Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

Title: Community-acquired Pneumonia Requiring Hospitalization among U.S. Adults

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

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Supplementary Methods

Study design, rationale, and overall conduct

The EPIC study was a competitive research cooperative agreement announced by the Centers for Disease Control and Prevention (CDC) in 2009. The study was conducted for 2.5 years based on the availability of research funds. Final sites were selected based on an objective review of site capability to conduct the study. The primary objective of the study was to determine the incidence and etiology of community-acquired pneumonia requiring hospitalization among U.S. adults. Several factors were assessed by the evaluation of external referees and while geographic representation was one of them, capacity to enroll a sufficient number of patients, collect and test specimens, and provide denominator data for incidence calculations were deemed most important to the success of the study.

The final study hospitals chosen to participate in the study represent a mix of hospital types. Northwestern Memorial Hospital, Rush University Medical Center, and Vanderbilt University Medical Center are all academic medical centers. John H. Stroger, Jr. Hospital of Cook County is a public urban teaching hospital and University of Tennessee Health Science Center/Saint Thomas Health is not-for-profit teaching community hospital.

The EPIC study was designed as a prospective, multicenter, population-based, active surveillance study. Together, CDC and site Principal Investigators wrote the study protocol, created the data collection instruments, and developed standard operating procedures. Annual site visits and investigator meetings took place from 2009 through 2013. Investigator and study

coordinator calls were held weekly to monitor study conduct (e.g. enrollment, laboratory procedures, etc.), troubleshoot issues, and maintain standardization among sites.

The initial design did not include control enrollment. However, after one complete season of case enrollment, CDC and site Principal Investigators, felt that because the EPIC study used real-time polymerase chain reaction (PCR) for the detection of respiratory viruses, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* from naso/oropharyngeal (NP/OP) swabs, it would be useful to enroll asymptomatic controls to help understand the significance of molecular detection of pathogens, especially human rhinovirus, which can be shed for >2weeks after an infection.[1] Thus, the inclusion of controls was intended to identify the background prevalence of these respiratory pathogens in the upper airway among asymptomatic adults and compare it with the prevalence of respiratory pathogens in enrolled adults hospitalized with pneumonia. With the resources available, only one adult study hospital was able to enroll a convenience sample of adults without pneumonia who presented for non-acute care in the general medicine clinic. While the emergency department (ED) was explored as a setting for enrollment, it was not feasible to enroll asymptomatic individuals from the ED, and thus we opted for a primary care clinic. While we present the overall proportion of detections in controls, our pathogen-specific incidence estimates were not adjusted based on detection in controls.

Detailed inclusion and exclusion criteria definitions

The Methods section in the manuscript outlines 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 (wbc >11,000/mm^3) or leukopenia (wbc <3000/mm^3).[2] Tachypnea was defined as respiratory rate >25 breaths/minute.

Adults who were immunocompetent and had recent hospitalization <28 days were excluded. Adults with immunosuppression and recent hospitalization <90 days were excluded; immunosuppression was defined as solid organ or hematopoietic stem cell transplant, cancer on chemotherapy, or steroid use (prednisone equivalent of 20 mg/day) for >30 days.

In addition to recent hospitalization, we excluded adults who had been enrolled in the EPIC study within the previous 28 days, or were functionally-dependent nursing home residents, or if they had a clear alternative diagnosis. Functional status of nursing home patients was assessed at time of screening using the Activities of Daily Living (ADL) Scale;[3] adults with score >7 were not considered independent and thus excluded. Examples of clear alternative diagnoses include admission for foreign body aspiration or known drug overdose. Lastly, we also excluded the following patients with specific conditions, either because they were considered to be severely immunosuppressed or they had a condition that is a known risk factor for non-community-acquired respiratory pathogens: tracheotomy, percutaneous endoscopic gastrostomy tube, cystic fibrosis, cancer with neutropenia, solid organ or hematopoietic stem cell transplant ≤90 days earlier, active graft versus host disease or bronchiolitis obliterans, or human immunodeficiency virus infection with CD4 cell count <200 cells/mm^3.[4] Neutropenia was defined as an absolute neutrophil count <500/mm^3.

Data collection and cleaning

Study staff at each site was extensively trained in screening, interviewing, and chart review prior to study initiation. CDC conducted annual site visits to each study site and performed yearly random audits to evaluate data quality and consistency of data collection from site to site.

Adults 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, and underlying medical conditions.

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.

Once all data was entered into the centralized database, systematic data checks were performed to detect outliers and errors, which were corrected to create a final dataset.

Vaccination verification

Vaccination data for 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, processing, and testing

Blood 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 most other specimens which were usually obtained for research purposes [urine, serology, sputum, and NP/OP swabs] and therefore after informed consent; in some cases, urine and sputum were also collected for clinical care but this was not standard at all hospitals. Blood for serology was collected in a Vacutainer tube and immediately stored at 4°C for 1-18 hours after collection before being centrifuged, aliquoted, and stored in Sarstedt tubes at -20°C. Urine was collected in a standard sterile specimen cup and refrigerated at 2-8°C. BinaxNOW urine antigen testing for *Legionella pneumophila* and *Streptococcus pneumoniae* occurred on site following the manufacturers recommendations.[5,6] Urine was refrigerated at 4°C for up to 48 hours after collection, and then aliquoted, and stored at -70°C.

NP/OP specimens were collected using sterile flocked Dacron (NP) or 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. PCR assays were performed on total nucleic acid extracted from NP/OP swabs at each site using CDC developed methods;[7-9] quality assurance and monitoring protocols maintained procedural standardization among sites.[10,11] For all NP/OP PCR assays, a cycle threshold value <40 was considered positive.

When available, sputum, endotracheal aspirates (ETA), bronchoalveolar-lavage (BAL) specimens, and pleural fluid (PF) were collected into sterile containers; gram stain and bacterial culture were performed on site per routine clinical care. Only bacterial culture from sputum and ETA of high quality (≤10 epithelial cells/low power field [lpf] and ≥25 white blood cells/lpf) were included.[12] However, legionella PCR results from a sputum specimen of any quality were included.[5,7] BAL specimens with significant growth of potential pathogens by semi-quantified (>3+ growth, moderate to heavy growth) or quantified ($>10^3$ colony forming units/ml) methods were included.[4,12]

After routine testing, PF was refrigerated at 4°C for up to 48 hours, aliquoted, and stored at -70°C. Frozen samples were then sent to CDC and once tested at CDC, were sent on dry ice to the University of Utah. PF PCR targeting *S. pneumoniae* (*lyt-A*) and *Streptococcus pyogenes* (*spy*) genes was performed at CDC.[13] PF specimens were then tested at the University of Utah for bacterial pathogens using the following PCR methods: 1) bench-top nested PCR assays[14] (*Haemophilus influenzae* [*rpoB, bexA*]), *Staphylococcus aureus* [*nuc, mecA*], *S. pneumoniae* [*lytA*], *S. pyogenes* [*speB*], and 2) FilmArray® blood culture panel[15] (*Acinetobacter baumanii, Escherichia coli, Fusobacterium, H. influenzae, Klebsiella pneumoniae*, pseudomonas, *S. aureus, S. pneumoniae, S. pyogenes,* viridans streptococci).

Frozen specimens (sera, NP/OP swabs, PF, urine, sputa), including bacterial isolates, were transported on dry ice to CDC for testing, quality control, and long-term storage at -70°C.

Laboratory quality control and validation of real-time PCR

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 completed an external quality assessment program provided by Quality Control for Molecular Diagnostics (QCMD) (http://www.qcmd.org/)[10,11] 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 and sputa 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, Neisseria 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 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.

For high-quality sputum bacterial cultures, the following were not considered clinically associated with community-acquired pneumonia: *Aspergillus* species, *Enterococcus* species, viridans group streptococci, and yeast.

Fungal and mycobacterial detections

Mycobacterium tuberculosis was considered a pathogen if detected in any acid-fast bacilli (AFB) sputum specimen. Nontuberculous mycobacterial (NTM) disease was assessed based on American Thoracic Society/Infectious Diseases Society of America guidelines;[16] NTM disease was included if there was a positive AFB culture with a NTM pathogen from at least two separate expectorated sputum samples (of any quality) or one positive culture from a BAL in the setting of radiographic findings consistent with pneumonia in an adult.[16] Fungal pathogens were determined per clinical guidelines.[17]

Influenza and other respiratory virus serology methods

All serology was performed at CDC on available paired acute-phase (obtained at enrollment) and convalescent-phase (obtained 3-10 weeks after enrollment) serum specimens. Serology for non-influenza respiratory viruses (adenovirus, human metapneumovirus, respiratory syncytial virus, and parainfluenza viruses 1,2,3 [PIV]) was performed using CDC developed indirect IgG enzyme immunassays.[18,19] A ≥4-fold rise in IgG antibody titer between the paired acute-phase and convalescent-phase serum specimens was considered evidence of current or recent infection. Due to antigenic cross-reactivity among PIV 1-3, 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 signal to the uninfected control cells or 2) differences in antibody levels identified among 4 or more virus assays indicating possible mismatches in the 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.[20] Influenza serology was performed on paired sera for the following strains circulating during 2010-2012: A/California/07/2009 (H1N1), A/Perth/16/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/4 (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 the non-specific agglutinins as needed. Two-fold dilutions of sera with a starting dilution of 1:10 were incubated with a standard amount of virus (4HAU/25 μL).[20] 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 the assay sensitivity.[21,22] 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) 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 the influenza B viruses. 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-phase and convalescent-phase 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.[23] If influenza serology results indicated seroconversion when vaccine was administered within 2 weeks (based on self/caregiver report or vaccine verification) before acute-phase serum collection, or between acute-phase and convalescent-phase 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 both HI and MN titer) virus with a convalescent titer ≥40 in adults who did not receive influenza vaccine or received vaccine more than 2 weeks prior to acute-phase serum collection and not between acute-phase and convalescent-phase sample collection.

Radiographic definitions and process

For enrollment into the study, adults were required to have chest radiography consistent with pneumonia within 48 hours of admission. The initial interpretation of the admission chest radiograph was made by clinicians providing care for the patient including but not limited to a resident, general medicine or sub-specialty attending, emergency room physician, or radiologist on call. The initial radiographic enrollment criteria were purposefully sensitive to avoid missing

any possible cases of pneumonia. However, it is known that there is variability in the clinical interpretation of chest radiography among clinicians and depending on their level of training in radiology.[24] To minimize these biases and increase the specificity of the case definition, independent confirmation by one board certified chest radiologist (study radiologists FC, EH) at each site who was blinded to demographic and clinical information was required for final inclusion in the study. Available chest radiographs prior to the current hospitalization were used for comparison. Each of the study radiologists used the same form for recording their interpretation. Radiographic pneumonia was defined as consolidation (a dense or fluffy opacity with or without air bronchograms), infiltrate (linear and patchy alveolar or interstitial densities), or pleural effusion.[24-26] Edema was defined as manifestation by Kerley A and B lines due to fluid in the interlobular space and was not included in the definition of infiltrate. Upon review of a 10% random sample of radiographs, inter-rater percent agreement between the two study radiologists was 86% (CI 81-89%).

The study design did assume that the treating clinicians radiographic read was sensitive and that the study radiologist read was more specific and thus, the study radiologist was screening out false-positives. This allows for the possibility that we missed radiographic cases that were not detected by the treating clinicians; however, there were not ample resources to review all radiographs initially deemed to be negative.

Counties under surveillance and incidence calculation inputs

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

1) Chicago site: John H. Stroger, Jr. Hospital of Cook County, Northwestern Memorial Hospital, Rush University Medical Center (all in Chicago, IL) – Illinois: Cook county

2) Nashville site: University of Tennessee Health Science Center/Saint Thomas Health, Vanderbilt University Medical Center (both in Nashville, TN) - Tennessee: Cheatham, Davidson, Dickson, Montgomery, Robertson, Rutherford, Sumner, Williamson, and Wilson counties

The source of market share data varied between 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 include dates of admission and discharge, discharge diagnoses, and patient date of birth along with gender, race, and ethnicity, among other variables. IL market share for the study hospitals was obtained through an IL Hospital Association CompData sharing agreement with the EPIC investigators for Cook County residents admitted to any IL hospital. 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 and were collected by age group.[27]

Enrollment city	Population* under surveillance, 2010	Population* under surveillance, 2011	Population* under surveillance, 2012	Percent market share, year 1†	Percent market share, year 2†
Chicago	3,969,474	3,992,029	4,015,945	8.7%	9.6%
Nashville	1,264,326	1,286,082	1,316,560	18.1%	18.7%

*****Population of adults ≥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 adults 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 ($P < 0.05$).

Supplemental Results

Supplemental Table S1: Characteristics of Hospitalized Adults with Community-acquired Pneumonia: Comparison of All Eligible Adults, All Enrolled Adults, and Adults with Radiographic Confirmation of Pneumonia

Characteristic	Eligible adults who were not enrolled[*] (n=943)	Enrolled adults who did not withdraw (n=2481)	Adults with radiographic pneumonia (n=2320)	Adults with radiographic pneumonia during incidence period[†] (n=2061)
Female sex – no. (%)	498 (53)	1276 (51)	1190 (51)	1070 (52)
Race and ethnicity – no. (%)				
Non-Hispanic white	443/922 (48)	1177 (47)	1086 (47)	971 (47)
Non-Hispanic black	329/922 (36)	955 (39)	898 (39)	789 (38)
Hispanic	99/922 (11)	254 (10)	243 (10)	216 (10)
Other	51/922 (6)	95 (4)	93 (4)	85 (4)
Age groups – no. (%)				
18-49 years	193 (20)[‡]	740 (30)	701 (30)	621 (30)

50-64 years	249 (26)‡	836 (34)	787 (34)	699 (34)
65-79 years	259 (27)‡	560 (23)	517 (22)	458 (22)
≥ 80 years	242 (26)‡	345 (14)	315 (14)	283 (14)
Illness onset to presentation – median days, (interquartile range, IQR)	NA§	4 (2-7) (n=2478)	4 (2-7) (n=2317)	4 (2-8) (n=2058)
Symptoms – no. (%)	NA			
Cough		2169 (87)	2029 (88)	1808 (88)
Dyspnea		1942 (78)	1808 (78)	1629 (79)
Fever/feverish		1677 (68)	1572 (68)	1406 (68)
Any underlying condition¶ – no. (%)	NA	1959 (79)	1817 (78)	1614 (78)
Chronic lung disease		1055 (43)	968 (42)	873 (42)
Chronic heart disease		882 (36)	810 (35)	708 (34)
Immunosuppression		739 (30)	685 (30)	606 (29)
Diabetes mellitus		656 (26)	597 (26)	532 (26)
Hospital indicators – no. (%)				
Length of stay – median days, IQR	3 (2-7)‡	3 (2-6)	3 (2-6)	3 (2-6)

CURB-65[‖] – median, IQR	NA	1 (0-2)	1 (0-2)	1 (0-2)
0-1 points (low risk)		1715 (69)	1614 (70)	1431 (69)
2 points (moderate risk)		491 (20)	444 (19)	401 (20)
3-5 points (high risk)		275 (11)	262 (11)	229 (11)
Pneumonia severity index[**] – median, IQR	NA	76 (52-103)	76 (52-103)	75 (52-102)
Class 1-3 (low risk)		1608 (65)	1510 (65)	1345 (65)
Class 4 (moderate risk)		652 (26)	606 (26)	534 (26)
Class 5 (high risk)		221 (9)	204 (9)	182 (9)
Intensive care unit admission	80/339 (24)	528 (21)	498 (21)	445 (22)
Invasive Mechanical ventilation	94 (10)[‡]	135 (5)	131 (6)	116 (6)
Death	51 (5)[‡]	53 (2)	52 (2)	44 (2)

[*]Data were available from 4 of 5 sites and includes 943/1146 (82%) non-enrolled but eligible patients. For ICU admission proportions, data were available for 2 of 5 sites and include 339/1146 (30%) non-enrolled but eligible patients.

[†]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)

[‡]P<0.001 when comparing eligible non-enrolled adults with those who were enrolled

[§]NA, data not available

[¶]Any underlying medical condition included chronic lung disease (asthma, chronic obstructive pulmonary disease, obstructive sleep apnea), chronic heart disease (i.e. coronary artery disease, congestive heart failure, but not hypertension), immunosuppression (either due to chronic condition or medication, malignancy [but not skin cancer], human immunodeficiency virus infection with CD4 count >200 cells/mm^3), diabetes mellitus, chronic kidney disease (with or without dialysis), neurological disorders (epilepsy, cerebral palsy, dementia, history of stroke), chronic liver disease (hepatitis, cirrhosis, hepatic failure), and splenectomy.

[‖]CURB-65 is a clinical prediction rule for community-acquired pneumonia mortality and is determined by presence of new onset confusion, urea >19 mg/dL, respiratory rate ≥30, systolic blood pressure <90 mmHg or diastolic blood pressure <60 mmHg, and age ≥65 years old; one point is allotted for presence of each factor for total of five.[28]

[**]Pneumonia severity index is a clinical prediction rule for community-acquired pneumonia related mortality based on gender, age, nursing home status, mental status, heart rate, respiratory rate, blood pressure, temperature, select underlying medical conditions, select laboratory values, and presence of pleural effusion.[29]

Supplemental Table S2: Bacterial Pathogen Detection and Timing of Inpatient and Outpatient Antibiotics* among Hospitalized Adults with Community-acquired Pneumonia with Available Specimen Collection Time

Specimen Type	Proportion collected before antibiotics	Positive detection before antibiotics	Positive detection after antibiotics	P value[†]
Blood Culture[‡] – No./Total (%)	1607/2069 (78)	106/1607 (7)	13/462 (3)	0.002
Urine antigen (*S. pneumoniae*) – No/Total (%)	221/1941 (11)	10/221 (5)	73/1720 (4)	0.86
Urine antigen (*L. pneumophila*) – No/Total (%)	226/1935 (12)	5/226 (2)	26/1709 (2)	0.44
Sputum Culture[§]– No/Total (%)	22/271 (8)	5/22 (23)	27/249 (11)	0.10
Pleural Fluid Culture – No./Total (%)	2/75 (3)	0/2 (0)	13/73 (18)	1.00
Pleural Fluid PCR – No./Total (%)	1/35 (3)	0/1 (0)	7/34 (21)	1.00

NP/OP swab PCR *(C. pneumonaie/ M. pneumoniae)* – No./Total (%)	245/2266 (11)	9/245 (4)	43/2021 (2)	0.13

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

[†]P value reflects comparisons between positive detections before and after antibiotics

[‡]Enterobacteriaceae were more frequently detected in blood cultures collected before antibiotics (2% vs. 0%, P=0.005). There were no significant differences in detection of *S. pneumoniae*, *S. pyogenes*, *S. aureus*, or *H. influenzae* in blood cultures collected before and after antibiotics.

[§]Based on high-quality criteria of ≤10 epithelial cells/lpf and ≥25 white blood cells/lpf

Supplementary Table S3: Number of Specific Pathogen Co-detection Combinations among Hospitalized Adults with Community-acquired Pneumonia*

Pathogen Detected†	HRV	Flu	S. pn.	HMPV	RSV	PIV	CoV	M. pn.	S. au.	AdV.	Leg.	Entero.	Other‡
HRV	161	6	14	2	2	2	6	2	0	1	1	0	2
Flu	6	108	4	1	0	3	1	1	3	1	0	0	2
S. pn.	14	4	80	7	2	7	2	0	0	0	1	0	1
HMPV	2	1	7	67	4	4	0	1	1	2	0	0	2
RSV	2	0	2	4	55	3	1	1	1	0	0	1	1
PIV	2	3	7	4	3	41	1	1	2	3	0	0	3
CoV	6	1	2	0	1	1	38	0	1	1	2	0	1
M. pn.	2	1	0	1	1	1	0	37	1	0	0	0	0
S. au.	0	3	0	1	1	2	1	1	24	0	0	3	2
AdV.	1	1	0	2	0	3	1	0	0	22	0	0	2
Leg.	1	0	1	0	0	0	2	0	0	0	28	0	0
Entero.	0	0	0	0	1	0	0	0	3	0	0	25	2
Other‡	2	2	1	2	1	3	1	0	2	2	0	2	57

*Among the 2259 with at least one specimen available for bacterial and viral testing.

†Human rhinovirus (HRV), influenza A/B (Flu), *S. pneumoniae* (S. pn.) , human metapneumovirus (HMPV), respiratory syncytial virus (RSV), parainfluenza viruses 1-3 (PIV), coronaviruses (CoV), *M. pneumoniae* (M. pn.), *S. aureus* (S. au.), adenovirus (AdV), *L. pneumophila* (Leg.), Enterobacteriaceae (Entero.)

‡76 other detections in 74 patients (12 *H. influenzae*, 9 *C. pneumoniae*, 8 *M. tuberculosis*, 8 pseudomonas, 7 *S. pyogenes*, 7 viridans group streptococci, 7 other Streptococcus spp., 4 nontuberculous mycobacterial species, 3 *Fusobacterium*, 3 *Pneumocystis jirovecii*, 1 each of bacteroides, coccidioides, histoplasma, pasteurella,1 *H. influenzae/Neisseria Meningitidis*,1 viridans group streptococci/other Streptococcus spp.).

Supplementary Table S4A/B: Number of Specific Pathogens Detected among Hospitalized Adults with Community-acquired Pneumonia by Sample Type*

Pathogen detected (total detected)	NP/OP PCR only (n=2247)	Serology only (n=854)	Both NP/OP PCR and serology (n=2259)
Human rhinovirus (n=194)	194	NA†	NA
Influenza A/B (n=132)	91	22/846	19
Human metapneumovirus (n=88)	63	9	16
Respiratory syncytial virus (n=68)	46	10	12
Parainfluenza viruses 1-3 (n=67)	45	11	11
Coronaviruses (n=53)	53	NA	NA
M. pneumoniae (n=43)	43	NA	NA
Adenovirus (n=32)	20	8	4

Pathogen detected (total detected)	Blood culture only (n=2070)	Urine antigen only (n=1923)	Sputum culture or PCR only	BAL culture only (n=83)	Pleural fluid culture only (n=71)	Pleural fluid PCR only (n=35)	More than one modality‡ (n=2259)
S. pneumoniae (n=115)	26	56§	8/267 high-quality by culture	3	1	0	21

S. aureus (n=37)	20	NA	10/267 high-quality by culture	4	0	0	3
L. pneumophila (n=32)	NA	25/1912	1/953 any quality by PCR	NA	NA	NA	6

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

†NA = not applicable

‡Detection by more than one modality: Among 21 *S. pneumoniae* detections by more than one modality, 16 were by blood culture and urine antigen, 4 by high-quality sputum culture and urine antigen, and 1 by pleural fluid PCR and urine antigen. Among 3 *S. aureus* detections by more than one modality, 1 was by high-quality sputum culture and pleural fluid culture, 1 by pleural fluid culture, pleural fluid PCR, and BAL culture, and 1 by blood culture and BAL culture. Among 6 *Legionella* detections by more than one modality, 6 were by sputum PCR and urine antigen.

§Two patients may be false-positive based on timing of pneumococcal vaccination and urine collection.[6]

Supplemental Table S5: Pathogen Detection among Hospitalized Adults with Community-acquired Pneumonia by Age Group*

Pathogen detected	18-49 years (n=681)	50-64 years (n=773)	65-79 years (n=506)	≥ 80 years (n=299)	All ages (n=2259)
Any detection	41%	36%	39%	34%	38%
Any co-detection†	5%	5%	4%	3%	5%
Human rhinovirus	11%	7%	8%	9%	9%
Influenza A/B	7%	6%	4%	5%	6%
S. pneumoniae	5%	5%	7%	3%	5%
Human metapneumovirus	4%	3%	5%	2%	4%
Respiratory syncytial virus	2%	3%	4%	4%	3%
Parainfluenza viruses 1-3	2%	4%	2%	4%	2%
Coronaviruses	2%	2%	3%	3%	2%
M. pneumoniae	4%	1%	1%	1%	2%
S. aureus	1%	2%	1%	1%	2%
Adenovirus	1%	2%	1%	1%	1%
L. pneumophila	1%	2%	1%	1%	1%
Enterobacteriaceae	1%	1%	2%	2%	1%

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

†Co-detection is defined as any combination of bacteria, virus, fungal, or mycobacterial detection

Supplemental Table S6: Pathogen Detection among Hospitalized Adults with Community-acquired Pneumonia by Pneumonia Severity Index (PSI) and Intensive Care Unit (ICU) Admission*

Pathogen detected	PSI I-III (n=1475)	PSI IV-V (n=784)	P value†	Non-ICU (n=1777)	ICU (n=482)	P value‡
Any detection	38%	37%	0.65	36%	45%	<0.001
Any co-detection§	5%	5%	0.66	4%	7%	0.003
Viral detection only	25%	21%	0.03	24%	22%	0.27
Bacterial detection only	10%	13%	0.006	9%	19%	<0.001
Bacterial-viral co-detection	3%	3%	0.90	2%	4%	0.04
Human rhinovirus	9%	7%	0.07	9%	8%	0.42
Influenza A/B	7%	4%	0.02	6%	6%	0.97
S. pneumoniae	4%	7%	0.02	4%	8%	<0.001
Human metapneumovirus	4%	3%	0.30	4%	3%	0.32
Respiratory syncytial virus	3%	4%	0.26	3%	4%	0.18
Parainfluenza viruses 1-3	3%	3%	0.95	3%	4%	0.04
Coronaviruses	2%	3%	0.64	2%	3%	0.57

M. pneumoniae	3%	1%	0.001	2%	1%	0.06
S. aureus	1%	3%	0.001	1%	5%	<0.001
Adenovirus	1%	1%	0.68	2%	<1%	0.05
L. pneumophila	2%	1%	0.06	2%	1%	0.43
Enterobacteriaceae	1%	2%	0.002	1%	3%	<0.001

*Among the 2259 with at least one specimen available for bacterial and viral testing

†P value comparing proportion of each detection between PSI I-III and PSI IV-V

‡P value comparing proportion of each detection between non-ICU and ICU admission

§Co-detection is defined as any combination of bacteria, virus, fungal, or mycobacterial detection

Supplementary Table S7: Comparison of Adults with Radiographic Pneumonia with Enrolled Controls*

Characteristic	All adults with radiographic pneumonia (n=2320)	Adults with radiographic pneumonia in Nashville during period of control enrollment (n=192)	Asymptomatic adult controls† (n=238)	All adult controls‡ (n=262)
Female	1190 (51)	114 (59)	129 (54)	143 (55)
Race and ethnicity				
Non-Hispanic white	1086 (47)	129 (67)	177 (74)	194 (74)
Non-Hispanic black	898 (39)	54 (28)	48 (20)	53 (20)
Hispanic	243 (10)	7 (4)	5 (2)	6 (2)
Other	93 (4)	2 (1)	8 (3)	9 (3)
Age median (IQR)	57 (46-71)	59 (50-72)	54 (41-65)	54 (40-64)
Age group				
18-49 years	701 (30)	43 (22)	97 (41)	110 (42)
50-64 years	787 (34)	75 (39)	80 (34)	88 (34)
65-79 years	517 (22)	47 (24)	54 (23)	56 (21)
≥ 80 years	315 (14)	27 (14)	7 (3)	8 (3)
Select underlying condition§ – no. (%)				

Chronic lung disease	799 (34)	87 (45)	32 (13)	36 (14)
Diabetes mellitis	545 (24)	55 (29)	42 (18)	46 (18)
Chronic heart disease	685 (30)	46 (24)	23 (10)	25 (10)
Immunosuppression	551 (24)	50 (26)	23 (10)	27 (10)

*Controls were only enrolled Vanderbilt University Medical Center from November 1, 2011-June 30, 2012

†There were only 5 detections among the asymptomatic controls and included: 2 human rhinovirus, 2 coronaviruses, and 1 human metapneumovirus

‡All adult controls include controls that subsequently developed symptoms within 14 days after enrollment

§Because controls were outpatients, underlying conditions was only assessed by patient interview for a limited number of conditions. Thus the underlying conditions depicted here are based on only patient interview for both controls and adults with radiographic pneumonia.

Supplementary Table S8: Comparison of Adults with Radiographic Pneumonia in the Study Cities

Characteristic	Chicago (n=1538)	Nashville (n=782)	P value
Female sex – no. (%)	776 (51)	414 (47)	0.26
Race and ethnicity – no. (%)			<0.001
Non-Hispanic white	580 (38)	506 (65)	
Non-Hispanic black	655 (43)	243 (31)	
Hispanic	224 (15)	19 (2)	
Other	79 (5)	14 (2)	
Age groups – no. (%)			0.02
18-49 years	487 (32)	214 (27)	
50-64 years	528 (34)	259 (33)	
65-79 years	315 (21)	202 (26)	
≥ 80 years	208 (14)	107 (14)	
Hospital indicators – no. (%)			
Length of stay – median days, IQR	3 (2-6)	3 (2-6)	0.87
CURB-65 – median, IQR	1 (0-2)	1 (0-2)	0.10
0-1 points (low risk)	1063 (71)	513 (68)	0.43
2 points (moderate risk)	284 (19)	147 (20)	
3-5 points (high risk)	160 (11)	92 (12)	
PSI – median, IQR	74 (51-102)	78 (54-105)	0.42
Class 1-3 (low risk)	995 (66)	480 (64)	0.49

Class 4 (moderate risk)	380 (25)	207 (28)	
Class 5 (high risk)	132 (9)	65 (9)	
ICU admission	327 (22)	155 (21)	0.55
Mechanical ventilation	88 (6)	38 (5)	0.48
Death	29 (2)	20 (3)	0.26
Pathogens Detected			
Any detection	559 (37)	294 (39)	0.36
Any co-detection[§]	84 (6)	26 (3)	0.03
Viral only	339 (23)	191 (25)	0.13
Bacterial only	158 (11)	89 (12)	0.33
Viral-Bacterial	46 (3)	13 (2)	0.06
Human rhinovirus	124 (8)	70 (9)	0.39
Influenza A/B	92 (6)	40 (5)	0.45
S. pneumoniae	72 (5)	43 (6)	0.34
Human metapneumovirus	60 (4)	28 (4)	0.77
Respiratory syncytial virus	44 (3)	24 (3)	0.72
Parainfluenza viruses 1-3	44 (3)	23 (3)	0.86
Coronaviruses	34 (2)	19 (3)	0.69
M. pneumoniae	33 (2)	10 (1)	0.16
S. aureus	27 (2)	10 (1)	0.42
Adenovirus	18 (1)	14 (2)	0.21
L. pneumophila	20 (1)	12 (2)	0.61
Enterobacteriaceae	24 (2)	7 (1)	0.20

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