**Supplementary Appendix**

Title: Incidence and Etiology of Community-acquired Pneumonia Requiring Hospitalization among U.S. Children

Running head: Community-acquired Pneumonia in U.S. Children

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**Supplementary Methods**

**Detailed inclusion and exclusion criteria definitions**

The main methods outline the inclusion and exclusion criteria. Here, we provide more detailed definitions of certain criteria as appropriate. Fever and hypothermia were defined as temperature ≥38˚C and <35.5˚C respectively. White blood cell count (wbc) was compared to baseline if available and defined as leukocytosis or leukopenia based on appropriate values for age. For children <5 years old, wbc >15,000/mm3 or <5500/mm3 and for children ≥5 years old, wbc >11,000/mm3 or <3000/mm3 were considered abnormal.1 Tachypnea was defined based on age: <2 months, >60 breaths/minute; 2 months to <12 months, >50 breaths/minute; 12 months to 5 years, >40 breaths/minute; and >5 years, >25 breaths/minute.1

Children with immunosuppression and hospitalization within the past 90 days were excluded; immunosuppression was defined as solid organ or hematopoietic stem cell transplant, cancer on chemotherapy, and steroid use (prednisone equivalent of 1 mg/kg) for >30 days. Children with neutropenia were also excluded; neutropenia was defined as an absolute neutrophil count <500/mm3.

**Data collection**

Children and/or their caregivers were interviewed by trained staff as soon as possible after enrollment (within 24 hours) using a standardized questionnaire that included demographics, clinical presentation, antimicrobial use prior to hospitalization, underlying medical conditions, and environmental risk factors such as smoke exposure.

Medical chart abstraction was performed after discharge to obtain information on admission and discharge timing, antimicrobials received during hospitalization, underlying medical conditions, vital signs, select laboratory results including routine microbiology, and outcomes including intensive care, mechanical ventilation, and death. Data on microbiological tests performed for clinical care and not distinctly for research purposes, such as tuberculosis and endemic mycoses, were collected.

**Vaccination verification**

Vaccination data for *H. influenzae* type b, influenza,and *S. pneumoniae* were self-reported and then verified through use of vaccine registries, medical record review (inpatient and outpatient), and non-traditional providers (pharmacy) when possible.

**Specimen collection and processing**

Blood (1-2 ml in infants and 5-20 ml in children based on weight) for culture was collected in BACTEC bottles, transported to the microbiology laboratory, and tested on site per routine methods. Initial blood cultures were usually obtained prior to enrollment as part of standard care, in contrast with all other specimens, which were obtained only for research purposes after enrollment. Whole blood (1-1.5 ml) was collected in an EDTA tube and transferred into Sarstedt tubes for storage at -20°C. Blood (2-5 ml based on weight) for serology was collected in a Vacutainer tube and immediately stored at 4°C for 1-18 hours after collection before being centrifuged to recover serum, aliquoted, and stored in Sarstedt tubes at -20°C. NP/OP specimens were collected using sterile flocked Dacron (NP) and wound rayon (OP) swabs with flexible shafts. NP swabs were passed through one nostril to the nasopharynx and rotated to collect epithelial tissue and absorb secretions and OP swabs were inserted into the posterior pharynx and tonsillar areas. NP/OP swabs were combined in 3 ml sterile universal transport medium and refrigerated at 4°C for up to 48 hours, aliquoted, and stored at -70°C.

When available, PF, ET aspirates, and BAL specimens were collected into sterile containers and tested on site per routine clinical care. After routine testing, PF was refrigerated at 4°C for up to 48 hours, aliquoted, and stored at -70°C. Only ET aspirates of high quality (≤10 epithelial cells/low power field [lpf] and ≥25 white blood cells/lpf) and BAL specimens with significant growth of potential pathogens by semi-quantified (moderate to heavy growth [>3+]) or quantified (≥103 colony forming units/ml) methods were included.2,3

Specimens (whole blood, sera, NP/OP swabs, PF), including bacterial isolates, were transported on dry ice to CDC for testing, quality control, and long-term storage. Once PF was tested at CDC, it was again frozen and sent on dry ice to the University of Utah.

**Pleural fluid PCR at the University of Utah**

PF specimens were tested at the University of Utah for bacterial pathogens using the following PCR methods: 1) bench-top nested PCR assays4 (*H. influenzae* [*rpoB, bexA*]), *S. aureus* [*nuc, mecA*]*, S. pneumoniae* [*lytA*]*, S. pyogenes* [*speB*])*,* and 2) FDA-approved FilmArray® blood culture panel5 (*Acinetobacter baumanii*, *Escherichia coli, H. influenzae, Klebsiella pneumoniae*, *S. aureus, S. pneumoniae, S. pyogenes,* viridans streptococci)*.*

**Laboratory quality control and validation**

CDC provided qualified PCR primers, probes, and reagents to each site, and CDC staff reviewed methods and trained laboratory staff at each site to standardize techniques. Prior to performing laboratory analyses, each site successfully completed an external quality assessment program provided by Quality Control for Molecular Diagnostics (QCMD) (http://www.qcmd.org/)6,7 and completed CDC influenza proficiency testing. A random sample of 10% of all NP/OP swabs from every month of the first year of the study, were also tested at CDC, the results of which demonstrated 99% agreement with site-specific data for all viral and atypical bacterial targets.

**Blood contaminants**

Certain bacteria isolated from blood were considered contaminants and unrelated to community-acquired pneumonia. These included *Aeroccocus*, *Alcaligenes faecalis*, *Bacillus*, *Citrobacter*, coagulase-negative *Staphylococcus, Corynebacterium, Enterococcus, Micrococcus, Neiserria subflava, Propionibacterium, Stomatococcus, Streptococcus bovis,* and *Veillonella.* *Candidemia* was not considered to be related to community-acquired pneumonia.

More virulent viridans streptococci (*S. anginosus, S. mitis*) were considered to be pathogens. Less virulent viridans streptococci (*S. salivarius* and viridans group streptococci without further speciation) were considered contaminants when isolated concurrently with a bacterial pathogen or if only one of two simultaneously collected blood cultures was positive. Otherwise, less virulent viridans group streptococci were considered to be pathogens.

**Influenza and other respiratory virus serology methods**

Serology for non-influenza respiratory viruses (AdV, HMPV, RSV and PIV) was performed using CDC developed indirect enzyme immunassays.8-9 A ≥4-fold rise in IgG antibody titer between paired acute and convalescent serum specimens was considered evidence of infection. Due to antigenic cross-reactivity among PIV 1-3, serological data from all three types were analyzed in aggregate. Results were considered inconclusive and excluded from the analysis if paired sera exhibited: 1) high background absorbance to uninfected control cells or 2) differences in antibody levels were identified among 4 or more viral assays indicating possible mismatches in serum pairs between patients.

For influenza serology, hemagglutination inhibition (HI) assays were performed for both influenza A and B viruses, and microneutralization (MN) assays were subsequently performed on all specimens that were originally positive for influenza B virus by HI assay.10 Influenza serology was performed on paired sera for the following strains circulating during 2010-2012: A/California/07/2009 (H1N1pdm09), A/Perth/16/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage).

HI assays were performed with 0.5% turkey red blood cells (RBCs). Serum samples were treated with receptor destroying enzyme and adsorbed with turkey RBCs to eliminate non-specific agglutinins as needed. Two-fold dilutions of sera at a starting dilution of 1:10 were incubated with a standard amount of virus (4HAU/25 µL).10 An HI titer was defined as the reciprocal of the highest serum dilution that completely inhibited hemagglutination. For influenza A virus HI assays, whole viruses were utilized; for influenza B virus HI assays, ether-treated antigens were used to improve assay sensitivity.11,12 All specimens that were positive for influenza B virus in the HI assay using ether-treated antigens were further tested by MN assay to verify specificity. The influenza B virus MN assays were performed by incubating two-fold serial dilutions of heat-inactivated sera (starting dilution 1:10), with 100 tissue culture infection dose 50 (TCID50) units of influenza B viruses. The virus-serum mixture was then used to infect Madin-Darby canine kidney (MDCK) cells. After 18-20 hours incubation, the presence of viral protein was detected by enzyme-linked immunosorbent assay (ELISA) with a monoclonal antibody specific to the nucleoprotein of influenza B. MN titers were defined as the reciprocal of the highest dilution of serum that gave 50% neutralization.

Influenza seroconversion was considered a 4-fold rise in titer for paired acute and convalescent sera with a convalescent titer achievement of ≥40. The final determination of influenza serology accounted for influenza vaccination status and timing based on data from self/caregiver report and the vaccine verification process.13 If influenza serology results indicated seroconversion when vaccine was administered within 2 weeks (based on self/caregiver report or vaccine verification) before acute serum collection, or between acute and convalescent serum collections, results were deemed inconclusive. Thus, influenza serology results were considered positive when there was ≥4-fold rise to influenza A (by HI titer) and/or B (by HI and MN titer) virus with a convalescent titer ≥40 in children who did not receive influenza vaccine or received vaccine more than 2 weeks prior to acute serum collection and not between acute and convalescent sample collection.

**Counties under surveillance and incidence calculation inputs**

States and counties under surveillance from each hospital were as follows:

1) Le Bonheur Children’s Hospital (Memphis, TN) – Arkansas: Crittenden, Mississippi; Mississippi: Desoto, Lafayette, Marshall, Panola, Tate, Tunica; Tennessee: Fayette, Lauderdale, Shelby, Tipton.

2) Monroe Carell Jr. Children’s Hospital at Vanderbilt (Nashville, TN) - Tennessee: Cheatham, Davidson, Dickson, Montgomery, Robertson, Rutherford, Sumner, Williamson, and Wilson.

3) Primary Children’s Hospital (Salt Lake City, UT) – Utah: Salt Lake.

Market share data varied among sites. The data source for TN counties was the TN Hospital Discharge Data System, an electronic data collection system that includes administrative data from all hospital admissions and emergency department visits in TN. By state mandate, data collected from all TN non-federal hospitals includes dates of admission and discharge, discharge diagnoses, and patient date of birth along with gender, race, and ethnicity, among other variables. For counties outside TN included in the Memphis catchment area (AR and MS), comparable market share data were not available; thus the assumption was made that the market share for AR and MS counties was the same as for TN counties in the Memphis catchment area. UT market share data were obtained through the UT Department of Health Indicator-Based Information System for Public Health, which features data derived from the National Center for Health Statistics through a collaborative agreement with the U.S. Census Bureau for the years 2000-2012. For market share, pneumonia admissions were based on International Classification of Diseases, 9th Revision, Clinical Modification discharge diagnosis codes 480-486, 487.0, and 510.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Enrollment hospital | Population\* under surveillance, 2011 | Population\* under surveillance, 2012 | Percent market share, year 1† | Percent market share, year 2† |
| Le Bonheur Children’s Hospital | 385, 382 | 383, 964 | 74.8% | 86.5% |
| Monroe Carell Jr. Children’s Hospital at Vanderbilt | 420, 289 | 425, 235 | 76.3% | 79.2% |
| Primary Children’s Hospital | 303, 213 | 306, 723 | 94.6% | 95.3% |

**\***Population of children < 18 years old

† Annual incidence rates were calculated from July 1, 2010 to June 30, 2011 (year 1) and July 1, 2011 to June 30, 2012 (year 2)

**Enrollment Validation**

To assess for selection bias during the enrollment process, we compared eligible children who were enrolled with those who were not enrolled. Variables included age, race and ethnicity, gender, and outcomes including length of stay, intensive care admission, mechanical ventilation, and death. Bivariate analysis was conducted using the chi-square, Fisher’s exact, or Wilcoxon rank-sum test for categorical and continuous variables as appropriate (P <0.05).

**Supplementary Table S1: Characteristics of Hospitalized Children with Community-acquired Pneumonia: Comparison of Eligible Children who were not Enrolled, Enrolled Children, and Children with Radiographic Confirmation of Pneumonia**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Characteristic | Eligible children who were not enrolled (n=1153)\* | Enrolled children who did not withdraw (n=2628) | Children with radiographic pneumonia(n=2358) | Children with radiographic pneumonia during incidence period(n=2012) |
| Female sex – no. (%) | 542 (47) | 1172 (45) |  1067 (45) | 909 (45) |
| Race and ethnicity – no. (%) Non-Hispanic white Non-Hispanic black Hispanic Other | 507 (44)438 (38)104 (9)†104 (9) | 1033 (39)896 (34)499 (19)200 (8) | 939 (40)781 (33)452 (19)186 (8) | 802 (40)690 (34)368 (18)152 (8) |
| Age groups – no. (%) 0-23 months 2-4 years 5-9 years 10-17 years | 471 (41)380 (33)184 (16)104 (9) | 1207 (46)642 (24)468 (18)311 (12) | 1055 (45)595 (25)422 (18)286 (12) | 871 (43)512 (25)378 (19)251 (12) |
| Illness onset to presentation – median days, (interquartile range, IQR) | NA‡ | 3 (1-6) | 3 (2-6) | 3 (2-6) |
| Symptoms – no. (%) Cough Fever/feverish Anorexia Dyspnea | NA | 2481 (94)2391 (91)1971 (75)1854 (71) | 2230 (94)2155 (91)1766 (75)1657 (70) | 1912 (95)1849 (92)1534 (76)1465 (73) |
| Any underlying condition§ – no. (%) Asthma Pre-term birth among children <2 yrs. Neurological disorder Congenital heart disease Chromosomal disorder | NA | 1338 (51)877 (33)251/1207 (21)216 (8)182 (7)144 (6) | 1197 (51)779 (33)218/1055 (21)199 (8)172 (7)138 (6) | 1028 (51)670 (33)177/871 (20)174 (9)150 (8)124 (6) |
| Hospital indicators – no. (%) Length of stay – median days, IQR Intensive care unit admission Mechanical ventilation Death | 2 (1-4)† 219 (19)69 (6)3 (<1) | 3 (2-4)538 (21)177 (7)4 (<1) | 3 (2-5)497 (21)166 (7)3 (<1) | 3 (2-5)447 (22)146 (7)3 (<1) |

\*Data was not available on 22 patients

† P<0.05 when comparing eligible non-enrolled children with those who were enrolled

‡ NA, data not available

§ Any underlying medical conditions included asthma, chromosomal disorders including Down syndrome, chronic kidney disease, chronic liver disease, congenital heart disease, diabetes mellitus, immunosuppression (either due to chronic condition or medication, malignancy [but not skin cancer], human immunodeficiency virus infection with CD4 count >200 cells/mm3), neurological disorders (including seizure disorder, cerebral palsy, scoliosis), pre-term birth (defined as gestational age <37 weeks at birth for those children who were <2 years old at time of hospitalization), and splenectomy. Only specific conditions that were prevalent in >5% of children are listed in the table.

**Supplementary Table S2A/B: Pathogen Detection among Hospitalized Children with Community-acquired Pneumonia by Sample Type\***

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen detected (total detected) | NP/OP PCR(n=2179) | Serology(n=1022) | More than one modality(n=2222) |
| Respiratory syncytial virus (n=622) | 593 | 195 | 166 |
| Human rhinovirus (n=606) | 606 | NA† | NA |
| Human metapneumovirus (n=285) | 252 | 108 | 75 |
| Adenovirus (n=248) | 222 | 42 | 16 |
| *M. pneumoniae* (n=178) | 178 | NA | NA |
| Parainfluenza viruses 1-3 (n=151) | 117 | 65 | 31 |
| Influenza A/B (n=149) | 88 | 84/961 | 23 |
| Coronaviruses (n=110) | 110 | NA | NA |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Pathogen detected (total detected) | Blood culture (n=2097) | Whole blood PCR (n=2031) | Pleural fluid culture (n=79) | Pleural fluid PCR (n=59) | ET aspirate (n=20) | BAL culture (n=23) | More than one modality |
| *S. pneumoniae* (n=79) | 22 | 50 | 9 | 24 | 1 | 2 | 23 |

**\***Among those with at least one specimen type available for both bacterial and viral testing.

†NA = not applicable

**Supplementary Table S3: Pathogen Detection among Hospitalized Children with Community-acquired Pneumonia by Age Group\***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Pathogen detected | <2 years (n=980) | 2-4 years (n=559) | 5-9 years (n=408) | 10-17 years (n=275) | All ages (n=2222) |
| Any detection | 88% | 84% | 72% | 66% | 81% |
| Any co-detection | 34% | 24% | 16% | 14% | 26% |
| Respiratory syncytial virus | 42% | 29% | 8% | 7% | 28% |
| Human rhinovirus | 29% | 25% | 30% | 19% | 27% |
| Human metapneumovirus | 14% | 17% | 10% | 4% | 13% |
| Adenovirus | 18% | 9% | 4% | 2% | 11% |
| *M. pneumoniae* | 2% | 5% | 16% | 23% | 8% |
| Parainfluenza viruses 1-3 | 7% | 8% | 6% | 4% | 7% |
| Influenza A/B | 6% | 5% | 9% | 11% | 7% |
| Coronaviruses | 6% | 6% | 3% | 4% | 5% |
| *S. pneumoniae* | 3% | 4% | 4% | 3% | 4% |

**\***Among those with at least one specimen available for bacterial and viral testing.

**Supplementary Table S4: Bacterial Pathogen Detection and Timing of Inpatient and OutpatientAntibiotics\* among Hospitalized Children with Community-acquired Pneumonia with Available Sample Collection Time**

|  |  |  |
| --- | --- | --- |
| Sample Type  | Positive detectionbefore antibiotics | Positive detection after antibiotics |
| Blood Culture – No./Total (%)  | 40/1426 (3) | 12/668 (2) |
| Whole Blood PCR –No./Total (%) | 15/817 (2) | 40/1197 (3) |
| Pleural Fluid Culture –No./Total (%) | 5/7 (71) | 29/78 (37) |
| Pleural Fluid PCR –No./Total (%) | 5/6 (83) | 45/57 (79) |
| NP/OP swab PCR –No./Total (%) | 34/620 (5) | 148/1584 (9) |

**\***Inpatient antibiotics based on medical chart and outpatient antibiotics based on self-report and limited to ≤5 days before hospitalization

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