virus also increases risk for secondary bacterial infections and is a major cause of childhood morbidity and mortality worldwide, data from developing countries is scarce.[37] In a previous Indonesian study of hospitalized patients with a severe acute respiratory infection (SARI), the prevalence of the influenza virus was 10.6% in children under 5 years old, and was never diagnosed during hospitalization.[38] PEER-PePPeS confirms the need for improved diagnostic strategies, management optimization, and influenza vaccination in children. Of note, our study was conducted before identification of COVID-19 in Indonesia[39], so did not address the role of COVID-19 in childhood pneumonia.

We also found that 66% of cases were caused by bacterial infection (bacteria only + mixed infection). Overall, *H. influenzae* non-type B was the most common bacteria implicated, followed by *K. pneumoniae* and *S. pneumoniae*. *H. influenzae* non-type B predominance was also observed in a Malaysian study, where 90% of enrolled children were vaccinated against Hib as part of the national immunization program.[24] With Indonesia’s moderate (56.4%) Hib vaccine coverage, high incidence of *H. influenzae* non-type B may represent its true prevalence or strains not covered by Hib.
This finding agrees with current data that non-typeable *H. influenzae* (NTHi) can cause significant illness, and argues for strengthening pediatric diagnostic laboratory capacity.

Our identification of *K. pneumoniae* as the second most common bacterial etiology is consistent with high carriage rates (~7%) in healthy Indonesian children. Carriage has been related to poor food and water sanitation and may give rise to pneumonia, especially in children with malnutrition.[41] Given *K. pneumoniae*’s potential for antibiotic resistance and high virulence of some strains, proactive detection and management strategies should be prioritized.[42]

The relatively low prevalence (15.4%) of *S. pneumoniae* in PEER-PePPes was surprising since carriage rates are high and PCV coverage low in Indonesia.[43] Low prevalence has also been reported from Malaysia, where PCV coverage is 8.7%[24] and in the PERCH study, reflecting temporal shifts in childhood pneumonia etiologies.[22] As only 4.8% of PEER-PePPeS subjects had received PCV, vaccination alone cannot account for the low *S. pneumoniae* prevalence. Antibiotic exposure prior to specimen collection may have reduced colonization density and lowered the yield of *S. pneumoniae* by both culture and PCR.[44] Moreover, our panel did not include *S. pneumoniae* paired serology, which may be useful to increase pneumococcal diagnosis in young children.[45] Nonetheless, *S. pneumoniae* remains an important etiological agent of severe/complicated CAP globally.[46] Our finding that *S. pneumoniae* was significantly associated with severe cases by the WHO classification system supports the need for ongoing surveillance, vaccination and prevention of transmission between adults and children.

Inclusion of several pathogen identification strategies in PEER-PePPes demonstrates the differential utility of assays and specimen types. Our findings highlight the value of molecular assays, especially in culture-negative cases where microorganisms may be nonrecoverable in culture due to prior antibiotics or presence of otherwise difficult to culture bacteria.[47,48] PCR is also less laborious and can identify genes associated with antibiotic resistance, though conventional culture methods are required to confirm phenotypic resistance.[49,50] Even with the limited PCR panels used in our study, molecular assays had greater sensitivity for identification of bacterial pathogens than blood or sputum culture.

Although sensitive for detection, PCR does not provide information regarding infectiousness or viability. Genome fragments from dead organisms may be detected, often at a low level, even after clinical resolution.[48] Furthermore, negative results may occur due to differential viral kinetics along the respiratory tract. Lower respiratory tract specimens, such as IS, should be sought as they originate from the site of infection.[12,13] Accordingly, we observed a higher yield from PCR on IS than NP specimens. We also found that the use of paired serologies increased the diagnostic yield and was
useful for pathogen confirmation, particularly in the setting of innocent bystander viruses and atypical bacteria.[12]

PEER-PePPeS used a comprehensive approach for pathogen detection to increase diagnostic yield. It also enrolled patients over a 27-month study period, facilitating assessment of seasonality. However, our study has several limitations. First, the relatively small sample size, and observational design may limit generalizability and causal inference. Second, most subjects (79.8%) received antibiotics before specimen collection in accordance with national guidelines. To address this, we enrolled subjects within 24 hours of admission, and specimens were collected as soon as possible to minimize the effects of antibiotics on culture results. Third, we did not enroll healthy control children, limiting the ability to estimate the adjusted population attributable fraction (aPaF) of each pathogen.[29,30] A healthy control group could have revealed baseline carriage rates, minimizing over-attribution of disease to non-pathogenic organisms.[21,22,29,30] Fourth, we did not collect lung aspirates or pleural fluid specimens, which are superior for determination of pneumonia etiology. [15] Fifth, several subjects had pneumonia of unknown etiology; this may have been due to administration of antibiotics before culture which could reduce sensitivity, poor IS quality, the limited panel of bacterial and viral pathogens tested, lack of fungal testing, or currently unrecognized causes of pediatric pneumonia.

In conclusion, the epidemiology of childhood CAP is constantly evolving in step with social and environmental factors and thus, should be regularly assessed. Our study found that *H. influenzae* non-type B and RSV were the most common pathogens causing hospitalized CAP among Indonesian children aged 2-59 months old, reflecting temporally dynamic etiologies of childhood CAP; studies from the 1970-1990s mainly detected *S. pneumoniae* and *H. influenzae* type B as the most important causes of childhood pneumonia in LMICs.[3–5] PCR on IS demonstrated the best sensitivity for pathogen identification. We recommend incorporating molecular assays for pathogen detection, preferably multiplexed point-of-care assays, into practice guidelines. Improvements in Indonesia’s lab infrastructure during the COVID-19 pandemic can be leveraged to facilitate use of molecular assays for evaluation of childhood CAP. Optimization of pathogen detection to understand changing childhood CAP epidemiology will also inform public policy on prevention and management.

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CONTRIBUTORS:

DL, HF, RT, YM, HK, AMN, ATA, CYL, HCL designed and conceptualized the study. DL, HF, RT, AB, C, MSA, DW, M, SD, AS performed clinical assessments and were responsible for data entry. DL, HF, RT, YM, HK, AMN, NL, AK, CYL designed the methodology for pathogen identification. YM, HK, AMN performed data analysis, interpretation and drafted the first manuscript. DL, HK, ATA, MK, AN, CYL, HCL assisted with manuscript writing, analysis, and interpretation of data. All authors contributed to manuscript development, edited for critical content, and have approved the final version.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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This study was approved by the Ethical Clearance Committee of Faculty of Medicine, Universitas Indonesia (No. 567/UN2.F1/ETIK/2017). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or guardians before enrollment.
DATA AVAILABILITY STATEMENT:

Data are available upon reasonable request. The anonymized data set will be shared following the signing of a data-sharing agreement, with permission of the ethical clearance committee, study authors, and all project partners, exclusively for non-commercial purposes. Please contact the corresponding author with any queries.

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**FIGURE CAPTIONS:**

**Figure 1.** Subject screening, enrolment, and monitoring flowchart. CAP, community-acquired pneumonia; RR, respiratory rate; CXR, chest X-Ray; CRP, C-reactive protein; PCT, procalcitonin; NP, nasopharyngeal; IS, induced sputum; WB, whole blood; BC, blood culture; IS, induced sputum culture; PCR, polymerase chain reaction.

**Figure 2.** Pathogen Distribution. (A) Overall proportion of identified viral/bacterial/mixed pathogen, (B) Viral/bacterial/mixed pathogens by age group, (C) Pattern of detection of the ten most identified pathogens, (D) Distribution of ten most identified pathogens by age group. *P*<0.05

**Figure 3.** Distribution of the (A) monthly count and (B) seasonal pattern of infection caused by *H. influenzae* non-type B, RSV, *K. pneumoniae*, *S. pneumoniae*, and Influenza virus during a 27-month study period.

**SUPPLEMENTARY INFORMATION:**

Details of Microbiological, Molecular and Serologic Methods, **Supplementary Table 1**

Antibiotic regimens administered prior to blood culture, **Supplementary Table 2**

Pathogen distribution by WHO severity classification status and mortality, **Supplementary Table 3**
Summary of fatal cases, **Supplementary Table 4.**

PEER-PEPPeS Study sites, **Supplementary Figure 1**

Proportion of Identified Pathogen in each Sites, **Supplementary Figure 2**

Proportion of Identified Pathogen between WHO Severity Status, **Supplementary Figure 3**
Figure 1. Subject screening, enrolment, and monitoring flowchart. CAP, community-acquired pneumonia; RR, respiratory rate; CXR, chest X-Ray; CRP, C-reactive protein; PCT, procalcitonin; NP, nasopharyngeal; IS, induced sputum; WB, whole blood; BC, blood culture; IS, induced sputum culture; PCR, polymerase chain reaction.
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381x381mm (300 x 300 DPI)
Figure 3. Distribution of the (A) monthly count and (B) seasonal pattern of infection caused by H. influenzae non-type B, RSV, K. pneumoniae, S. pneumoniae, and Influenza virus during a 27-month study period.

381x177mm (300 x 300 DPI)
1   SUPPLEMENTARY MATERIALS.

2

3 Supplementary Table 1. Microbiological, Molecular and Serologic Methods

| No. | Assays            | Procedures                                                                                                                                 |
|-----|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| 1.  | Gram stain        | Gram-stained smears were obtained from the most purulent portion of each induced sputum specimen. The good quality specimen was defined as <10 squamous epithelium per low-power field (magnification, 100×) \(^1\). The procedure of the Gram stain required four basic steps that include applied a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram’s Iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstained with safranin \(^2\). The Gram-stained smears interpreted as follows: Gram-positive lancet-shaped diplococci (GPDC) suggest *Streptococcus pneumoniae*; Gram-positive diplococci (GPDC) or cocci in chains suggest *Streptococcus pyogenes*; Gram-positive cocci in clusters (GPC-cluster) suggest *Staphylococcus aureus*; Gram-negative coccobacilli (GNCB) suggest *Hemophilus influenzae*, *Bordetella pertussis* or *Acinetobacter baumannii*; Gram-negative diplococci (GNDC) suggest *Moraxella catarrhalis*; large Gram-negative rods (GNR-large) suggest *Klebsiella pneumoniae* or *Escherichia coli*; and small Gram-negative rods (GNR-small) suggest *Pseudomonas aeruginosa* \(^3\). |
| 2.  | Induced Sputum Culture | The most purulent portion of induced sputum was inoculated onto sheep blood, chocolate, and MacConkey agars, streaked out using a standard 4-quadrant streaking method, and incubated at 35°C for 48 hours. Cultures were examined at 24 hours and 48 hours, and predominant bacteria were identified and quantified according to the farthest quadrant with visible colonies (first quadrant, scanty; second quadrant, 1+; third quadrant, 2+; fourth quadrant, 3+) \(^4\). Then, the predominant bacteria isolates were inoculated into the appropriate VITEK identification strip using the VITEK® 2 COMPACT (BioMérieux, Germany). Briefly, a bacterial suspension was adjusted to a McFarland standard of 0.50 in a solution of 0.45 % sodium chloride using DensiLameter. The time between preparation of the solution and filling of the card was always less than 1 h. Analysis was done using the identification card and automatically read every 15 min. Bacteria identification and antibiotic susceptibility testing results were analyzed using the VITEK 2 software according to the manufacturer’s instructions \(^5\). |
| 3.  | Blood Culture     | Up to 2 mL of blood samples (2 bottle sets) were collected and sent to the site laboratory with standardized procedures. Blood cultures were incubated for at least 5 days, unless positive, using automated systems (BacT/ALERT in Tangerang Hospital; BACTEC at other sites) \(^6\). Organisms were identified according to standard microbiological methods as described in induced sputum culture section. The following organisms were considered to be contaminants when identified in blood cultures: Coagulase-negative *staphylococci*, *Micrococcus* spp., *Propionibacterium* spp., Alpha-hemolytic streptococci (except
| No. | Assays                                                                 | Procedures                                                                                                                                                                                                                                                                                                                                 |
|-----|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 4.  | Viral RNA Extraction                                                   | Viral RNA was extracted from viral transport media (VTM) containing respiratory swab as well as sputum, using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Briefly, 140 µl of VTM or sputum coat was lysed in 560 of carrier RNA-containing AVL buffer, followed by the binding of viral RNA to the QIAamp membrane. Contaminants were removed from viral RNA in two separate washing steps using two different wash buffers, AW1 and AW2. Viral RNA was eluted in 60 µl of AVE buffer and kept in -80°C if not directly used. |
| 5.  | Bacterial DNA Extraction                                               | Bacterial DNA was extracted from viral transport media (VTM) containing respiratory swab as well as sputum, using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Briefly, 20 µl of QIAGEN Protease and 200 µl of VTM or sputum coat was lysed in 200 of AL buffer, followed binding of DNA to the QIAamp membrane. Contaminants were removed from DNA in two separate washing steps using two different wash buffers, AW1 and AW2. Bacterial DNA was eluted in 200 µl of AE buffer and kept in -80°C if not directly used. |
| 6.  | qPCR for Respiratory Viruses                                            | The realtime PCR for respiratory virus detection was done followed the protocol of Beld et al., 2004 and Jansen et al., 2011. Positive control is a synthetic plasmid carrying the nucleotide sequence of the detection target. Primers, probes, and positive controls were synthesized and purified by an outside vendor (Integrated DNA Technologies, Iowa, US). Realtime PCR was done using the TaqManTM Fast Virus 1-Step Master Mix (Thermo Fisher Scientific; Cat#: 4444432) in an Applied Biosystems 7500 Fast Realtime PCR System (Thermo Fisher Scientific, MA, US). The reaction mixture composition was 1X TaqManTM Fast Virus 1-Step Master Mix, 0.5 µM of each primer, 0.25 µM probe, and 4 µl RNA, in a total 20 µl volume. The cycle condition was 50°C reverse transcription for 5 minutes, 95°C initial denaturation for 20 seconds, followed by 45 cycles of denaturation (95°C, 3 seconds) and annealing/elongation (55°C, 30 seconds). Realtime PCR works correctly when the positive control demonstrates the amplification curve and the template-free (negative) control demonstrates no amplification curve (no Ct values). |
| 7.  | qPCR for Respiratory Bacteria                                           | In real-time PCR (qPCR) a portion of bacterial DNA genome specific to the pathogen(s) of interest is amplified using a specific pair of primers and probes for each bacteria, that were selected from the available literature. A detector (TaqMan® probe) is used in the reaction. Mastermix is prepared in a 1.5-ml tube for total reaction. qPCR assays were carried out in a total volume of 20 µl, comprising 10 µl of TaqMan® Fast Universal PCR Master Mix, 1.4 µl of nuclease-free water (Promega), 3.6 µl of oligonucleotide mixtures, and 4 µl of |
| No. | Assays                  | Procedures                                                                                                                                                                                                                                                                                                                                 |
|-----|-------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 8.  | Serology Test           | Assays were obtained from SERION ELISA classic kit (Institut Virion/Serion Laboratories, Germany) and used according to the insert of SERION kit. SERION ELISA classic is a qualitative and quantitative immunoassay for detecting human antibodies in serum or plasma with their corresponding antigen. The indirect enzyme immunoassay kit in this study was coated with specific antigens of the pathogen of interest. Patient sera are diluted in a rheumatoid factor and then diluted in Sample Diluent (containing phosphate with tween 20 and Bromphenol blue) and incubated in the coated microwells to bind serum antibody to the solid-phase antigen. The microwells are then washed to remove unreacted serum proteins, and enzyme conjugate (anti-human IgA, IgG, or IgM APC_Alkaline phosphatase) is added to label the bound antibody. After further incubation, the microwells are washed to remove unbound APC Conjugate. The pNPP (para-nitrophenyl phosphate) substrate is then added to quantitate the Conjugate-bound p-nitrophenyl phosphate portion. The colorless substrate p-nitrophenyl phosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the serum antibody. This timed reaction is interrupted with a Stop Solution (sodium hydroxide). Color intensity (Absorbance) is measured at a wavelength of 405nm on a microtiter plate reader or spectrophotometer within 15 minutes of adding the stop solution. Antibody activities are calculated by the SERION evaluation software.                                                                 |

**Footnote References:**

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### Supplementary Table 2. Antibiotic regimens administered prior to blood culture

| Antibiotic Regimen, (Dose) | All sites (N=188), Administered Dose(s) prior to blood culture, N (%) | Semarang (N=47), Administered Dose(s) prior to blood culture, N (%) | Yogyakarta (N=52), Administered Dose(s) prior to blood culture, N (%) | Tangerang (N=89), Administered Dose(s) prior to blood culture, N (%) |
|---------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Ampicillin (50 mg/kg IV q6hr) + Gentamicin (2 – 7.5 mg/kg IV q24hr) | 65 (34.6) | 25 (53.2) | 40 (76.9) | 0 (0) |
| 1x: 45 (24.0) | 1x: 20 (42.6) | 1x: 25 (48.1) | 2x: 20 (10.6) | 2x: 15 (28.8) |
| 2x: 20 (10.6) | 2x: 5 (10.6) | 2x: 5 (10.6) | 2x: 5 (10.6) | 2x: 5 (10.6) |
| Cefotaxime (50 – 100 mg/kg IV q6hr) | 32 (17.0) | 0 (0) | 0 (0) | 32 (36.0) |
| All received 1 dose | All received 1 dose | All received 1 dose | All received 1 dose |
| Ceftriaxone (50 mg/kg IV q12hr) | 27 (14.4) | 0 (0) | 0 (0) | 27 (30.3) |
| All received 1 dose | All received 1 dose | All received 1 dose |
| Ampicillin (50 mg/kg IV q6hr) | 14 (7.4) | 5 (10.6) | 9 (17.3) | 0 (0) |
| 1x: 10 (5.3) | All received 1 dose | 1x: 5 (9.6) | All received 1 dose |
| 2x: 4 (2.1) | 2x: 4 (2.1) | 2x: 4 (2.1) |
| Gentamicin (2 – 7.5 mg/kg IV q24hr) | 3 (1.6) | 3 (6.4) | 0 (0) | 0 (0) |
| 1x: 2 (1.1) | 1x: 2 (1.1) | 1x: 2 (1.1) |
| 2x: 1 (0.5) | 2x: 1 (0.5) | 2x: 1 (0.5) |
| Ceftazidime (50 – 100 mg/kg IV q8hr) | 3 (1.6) | 0 (0) | 0 (0) | 3 (3.4) |
| All received 1 dose | All received 1 dose |
| Cefamandole (50 – 100 mg/kg IV q12hr) | 2 (1.1) | 2 (4.3) | 0 (0) | 0 (0) |
| 1x: 1 (0.5) | 1x: 1 (0.5) | 1x: 1 (0.5) |
| 2x: 1 (0.5) | 2x: 1 (0.5) | 2x: 1 (0.5) |
| Ceftriaxone (50 mg/kg IV q12hr) + Gentamicin (2 – 7.5 mg/kg IV q24hr) | 2 (1.1) | 2 (4.3) | 0 (0) | 0 (0) |
| All received 1 dose | All received 1 dose | All received 1 dose |
| Amikacin (15 mg/kg IV q8hr) + Cefotaxime (50 – 100 mg/kg IV q6hr) | 1 (0.5) | 1 (2.1) | 0 (0) | 0 (0) |
| All received 1 dose | All received 1 dose |
| Amoxicillin syrup (40 mg/kg PO q12hr) | 1 (0.5) | 1 (2.1) | 0 (0) | 0 (0) |
| All received 1 dose | All received 1 dose |

IV = intravenous; PO = peroral; qXhr = given at X hour intervals.
**Supplementary Table 3.** Pathogen distribution by WHO severity classification status and mortality.

| Pathogens          | WHO Classification System | p-value | Mortality Outcome | p-value |
|--------------------|----------------------------|---------|-------------------|---------|
|                    | Severe (N=89) | Non-severe (N=99) | Died (N=19) | Alive (N=169) |
| H. influenzae non-type b | 31 (34.8%) | 42 (42.4%) | 8 (42.1%) | 65 (38.5%) |
| RSV                | 25 (28.1%) | 26 (26.3%) | 2 (10.5%) | 49 (29.0%) |
| K. pneumoniae      | 15 (16.9%) | 28 (28.3%) | 6 (31.6%) | 37 (21.9%) |
| S. pneumoniae      | 19 (21.3%) | 10 (10.1%) | 1 (5.2%) | 28 (16.6%) |
| Influenza virus    | 9 (10.1%) | 16 (16.2%) | 3 (15.8%) | 22 (13.0%) |
| S. aureus          | 8 (9.0%) | 12 (12.1%) | 0 (0.0%) | 20 (11.8%) |
| PIV                | 8 (9.0%) | 9 (9.1%) | 1 (5.3%) | 16 (9.5%) |
| hMPV               | 6 (6.7%) | 5 (5.1%) | 1 (5.3%) | 10 (5.9%) |
| Rhinovirus         | 7 (7.9%) | 3 (3.0%) | 1 (5.3%) | 9 (5.3%) |
| B. pertussis       | 4 (4.5%) | 3 (3.0%) | 2 (10.5%) | 5 (3.0%) |

Differences in categorical variables were compared using Pearson χ² or Fisher’s exact test when the expected values in any of the contingency table cells were below 5.

**Supplementary Table 4.** Summary of fatal cases.

| Case, Site, Gender (Age, mo) | Medical History | Signs and Symptoms (SS), Vital Signs (VS), Laboratory parameter (Lab) at admission | CXR | Causative Pathogen | ABX during Hospitalization | Hospitalization status | Cause of Death |
|-----------------------------|----------------|---------------------------------------------------------------------------------|-----|-------------------|----------------------------|------------------------|----------------|
| R01, SMG, Male (4)          | Recurrent pneumonia, congenital heart disease, severe malnutrition | $SS$: Cough, fever, dyspnea, chest indrawing, intercostal retraction, rhonchi $VS$: 38°C, RR 44/min, SpO₂ 97% $Lab$: Hb 9.6 g/dL, WBC 24.1 ×10⁹/L, PCT 3.50 ×10⁹/L, NLR 4.63, CRP 25.70 mg/L, PCT 2.41 mg/mL | Alveolar infiltrate | Rhinovirus, H. influenzae non-type b | Gentamicin, Ceftriaxone, Sulbactam | On mechanical ventilator, ICU admission (25 days) | Died on Day-26 |
| R02, SMG, Female (23)       | Recurrent pneumonia, congenital heart disease, incomplete NIP (DPT-Hib), malnutrition, developmental delay | $SS$: Cough, fever, dyspnea, chest indrawing, intercostal retraction, rhonchi $VS$: 37.5°C, RR 56/min, SpO₂ 95% $Lab$: Hb 10.6 g/dL, WBC 14.1 ×10⁹/L, PCT 4.05 ×10⁹/L, NLR 9.63, CRP 14.90 mg/L, PCT 0.37 ng/mL | Alveolar and interstitial infiltrates | Influenza A (H1N1) | Gentamicin, Amikacin, Ceftriaxone, Cefotaxime | On mechanical ventilator, ICU admission (9 days) | Died on Day-21 |
| R03, SMG, Female (11)       | Low birth weight, congenital heart disease, incomplete NIP (Measles), severe malnutrition, developmental delay | $SS$: Cough, fever, dyspnea, chest indrawing, intercostal retraction, rhonchi $VS$: 38.3°C, RR 45/min, SpO₂ 96% $Lab$: Hb 8.1 g/dL, WBC 15.9 ×10⁹/L, PCT 677.1 ×10⁹/L, NLR 1.87 | Alveolar and interstitial infiltrates | Influenza A (H3N2), B. pertussis, H. influenzae non-type b, K. pneumoniae | Gentamicin, Ceftriaxone, Astamycin | On nasal cannula, Died on Day-19 | Cardiopulmonary failure |
| R04, SMG, Male (45)          | Recurrent pneumonia, frontomethaphysial dysplasia syndrome, | $SS$: Cough, fever, dyspnea, nasal flaring, intercostal retraction, rhonchi, wheezing $VS$: 36.7°C, RR 40/min, SpO₂ 99% | Alveolar infiltrate | Unknown | Gentamicin | On nasal cannula, Died on Day-2 | Respiratory failure |
| Case, Site, Gender (Age, mo) | Medical History | Signs and Symptoms (SS), Vital Signs (VS), Laboratory parameter (Lab) at admission | CXR | Causative Pathogen | ABX during Hospitalization | Hospitalization status | Cause of Death |
|----------------------------|-----------------|---------------------------------------------------------------------------------|-----|-------------------|---------------------------|------------------------|---------------|
| 1R5, SMG, Male (5)         | Premature birth, low birth weight, recurrent pneumonia, congenital heart disease, incomplete NIP (DPT-Hib) | Lab: Hb 11.7 g/dL, WBC 11.3 ×10^9/L, PLT 277 ×10^9/L, NLR 0.98, CRP 0.30 mg/L, PCT 0.05 mg/L | Alveolar infiltrate | K. pneumonia | Ampicillin, Gentamicin | ICU admission (1 day) | Died on day 6 | Cardiopulmonary failure |
| 1R6, SMG, Female (3)      | Recurrent pneumonia, incomplete NIP (DPT-Hib), malnutrition | Lab: Hb 8.2 g/dL, WBC 16 ×10^9/L, PLT 499 ×10^9/L, ANC 6.7, NLR 0.76, CRP 13.10 mg/L, PCT 0.28 mg/L | Alveolar infiltrate | Unknown | Ampicillin, Gentamicin, Vancomycin | On mechanical ventilator/ ICU admission (7 days) | Died on day 18 | Septic shock, respiratory failure |
| 1R7, YGY, Female (10)    | Congenital heart disease, incomplete NIP (DPT-Hib, and Measles), severe malnutrition, developmental delay | Lab: Hb 10.1 g/dL, WBC 12.1 ×10^9/L, PLT 415 ×10^9/L, ANC 6.0, NLR 1.15, CRP 4.90 mg/L, PCT 0.11 mg/L | Alveolar and interstitial infiltrates | HMPV, RSV A | Ampicillin, Ceftriaxone | On nasal cannula Hospital discharge on day 10 | Died on day 29 (outside hospitalization) | Acute Respiratory Distress Syndrome |
| 1R8, YGY, Female (3)      | Low birth weight, congenital heart disease, incomplete NIP (DPT-Hib), severe malnutrition | Lab: Hb 9.7 g/dL, WBC 11.3 ×10^9/L, PLT 115 ×10^9/L, ANC 7.0, NLR 1.92 | Alveolar and interstitial infiltrates | Unknown | Ampicillin, Ceftriaxone | On nasal cannula Died on day 15 | Aspiration, mucous hypersecretion |
| 1R9, YGY, Female (5)      | Congenital heart disease, incomplete NIP (DPT-Hib), severe malnutrition | Lab: Hb 10.3 g/dL, WBC 26.9 ×10^9/L, PLT 788 ×10^9/L, ANC 18.5, NLR 2.97 | Alveolar and interstitial infiltrates, pleural effusion | H. influenzae non-type b, K. pneumonia | Ampicillin, Gentamicin | On nasal cannula | Died on day 18 | Unknown death |
| 1R10, YGY, Male (6)      | Recurrent pneumonia, congenital heart disease, tuberculosis, incomplete NIP (DPT-Hib) | Lab: Hb 11.6 g/dL, WBC 13.3 ×10^9/L, PLT 189 ×10^9/L, ANC 3.7, NLR 0.48, CRP 4.90 mg/L, PCT 0.08 mg/mL | Alveolar and interstitial infiltrates | Unknown | Ampicillin, Ceftriaxone | On non-rebreather mask | Died on day 4 | Septic shock |
| 1R11, TRG, Female (5)    | Premature birth, developmental delay | Lab: Hb 8.5 g/dL, WBC 12.1 ×10^9/L, PLT 208 ×10^9/L, ANC 8.6, NLR 3.23, CRP 0.93 mg/L, PCT 0.74 mg/mL | Alveolar infiltrate | A. baumannii (MDR) | Cefotaxime | On nasal cannula Hospital discharge on day 7 | Died on day 17 (outside hospitalization) | Unknown death |
| 1R12, TRG, Female (2)    | Incomplete NIP (DPT-Hib) | Lab: Hb 10.5 g/dL, WBC 13.6 ×10^9/L, PLT 289 ×10^9/L, ANC 10.2, NLR 3.95, CRP 275.30 mg/L, PCT 0.70 mg/mL | Alveolar and interstitial infiltrates | Unknown | Ceftriaxone, Ceftazidime, Azithromycin | On Nasal cannula | Died on day 8 | Sepsis |
| 1R13, TRG, Female (2)    | Incomplete NIP (DPT-Hib) | Lab: Hb 7.8 g/dL, WBC 21.2 ×10^9/L, PLT 563 ×10^9/L, ANC 16.5, NLR 3.9, CRP 280.30 mg/L, PCT 0.09 mg/mL | Alveolar and interstitial infiltrates, pleural effusion | Influenza B, 5 mets (MDR) | Ceftazidime | On non-rebreather mask ICU admission (3 days) | Died on day 3 | Respiratory Failure |
| Case, Site, Gender (Age, mo) | Medical History | Signs and Symptoms (VS), Vital Signs (VS), Laboratory parameter (Lab) at admission | CXR | Causative Pathogen | ABX during Hospitalization | Hospitalization status | Cause of Death |
|---------------------------------|-----------------|---------------------------------------------------------------------------------|-----|-------------------|--------------------------|------------------------|----------------|
| #14, TRG, Female (2)            | Congenital heart disease, incomplete NIP (DPT-Hib), severe malnutrition | **VS:** Cough, fever, dyspnea, nasal flaring, chest indrawing, intercostal retraction, rhonchi, wheezing  
**VS:** 37.3 °C, RR 60x/min, SpO₂ 76%  
**Lab:** HB 9.5 g/dL, WBC 17.7 ×10⁹/L, PLT 296 ×10⁹/L, ANC 8.8, NLR 3.42, CRP 0.70 mg/L, PCT 0.02 ng/mL. | Interstitial infiltrate | Unknown | Cefotaxime | On Simple mask  
Died on day 2 | Respiratory Failure |
| #15, TRG, Male (9)              | Incomplete NIP (Measles) | **VS:** Cough, fever, dyspnea, nasal flaring, chest indrawing, intercostal retraction, rhonchi  
**VS:** 37 °C, RR 40x/min, SpO₂ 89%  
**Lab:** HB 6.4 g/dL, WBC 25.7 ×10⁹/L, PLT 801 ×10⁹/L, ANC 18.5, NLR 3.45, CRP 33.35 mg/L, PCT 0.34 ng/mL. | Interstitial infiltrate | H. influenzae non-type b | Cefotaxime, Ceftriaxone, Meropenem | On mechanical ventilator  
ICU admission (8 days)  
Died on day 12 | Meningoencephalitis, Respiratory Failure |
| #16, TRG, Female (4)            | Premature birth, low birth weight, congenital heart disease, incomplete NIP (DPT-Hib) | **VS:** Cough, fever, dyspnea, diarrhea, chest indrawing, intercostal retraction, rhonchi  
**VS:** 38 °C, RR 32x/min, SpO₂ 85%  
**Lab:** HB 9.2 g/dL, WBC 16.8 ×10⁹/L, ANC 9.4, NLR 2.42, CRP 2.46 mg/L, PCT 2.24 mg/mL. | Alveolar and interstitial infiltrates, pleural effusion | H. influenzae non-type b, K. pneumoniae | Cefotaxime, Gentamicin, Ceftriaxone | On nasal cannula  
Died on day 11 | Unknown death |
| #17, TRG, Female (20)          | Developmental delay, incomplete NIP (DPT-Hib) | **VS:** Cough, fever, dyspnea, diarrhea, chest indrawing, intercostal retraction, rhonchi  
**VS:** 36.3 °C, RR 40x/min, SpO₂ 75%  
**Lab:** HB 7.0 g/dL, WBC 15.2 ×10⁹/L, PLT 668 ×10⁹/L, ANC 9.9, NLR 2.13, CRP 55.10 mg/L. | Alveolar and interstitial infiltrates, pleural effusion | H. influenzae non-type b, K. pneumoniae | Cefotaxime, Gentamicin, Ceftriaxone | On mechanical ventilator  
ICU admission (3 days)  
Died on day 8 | Septic shock, Cardiopulmonary failure |
| #18, TRG, Male (4)              | Low birth weight, developmental delay, recurrent pneumonia, incomplete NIP (DPT-Hib), severe malnutrition | **VS:** Cough, fever, dyspnea, nasal flaring, chest indrawing, intercostal retraction, rhonchi  
**VS:** 36.7 °C, RR 50x/min, SpO₂ 92%  
**Lab:** HB 11.6 g/dL, WBC 20.5 ×10⁹/L, PLT 413 ×10⁹/L, ANC 11.9, NLR 2.52, CRP 16.80 mg/L, PCT 20.1 mg/mL. | Alveolar and interstitial infiltrates, pleural effusion | PIV 3, H. influenzae non-type b, S. pneumoniae | Cefazidime | On non-rebreather mask  
Died on day 3 | Respiratory failure |
| #19, TRG, Male (15)              | Incomplete NIP (DPT-Hib and Measles) | **VS:** Cough, fever, dyspnea, rhonchi  
**VS:** 37.8 °C, RR 52x/min, SpO₂ 88%  
**Lab:** HB 9.4 g/dL, WBC 23.6 ×10⁹/L, PLT 786 ×10⁹/L, CRP 3.30 mg/L, PCT 0.07 ng/mL. | Interstitial infiltrate | RV B, B. pertussis, H. influenzae non-type b | Cefotaxime | On nasal cannula  
Hospital discharged on day 5  
Died on day 20 (outside hospitalization) | Unknown death |

Abbreviations: SMG: Semarang site; YGY: Yogyakarta site; TGR: Tangerang site; NIP: mandatory National Immunization Program; DPT-Hib: a combined vaccine of adsorbed diphtheria, tetanus toxoids, acellular pertussis and of Hemophilus influenzae type b conjugate vaccines; CXR: chest X-ray; ABX: Antibiotics; RSV: Respiratory Syncytial Virus; hMPV: Human Metapneumovirus; PIV: Parainfluenza Virus; MDR: Multidrug resistance.
**Supplementary Figure 1. PEER-PePPeS Study sites**

1. Kariadi Hospital, Semarang
   - *Satellite sites*: Adhyatma Hospital and Bhakti Wira Tamtama Hospital
2. Sardjito Hospital, Yogyakarta
3. Tangerang District Hospital, Tangerang
   - *Satellite site*: An-Nisa Hospital

**Supplementary Figure 2. Proportion of Identified Pathogen in each Sites.** (A) Semarang, (B) Yogyakarta, and (C) Tangerang
Supplementary Figure 3. Proportion of Identified Pathogen between WHO Severity Status. (A) Non-severe Pneumonia, (B) Severe Pneumonia.
STROBE Statement—Checklist of items that should be included in reports of cohort studies

| Paragraph/ Line number | Recommendation |
|------------------------|----------------|
| **Title and abstract** | Page 1-2       |
| (a) Indicate the study’s design with a commonly used term in the title or the abstract |
| (b) Provide in the abstract an informative and balanced summary of what was done and what was found |

| **Introduction** | |
|------------------|----------------|
| Background/rationale | Introduction, paragraph 1-3 (Page 3-4) |
| Explain the scientific background and rationale for the investigation being reported |
| Objectives | Introduction, paragraph 4 (Page 4) |
| State specific objectives, including any prespecified hypotheses |

| **Methods** | |
| Study design | Methods, paragraph 1 (Page 4) |
| Present key elements of study design early in the paper |
| Setting | Methods, paragraph 1 and 4 (Page 4-5) |
| Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants | Methods, paragraph 4 (Page 5) |
| (a) Give the eligibility criteria, and the sources and methods of selection of participants |
| Variables | Methods, paragraph 2, 3, and 5 (Page 4-5) |
| Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/measurement | Methods, paragraph 5, 6, 7, and 8 (Page 5-6) |
| For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias | Methods, paragraph 9 and 10 (Page 6-7) |
| Describe any efforts to address potential sources of bias |
| Study size | Methods, paragraph 1 and 4 (Page 4-5) |
| Explain how the study size was arrived at |
| Quantitative variables | Methods, paragraph 9 (Page 6) |
| Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | Methods, paragraph 9 (Page 6) |
| (a) Describe all statistical methods, including those used to control for confounding |
| (b) Describe any methods used to examine subgroups and interactions |
| (c) Explain how missing data were addressed |
| (d) If applicable, describe analytical methods taking account of sampling strategy |
| (e) Describe any sensitivity analyses |

| **Results** | |
| Participants | Results, paragraph 1 (Page 7) and Flow diagram/Fig. 1 |
| (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed |
| (b) Give reasons for non-participation at each stage |
| (c) Consider use of a flow diagram |
| Descriptive data | Results, paragraph 2 and 3. Table 1. (Page 7-9) |
| (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders |
| (b) Indicate number of participants with missing data for each variable of interest |
| Outcome data | Results, paragraph 4 and 5 (Page 9-10) |
| Report numbers of outcome events or summary measures |
| Main results | Results, paragraph 5, 7, and 9. Table 2. (Page 10-11) |
| (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval) |
12). Fig. 2 and Fig. 3  interval). Make clear which confounders were adjusted for and why they were included

(b) Report category boundaries when continuous variables were categorized

(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

| Other analyses | Results, paragraph 6 (Page 10). Results, paragraph 8. (Page 11-12). |
|----------------|------------------------------------------------------------------|
|                 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses |

**Discussion**

| Key results     | Discussion, paragraph 1 (Page 12). |
|-----------------|------------------------------------|
|                 | Summarise key results with reference to study objectives |

| Limitations     | Discussion, paragraph 11 (Page 15). |
|-----------------|-------------------------------------|
|                 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |

| Interpretation  | Discussion, paragraph 2-10 (Page 12-15). |
|-----------------|-----------------------------------------|
|                 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence |

| Generalisability | Discussion, paragraph 3-8 (Page 13-14). Discussion, paragraph 12 (Page 15). |
|------------------|--------------------------------------------------------------------------------|
|                  | Discuss the generalisability (external validity) of the study results |

**Other information**

| Funding         | Funding statement (Page 16). |
|-----------------|------------------------------|
|                 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |

*Give information separately for exposed and unexposed groups.*

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.