MAJOR ARTICLE

Bacterial Complications of Respiratory Tract Viral Illness: A Comprehensive Evaluation

Ann R. Falsey,[1,3] Kenneth L. Becker,[4,5] Andrew J. Swinburne,[3] Eric S. Nylen,[4,5] Maria A. Formica,[3] Patricia A. Hennessey,[3] Mary M. Criddle,[3] Derick R. Peterson,[2] Andrea Baran,[2] and Edward E. Walsh[1,3]

[1]Department of Medicine and [2]Biostatistics and Computational Biology, University of Rochester, and [3]Rochester General Hospital, New York; and [4]Veterans Affairs Medical Center and [5]George Washington University, Washington, D.C.

Background. Respiratory tract infection is one of the most common reasons for hospitalization among adults, and recent evidence suggests that many of these illnesses are associated with viruses. Although bacterial infection is known to complicate viral infections, the frequency and impact of mixed viral-bacterial infections has not been well studied.

Methods. Adults hospitalized with respiratory illness during 3 winters underwent comprehensive viral and bacterial testing. This assessment was augmented by measuring the serum level of procalcitonin (PCT) as a marker of bacterial infection. Mixed viral-bacterial infection was defined as a positive viral test result plus a positive bacterial assay result or a serum PCT level of $\geq$ 0.25 ng/mL on admission or day 2 of hospitalization.

Results. Of 842 hospitalizations (771 patients) evaluated, 348 (41%) had evidence of viral infection. A total of 212 hospitalizations (61%) involved patients with viral infection alone. Of the remaining 136 hospitalizations (39%) involving viral infection, results of bacterial tests were positive in 64 (18%), and PCT analysis identified bacterial infection in an additional 72 (21%). Subjects hospitalized with mixed viral-bacterial infections were older and more commonly received a diagnosis of pneumonia. Over 90% of hospitalizations in both groups involved subjects who received antibiotics. Notably, 4 of 10 deaths among subjects hospitalized with viral infection alone were secondary to complications of *Clostridium difficile* colitis.

Conclusions. Bacterial coinfection is associated with approximately 40% of viral respiratory tract infections requiring hospitalization. Patients with positive results of viral tests should be carefully evaluated for concomitant bacterial infection. Early empirical antibiotic therapy for patients with an unstable condition is appropriate but is not without risk.

Keywords. virus; bacterial infection; procalcitonin; pneumonia.

Respiratory tract infections are one of the most common reasons for hospital admission among adults. Recent evidence indicates that a significant proportion of these illnesses are associated with viruses [1–3]. Although influenza virus remains indisputably the most significant viral respiratory pathogen in adults, a number of other common respiratory viruses contribute to the substantial burden of disease in older populations. Sensitive molecular techniques are evolving rapidly, and point-of-care testing for respiratory viruses will soon be possible [4]. While these tools will provide a more accurate assessment of the impact of respiratory viruses, it is unclear whether these new diagnostic tests will affect patient management, because of overriding concerns about possible bacterial coinfection, particularly in individuals requiring hospitalization [5, 6].

The occurrence of staphylococcal and pneumococcal pneumonia as a complication during influenza pandemics, including the recent 2009 influenza A(H1N1) pandemic, is well recognized [7, 8]. In vitro and animal studies demonstrate viral-bacterial synergy promoted by enhanced bacterial adherence and immune-mediated interactions [9]. Although temporal associations between

Received 18 December 2012; accepted 11 February 2013; electronically published 9 May 2013.

Correspondence: Ann R. Falsey, MD, Infectious Disease Unit, Rochester General Hospital, 1425 Portland Ave, Rochester, NY, 14621 (ann.falsey@rochestergeneral.org).

The Journal of Infectious Diseases 2013;208:432–41

DOI: 10.1093/infdis/jit190

bacterial pneumonia and seasonal viral respiratory tract infection have been reported, there are few specific data on the risk of bacterial complications of seasonal influenza and other virus infections [1, 10, 11]. The study of bacterial lung infection has been hampered by the lack of specific and sensitive tests for invasive disease. Results of blood cultures are infrequently positive, and sputum may be contaminated by upper airway tract secretions, leading to overdiagnosis of infection [12]. In most cases, clinical, laboratory, and radiographic findings do not reliably distinguish viral from bacterial infections [13]. Newer tests such as urinary antigen analysis and polymerase chain reaction (PCR) can augment traditional microbiologic testing but are not available for all organisms. Thus, clinicians continue to struggle with ruling out bacterial respiratory tract infection in patients with viral infection, and antibiotics are almost universally used in hospitalized patients [6].

Recently, serum biomarkers such as procalcitonin (PCT) have been evaluated as surrogate indicators of bacterial infection. PCT, a calcitonin precursor normally produced in neuroendocrine cells of the thyroid and lung, is secreted by cells throughout the body in response to bacterial infections [14]. Serum PCT levels of <0.25 ng/mL are uncommon with invasive bacterial disease, and PCT determinations have been used as a guide to antibiotic therapy in patients with respiratory tract illnesses, including community-acquired pneumonia (CAP) and acute exacerbation of chronic obstructive pulmonary disease (COPD), without an increased frequency of adverse outcomes [15].

We sought to more accurately define the incidence and impact of bacterial coinfection in patients hospitalized with documented viral infection, using a panel of bacteria-specific diagnostic tests augmented by measurement of the serum PCT level.

METHODS

Patient Population

The study was performed at Rochester General Hospital in Rochester, New York. Adults aged ≥21 years admitted through the emergency department with diagnoses compatible with acute respiratory tract infection were recruited from 1 November through 30 May during 3 winters (2008–2011). Patients were screened within 24 hours of hospitalization, excluding those given antibiotics prior to admission or with immunosuppression, cavitary lung disease, or witnessed aspiration. Informed consent was obtained from subjects or their legal guardians. The study was approved by the University of Rochester and Rochester General Hospital institutional review boards.

Illness Evaluation

At enrollment, demographic, clinical, and laboratory information was collected. To provide uniformity, a primary clinical admitting diagnosis was assigned by a pulmonary specialist after examination of each subject and review of laboratory and radiographic findings. All subjects underwent testing for bacterial pathogens, including blood culture, sputum culture and Gram stain, *Streptococcus pneumoniae* urine antigen and pneumococcal serologic testing, and PCR of sputum and nose and throat swab specimens for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*. Sputum was induced with normal saline if subjects were unable to expectorate a sample that was considered adequate on the basis of standard criteria. Nose and throat swab and sputum specimens were tested for viruses by reverse transcription PCR (RT-PCR), and sera were collected on admission and 4–6 weeks later for viral and pneumococcal serologic testing. Serum was also collected on hospital day 2 for PCT measurement.

Laboratory Methods

Standard Microbiological Testing

Blood cultures, sputum Gram stain and culture, influenza virus antigen testing, and viral cultures were performed by the Rochester General Hospital clinical laboratory, and results were available to clinicians for patient care. Sputum samples were plated on blood, chocolate, and MacConkey agar. *Legionella* testing (by sputum culture and urinary antigen assay) was performed at the discretion of the treating physician. Single blood cultures positive for organisms consistent with skin flora (ie, coagulase-negative staphylococcus, corynebacterium, α hemolytic streptococci, and *Propionibacterium acnes*) were considered contaminants. Sputum cultures were only considered positive if more than a rare pathogenic bacteria grew from an adequate sample with the exception of Legoinella.

S. pneumoniae Serologic Analysis

Pneumococcal surface protein A antigens covering families 1 and 2, obtained from the University of Alabama Bacterial Respiratory Pathogen Reference Laboratory, were used in an enzyme immunoassay [16]. A ≥4-fold rise in titer was considered evidence of infection.

Urinary Antigen Testing for S. pneumoniae

Urine samples were assayed for pneumococcal antigen, using the Binax NOW urine assay (Binax, Scarborough, ME).

PCT Level

PCT levels were measured by resolved amplified cryptate emission technology (Kryptor PCT, Brahms, Henningsdorf, Germany). The functional sensitivity of the assay is 0.06 ng/mL (mean normal level [±SD], 0.033 ± 0.003 ng/mL) [17].

Viral Serologic Analysis

Immunoglobulin G titers in acute- and convalescent-phase serum specimens were determined using established methods for influenza A and B viruses, respiratory syncytial virus,

human metapneumovirus, parainfluenza types 1–3, and human coronaviruses 229E and OC43 [18]. A ≥4-fold rise in the viral specific immunoglobulin G level was considered evidence of infection.

Real-Time PCR Analysis

Assays for respiratory syncytial virus, human metapneumovirus, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* were performed using published methods [19–21]. Human rhinovirus assays were performed on the basis of methods published by Lu et al, with the following modification of the forward primer (5′-CYGCCZGCGTGGY-3′) [22]. Primers and probes for other viruses were as follows: influenza A virus (Matrix gene), influenza B virus (NS1 gene), human coronaviruses 229E and OC43 (polymerase gene), and parainfluenza viruses 1–3 (nucleocapsid gene). Sequences will be supplied on request.

Definitions

Virus Infection Alone

Virus infection alone was defined as nose and throat swab samples or a sputum sample positive for any virus by one of the following assays: (1) RT-PCR (for all viruses), (2) a rapid influenza antigen test, or (3) serologic analysis (for all viruses, with exception of those detected during illness coinciding with influenza vaccination). Additional criteria involved negative results of all tests for bacteria and serum PCT values of <0.25 ng/mL on admission and day 2.

Bacterial Infection Alone

Bacterial infection alone was defined on the basis of negative results of viral diagnostic tests and any of the following: (1) a positive blood culture result, (2) a culture of an adequate sputum sample that was positive for a respiratory pathogen, (3) a urinary antigen test positive for *S. pneumoniae* or *Legionella pneumophila*, (4) a serologic assay positive for pneumococci, (5) a PCR assay positive for *M. pneumoniae* or *C. pneumoniae*, or (6) a serum PCT level of ≥0.25 ng on admission or hospital day 2.

Mixed Viral-Bacterial Infection

A mixed viral-bacterial infection met the definitions for bacterial infection and viral infection.

Statistical Methods

Continuous variables were compared using the nonparametric Wilcoxon test, and categorical variables were compared using the Fisher exact test. Pearson correlation coefficients were used to summarize dependencies between pairs of continuous variables. For univariate analysis comparing mixed viral-bacterial infection with viral infection alone, the false-discovery rate was used to account for multiple comparisons [23]. Multiple logistic regression analysis was used to model the outcome (mixed viral-bacterial infection vs viral infection alone) as a function of a subset of candidate predictors, including age of ≥65 years, KATZ functional score, symptoms for ≥7 days, temperature of ≥38°C, pulse rate, systolic blood pressure of ≥100 beats/min, CURB-65 score, oxygen saturation level of ≥ 85%, peripheral white blood cell count (WBC) of ≥12 000 cells/mL, anion gap, $\log_2$ blood urea nitrogen level, sex, diabetes mellitus, congestive heart failure, COPD, chronic renal failure, active smoking, steroid use, influenza vaccination, clinical admission diagnoses (pneumonia, acute exacerbation of COPD, bronchitis, congestive heart failure, asthma, viral infection/influenza, or other diagnosis), nasal congestion, sputum production, confusion, wheezing, rales, positive viral PCR findings, and radiographic findings (ie, no acute disease or infiltrate). Variables not included because of missing values included pneumococcal vaccination status, percentage of neutrophils or band forms in peripheral blood, and basal metabolic index.

RESULTS

During the 3-year study period, 2217 hospitalizations with appropriate admission diagnoses were screened. Hospitalizations were excluded for the following reasons: prior antibiotic use (in 354), immunosuppression (in 178), witnessed aspiration (in 26), and follow up not possible (in 71); 54 hospitalizations were excluded for other reasons. Of the 1534 hospitalizations eligible for inclusion, 368 involved patients who were not able to give consent, and 324 involved patients who refused to participate. Thus, 842 hospitalizations involving 771 patients who consented to participate were included in the study (Figure 1). A microbiologic diagnosis was made in 447 (53%) of 842 hospitalizations, of which 99 (12%) involved bacterial infection alone and 348 (41%) had evidence of viral infection. Of those involving viral infection, 212 (61%) had viral infection alone, 64 (18%) had evidence of mixed viral-bacterial infection on the basis of specific bacterial testing, and 72 (21%) were identified on the basis of an elevated serum PCT level. This report will focus on the 348 hospitalizations with documented viral infection (Table 1).

Viral diagnosis was made on the basis of RT-PCR findings for 74% of hospitalizations and on the basis of serologic tests alone for the remaining 26% (Table 1). Influenza A virus was the most common virus and accounted for 10% of hospitalizations, followed by respiratory syncytial virus (7%) and human coronavirus OC43 (7%). Influenza B virus was the least frequent virus detected (0.8%). Thirty-eight hospitalizations (5%) were associated with multiple viruses. The primary clinical diagnoses associated with virus infection were acute exacerbation of COPD (in 26% of hospitalizations), pneumonia (in 21%), acute bronchitis (in 18%), and asthma exacerbation (in 18%). The clinical admission diagnoses were similar for most pathogens, although the rate of pneumonia was lowest for human

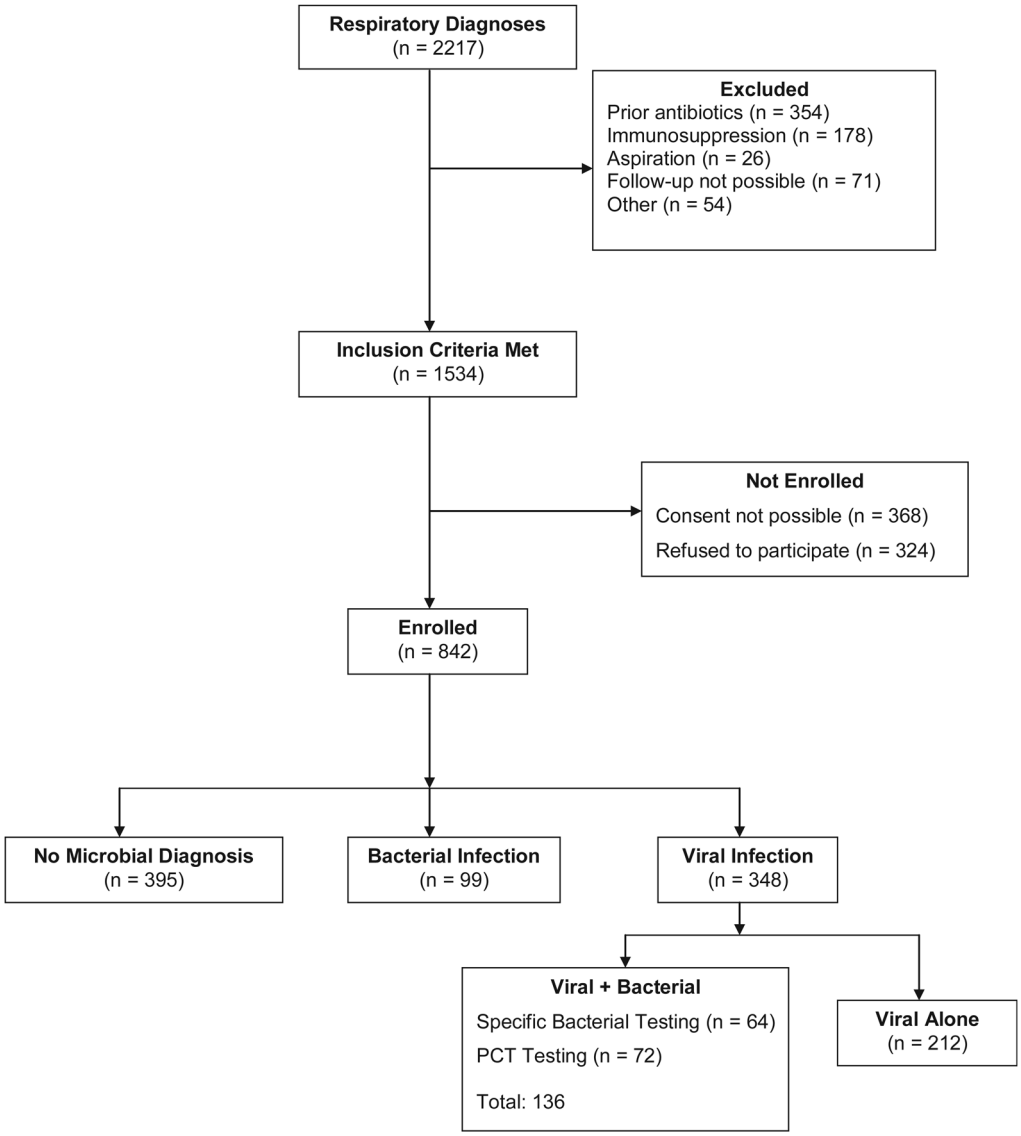

Figure 1. Flow diagram of subject recruitment, enrollment, and categorization.

rhinovirus (in 10% of hospitalizations) and highest for human metapneumovirus (in 31%) and human coronavirus OC43 (in 30%). There were no significant differences in the rates of mixed viral-bacterial infection by viral pathogen, including 2009 pandemic A(H1N1) (bacterial co-infection rate was 35%).

For the 64 hospitalizations involving mixed viral-bacterial infections in which specific bacterial pathogens were documented, diagnosis was confirmed with blood culture (in 7 hospitalizations), sputum culture (in 35), pneumococcal urinary antigen testing (in 15), and pneumococcal serologic testing (in 19). Atypical bacteria were rarely identified (*M. pneumoniae* [in 2 hospitalizations] and *C. pneumoniae* [in 0]) during the first 2 years, and therefore testing was discontinued in the third year. *S. pneumoniae* accounted for 35 of 64 bacterial diagnoses (55%; Figure 2).

Of the 348 hospitalizations involving viral infection, 344 (99%) had either an admission or day 2 PCT measurement, and 317 (91%) had both values available. Correlation between day 1 and 2 PCT levels was high ($R = .90$; $P = .0001$). Overall, 105 of the 344 hospitalizations (31%) with at least 1 PCT measurement involved patients with levels of ≥ 0.25 ng/mL (Table 1). On the basis of a positive bacterial test result or a PCT level of ≥ 0.25 ng/mL on admission or day 2, 136 of 348 hospitalizations (39%) involving viral illnesses had evidence of mixed viral-bacterial infection (Table 1). Of these, 33 involved both positive results of bacterial tests and elevated PCT levels, 31 involved only positive results of bacterial tests, and 72 involved only elevated PCT levels. Of note, despite vigorous attempts to collect adequate sputum in a timely fashion, we were frequently unable to procure adequate sputum within 6 hours

Table 1. Incidence of Viral Infection and Bacterial Coinfection Among Hospitalizations Involving Lower Respiratory Tract Infection

Pathogen	2008–2009 (n = 282)	2009–2010 (n = 274)	2011–2011 (n = 286)	Total (n = 842)	Virus Positivity by PCR	Bacteria-Specific Diagnosis	PCT level ≥ 0.25 ng/mL on d 1 or 2	Bacteria- Specific Diagnosis or PCT Level ≥ 0.25
Influenza A virus	24	21	38	83 (10)	48 (58)	10 (12)	28 (34)	33 (40)
Influenza B virus	4	2	1	7 (0.8)	4 (57)	1 (14)	3 (43)	3 (43)
RSV	20	24	11	55 (7)	42 (76)	10 (18)	14 (25)	17 (31)
HMPV	22	9	7	38 (5)	32 (84)	10 (26)	9 (24)	15 (39)
HCoV-OC43	35	1	20	56 (7)	46 (82)	8 (14)	19 (34)	22 (39)
HCoV-229E	7	8	1	16 (2)	11 (69)	3 (19)	6 (38)	7 (44)
PIV 1–3	12	11	1	24 (3)	16 (67)	7 (29)	9 (38)	12 (50)
Rhinovirus	10	10	10	30 (4)	30 (100)	5 (17)	6 (20)	9 (30)
Adenovirus[a]	1	0	0	1 (0.1)	NA	0	0	0
Viral- bacterial coinfection	13	8	17	38 (5)	27 (71)	10 (26)	10 (26)	18 (47)
Overall	148	94	106	348 (41)	256 (74)	64 (19)	105 (30)	136 (39)

Data are no. or no. (%) of hospitalizations.

Abbreviations: HCoV-OC43, human coronavirus OC43; HCoV-229E, human coronavirus 229E; HMPV, human metapneumovirus; NA, not applicable; PCR, polymerase chain reaction; PCT, procalcitonin; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

[a] Based on viral culture from the clinical laboratory. PCR for adenovirus was not performed.

of antibiotic administration. Thus, in 69% of the hospitalizations with bacterial infection defined on the basis of PCT level alone, the negative results of sputum cultures were considered unreliable, compared with 51% for the group as a whole.

Hospitalizations associated with elevated PCT levels with or without positive results of bacterial tests involved patients who were similar with respect to age, rate of pneumonia, physical examination findings, and severity of illness score. In contrast, hospitalizations associated with low PCT values and positive bacterial test results involved patients who were younger, had lower severity of illness scores, and were less likely to receive a diagnosis of pneumonia (19% vs 44%; $P = .02$) and more likely to receive a diagnosis of asthma or COPD exacerbations (55% vs 26%; $P = .006$) as compared to those associated with elevated PCT values.

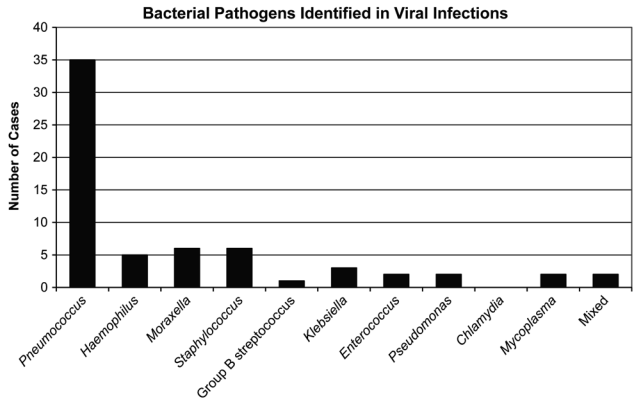

Figure 2. Number of specific bacterial pathogens identified in subjects with documented viral infection.

Clinical and laboratory variables were compared according to bacterial test positivity (Table 2). Not surprisingly, hospitalizations associated with positive blood culture results involved patients with higher severity of illness scores and abnormal findings of more laboratory tests, compared with those associated with positive results of other bacterial tests or those associated with no positive bacterial test results. No consistent differences between the groups were noted with respect to admission diagnoses or radiologic findings. Bacteremic hospitalizations involved a significantly greater percentage of PCT measurements of >0.25 ng/mL, compared with those involving no positive results of bacterial tests (86% vs 25%; $P = .002$). Of note, the single hospitalization involving a bacteremic subject with a low PCT level yielded a clinical diagnosis of bronchitis and 1 blood culture positive for multiple organisms (enterococci, *Staphylococcus aureus*, and coagulase-negative staphylococci), raising the possibility of blood culture contamination. In addition, median PCT values were higher for groups with positive bacterial test results as compared to those with no positive bacterial test results (bacteremia, $P = .001$; urine antigen testing, $P = .001$; pneumococcal serologic testing, $P = .001$; and sputum culture alone, $P = .055$).

We compared subject and illness characteristics among hospitalizations involving viral infection alone to those involving mixed viral-bacterial infections, using the combination of a positive microbiologic test and/or serum PCT level of >0.25 ng to define bacterial infection. A number of clinical features were significantly different by univariate analysis, as shown in

Table 2. Characteristics of Hospitalizations Involving Subjects With Viral Illnesses, by Bacterial Test Positivity

Characteristic	Blood Culture (n = 7)	Urine Antigen Test[a] (n = 14)	Pneumococcal Serologic Test[a] (n = 18)	Sputum Culture Alone (n = 28)	No Positive Test Result (n = 285)
Clinical admission diagnosis					
Pneumonia	1 (14)	7 (50)	7 (39)	8 (29)	54 (19)
Exacerbation of COPD	1 (14)	2 (14)	3 (17)	10 (36)	80 (28)
Acute bronchitis	2 (29)	2 (14)	1 (6)	2 (7)	51 (18)
Congestive heart failure	0	1 (7)	2 (11)	1 (4)	17 (6)
Asthma	0	2 (14)	2 (11)	7 (25)	52 (18)
Viral infection/influenza	0	0	1 (6)	0	26 (9)
Other	3 (43)	0	2 (11)	0	5 (2)
Physical examination finding					
Confusion	1 (14)	1 (7)	3 (17)	2 (7)	31 (11)
Wheezes	4 (57)	7 (50)	6 (33)	19 (68)	165 (57)
Rales	5 (71)	8 (57)	7 (39)	11 (39)	98 (34)
Temperature	37.4 ± 1.5	37.6 ± 0.9	37.4 ± 0.9	37.3 ± 1.1	37.5 ± 1.0
Pulse	124 ± 23	113 ± 17	114 ± 17	106 ± 20	105 ± 46
Systolic BP, mm Hg	106 ± 30	116 ± 30	120 ± 25	118 ± 21	122 ± 21
CURB-65 score	2.6 ± 1.3	1.7 ± 1.3	1.9 ± 1.3	1.8 ± 1.2	1.6 ± 1.2
Laboratory finding					
WBC count, × 10^3 cells/μL	14.7 ± 8.8	12.8 ± 6.2	13.3 ± 5.8	12.6 ± 4.8	9.9 ± 5.0
Bands, %	14.2 ± 0.0	12.8 ± 17.2	8.0 ± 15.8	5.6 ± 10.1	2.9 ± 6.6
Anion gap, mEq/L	11.2 ± 5.3	10.0 ± 2.6	10.6 ± 2.3	9.2 ± 3.5	8.7 ± 2.7
PCT level > 0.25 ng/mL[b]	6 (86)	9 (64)	11 (61)	11 (39)	72 (25)
Highest PCT level on d 1 or 2, median (IQR)[c]	15.5 (0.7–20.5)	0.4 (0.2–13.7)	1.2 (0.2–4.5)	0.2 (0.1–0.8)	0.1 (0.1–0.3)
Radiographic finding					
No acute disease	3 (43)	5 (36)	8 (44)	11 (39)	144 (50)
Infiltrate	2 (29)	6 (43)	5 (28)	13 (46)	99 (34)

Data are no. (%) of hospitalizations or mean ± SD, unless otherwise indicated. Data do not include 1 subject with bacterial infection diagnosed on the basis of mycoplasma-positive polymerase chain reaction analysis.

Abbreviations: BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; PCR, polymerase chain reaction; PCT, procalcitonin; WBC, white blood cell.

[a] Hospitalizations involving subjects with positive blood culture results were excluded. Five hospitalizations involved subjects with both urine antigen and serologic tests positive for pneumococcus and are included in both groups.

[b] Comparison of no positive test results vs positive blood culture results ($P = .002$), urine antigen test results ($P = .003$), serologic test results ($P = .002$), and sputum-alone test results ($P = .12$), with an overall 5-group P value of <.0001, by the Fisher exact test.

[c] Comparison of no positive test results vs positive blood culture results ($P = .001$), urine antigen test results ($P = .001$), serologic test results ($P = .001$), and sputum-alone test results ($P = .055$), with an overall 5-group P value of <.0001, by the Kruskal-Wallis test.

Table 3. Among hospitalizations associated with mixed viral-bacterial infections, subjects were older, had higher rates of chronic renal failure, more commonly received diagnoses of pneumonia, and less commonly received diagnoses of acute exacerbation of COPD and asthma. These subjects also had significantly lower systolic blood pressures, as well as significantly higher mean pulse rates, peripheral white blood cell counts, band forms, anion gaps, and blood urea nitrogen levels, although there was substantial overlap in these values. Multivariate analysis shown in Table 4 indicates that the factors that were mostly strongly predictive of mixed viral-bacterial infection ($P < .01$) were peripheral white blood cell count of >12 000 cells/mL (odds ratio [OR], 3.8), anion gap (OR, 1.2 per unit change), COPD (OR, 2.9), chronic renal failure (OR, 10.7), and infiltrate on chest radiograph (OR, 2.5).

Although severity of illness scores (CURB-65) were significantly higher among hospitalizations involving mixed viral-bacterial infection, rates of intensive care use, length of stay, and in-hospital mortality were not significantly different between the 2 groups (Table 5). At 1-month of follow-up, patients hospitalized with mixed viral-bacterial infection continued to require a higher level of medical care more frequently than those hospitalized without bacterial infections, but by 3 months outcomes of the 2 groups were similar.

Table 3. Characteristics of Hospitalizations Involving Viral Infection Alone Versus Mixed Viral-Bacterial Infections

Characteristic	Missing Value	Viral Alone (n = 212)	Mixed Viral-Bacterial (n = 136)	FDR[a]
Age, y	0	62.7 ± 17.4	67.4 ± 16.5	0.03
Female sex	0	127 (60)	74 (54)	0.44
Diabetes mellitus	1	76 (36)	53 (39)	0.75
Congestive heart failure	0	50 (24)	38 (28)	0.48
COPD	1	79 (37)	59 (44)	0.38
Chronic renal failure	2	2 (1)	16 (12)	<0.0001
Active smoker	1	70 (33)	36 (27)	0.35
KATZ functional score		0.5 ± 1.8	0.9 ± 2.5	0.34
Chronic corticosteroid use	2	27 (13)	19 (14)	0.84
Influenza vaccination	8	153 (74)	88 (67)	0.29
Pneumococcal vaccination	47	102 (56)	80 (68)	0.07
Days of symptoms PTA	0	4.24 ± 4.07	4.52 ± 6.01	0.48
Clinical admission diagnosis				
Pneumonia	0	22 (10)	52 (38)	<0.0001
Acute exacerbation of COPD	0	68 (32)	28 (21)	0.04
Acute bronchitis	0	42 (20)	16 (12)	0.10
Congestive heart failure	0	13 (6)	7 (5)	0.89
Asthma	0	48 (23)	15 (11)	0.02
Viral infection/ influenza	0	17 (8)	10 (7)	1.0
Other	0	2 (1)	8 (6)	0.03
Symptom				
Nasal congestion	2	135 (64)	65 (49)	0.02
Sputum production	0	166 (78)	107 (79)	1.0
Dyspnea	0	198 (93)	127 (93)	1.0
Physical examination finding				
Confusion	1	20 (9)	17 (13)	0.48
Wheezes	0	138 (65)	63 (46)	0.002
Rales	0	60 (28)	66 (49)	0.0009
Temperature, °C	0	37.3 ± 1.0	37.6 ± 1.1	0.06
Pulse	0	101 ± 19	114 ± 63	0.001
Systolic BP, mm Hg	0	123 ± 19	118 ± 25	0.02
BMI	67	32.4 ± 10.2	29.1 ± 9.0	0.02
CURB-65 score	0	1.4 ± 1.1	2.1 ± 1.2	<0.0001
Laboratory finding				
SaO_2 level, %	0	92.9 ± 4.5	91.6 ± 6.4	0.44
WBC count, × 10^3 cells/μL	2	9.0 ± 3.8	12.5 ± 6.4	<0.0001
Neutrophils, %	32	72 ± 13	76 ± 12	0.002
Bands, %	62	2.4 ± 6.6	6.0 ± 10.1	0.001
Anion gap, mEq/L	5	8.4 ± 2.4	9.8 ± 3.2	<0.0001
BUN level, mg/dL	3	18.5 ± 12.2	24.9 ± 15.4	<0.0001
Viral PCR positive		159 (75)	96 (71)	0.48
Radiographic finding				
No acute disease	1	121 (57)	49 (36)	0.0005
Infiltrate	1	55 (26)	69 (51)	<0.0001

Data are no. or no. (%) of hospitalizations or mean ± SD.

Abbreviations: BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; COPD, chronic obstructive pulmonary disease; PCR, polymerase chain reaction; SaO_2, oxygen saturation; WBC, white blood cell.

[a] The false-discovery rate (FDR), accounting for all 39 univariate tests, was used to compare variables. Unadjusted *P* values were smaller.

For 90% of hospitalizations deemed to involve viral infection alone, patients were treated with antibiotics, as were patients in 92% of those judged to involve mixed viral-bacterial infection (Table 5), although patients hospitalized with bacterial infection were treated longer than those hospitalized with viral infection alone (mean treatment duration [±SD], 6.2 ± 9.0 vs 4.2 ± 4.6; false-discovery rate = .04). Potential antibiotic-related adverse events were significantly more common in the mixed viral-bacterial group (74 [54%], compared with 71 [33%] for viral infection alone; false-discovery rate = .002). Notably, 4 of 10 deaths among patients hospitalized with viral infection alone were secondary to complications of *Clostridium difficile* colitis.

Table 4. Multivariate Analysis of Factors Predictive of Mixed Viral-Bacterial Infections

Covariate	Odds Ratio (95% CI)	*P*
SaO_2 level < 85%	3.5 (1.3–9.9)	.02
WBC count ≥12.0 × 10^3 cells/μL	3.8 (2.1–7.1)	<.0001
Pulse rate 10 beats/min	1.2 (1.0–1.4)	.04
Anion gap	1.2 (1.1–1.3)	.003
COPD	2.9 (1.4–5.7)	.003
Chronic renal failure	10.7 (2.0–58.1)	.006
Pneumonia admission diagnosis	4.3 (1.0–19.2)	.05
COPD admission diagnosis	1.0 (.2–4.4)	1.0
Bronchitis admission diagnosis	1.5 (.3–6.7)	.6
Asthma admission diagnosis	1.9 (.4–9.1)	.40
Viral infection/influenza admission diagnosis	4.6 (.9–23.5)	.07
Other admission diagnosis	7.81 (.8–72.1)	.07
Rales on examination	1.9 (1.0–3.6)	.04
Infiltrate on chest radiograph	2.5 (1.3–4.9)	.007

Abbreviations: BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; SaO_2, oxygen saturation; WBC, white blood cell.

Table 5. Illness Outcomes

Variable	Viral Alone	Mixed Viral-Bacterial	FDR[a]
In hospital			
ICU admission	13 (6)	19 (14)	0.08
Respiratory failure	9 (4)	12 (9)	0.26
Death	2 (1)	3 (2)	0.60
Length of stay, d	6.4 ± 9.5	12.3 ± 43.4	0.16
Length of illness, d	19.4 ± 11.2	22.4 ± 11.3	0.08
Antibiotic use			
Any inpatient antibiotics	191 (90)	125 (92)	0.83
Duration of inpatient use, d	4.2 ± 4.6	6.2 ± 9.0	0.04
Discharged receiving oral antibiotics	123 (58)	88 (65)	0.38
Antibiotic complications	71 (33)	74 (54)	0.002
1 mo of follow-up[b]			
C. difficile infection	5 (3)	3 (2)	0.83
Return to baseline health	122 (63)	61(49)	0.10
Higher level of care needed	17 (9)	17 (14)	0.04
Repeat hospitalization	35 (18)	21 (17)	0.98
Death	4 (2)	3 (2)	1.0
3 mo of follow-up[c]			
C. difficile infection	2 (1)	5 (5)	1.0
Higher level of care needed	8 (5)	8 (8)	0.63
Repeat hospitalization	36 (21)	25 (23)	0.83
Death	4 (2)	6 (6)	0.36
Total deaths at 3 mo	10 (5)	12(9)	0.36

Data are no. (%) of hospitalizations or mean ± SD for 212 hospitalizations involving viral infection alone and 136 involving mixed bacterial-viral infection, unless otherwise indicated.

Abbreviations: *C. difficile, Clostridium difficile*; ICU, intensive care unit.

[a] The false-discovery rate (FDR) accounts for all 19 univariate tests in this table. Unadjusted *P* values are smaller.

[b] Data are for 194 hospitalizations involving viral infection alone and 124 involving mixed bacterial-viral infection.

[c] Data are for 175 hospitalizations involving viral infection alone and 107 involving mixed bacterial-viral infection.

DISCUSSION

Our study is the largest published prospective assessment of bacterial coinfection in patients with viral respiratory disease requiring hospitalization. Using comprehensive bacterial testing plus the serum biomarker PCT to define bacterial infection, our data indicate that nearly 40% of viral-associated hospitalizations have evidence of concomitant bacterial infection. Recent studies of 2009 pandemic influenza (A)H1N1 showed overall rates of bacterial coinfection of 20%–24% among critically ill children and adults and up to 50% among those with fatal illness [24, 25]. Much less is known regarding the bacterial complications for other viral respiratory tract infections, particularly those in adults. Our data indicates that rates and types of bacterial infections with non–influenza viruses are similar to those observed with influenza. The high rate of mixed viral-bacterial infection identified in our study reflects the use of the serum biomarker PCT to augment comprehensive bacterial testing. In most previous studies reporting bacterial complications of viral infection, the bacterial testing was neither comprehensive nor systematic and frequently was left to the discretion of the clinician caring for the patient [26–28].

Although a number of clinical and laboratory variables were associated with mixed bacterial-viral infections, the absolute differences in individual variables from hospitalizations involving viral infection alone were small and unlikely to be helpful for patient management. Nevertheless, in our study, subjects hospitalized with mixed bacterial-viral infections were more ill than those hospitalized with viral infection alone, and administration of early appropriate antibiotics to these patients is important. However, it is also noteworthy that 61% of hospitalizations involving viral infection had no evidence of bacterial infection. Most of these individuals had normal chest radiograph findings and were hemodynamically stable, yet 90% received a course of antibiotic treatment. Four deaths due to complications from *C. difficile* colitis occurred in this group. Once considered a nuisance, *C. difficile* colitis has now evolved into a deadly syndrome with high mortality rates and relapse rates of 20%–30% [29]. These data highlight the critical need for better methods to safely reduce unnecessary use of antibiotics.

Recent European studies suggest that PCT-guided algorithms are a reasonable alternative to traditional microbiology-guided antibiotic therapy [15]. In 6 trials involving >2500 subjects with CAP or acute exacerbation of COPD who were randomly assigned to receive standard care or PCT-guided antibiotic treatment, there were no discernable adverse outcomes, and antibiotic use was significantly decreased, especially in those with non-pneumonic respiratory tract infections. In our study, serum PCT levels of ≥0.25 ng/mL correlated with radiographic pneumonia and higher severity of illness scores, which has been previously noted and is consistent with PCT level as a surrogate for invasive bacterial infection [30]. In addition, higher mean PCT levels were noted in patients who received a diagnosis of bacterial infections by a variety of methods, including blood and sputum cultures as well as pneumococcal urine antigen and serologic testing, suggesting that elevated PCT levels should heighten a clinician's suspicion for bacterial infection. However, PCT levels may not correlate as well with less invasive bacterial infections, such as bronchitis and acute exacerbation of COPD, and thus in some patients a low PCT level may not rule out the presence of bacterial infection [31, 32]. Although issues of bacterial colonization in this population complicate interpretation, examination of the sputum may continue to be important in patients with severe underlying lung disease.

This study has a number of limitations. First, our results are only applicable to adults, and the rates of mixed bacterial-viral

infections may be quite different in children. In addition, our results may not be applicable to all adults admitted with respiratory tract infections, since most illnesses in our study were not severe, as reflected by the low mortality rate. The inability to obtain consent from some of the most critically ill patients may have underestimated the rate of mixed viral-bacterial infections. Also, despite vigorous attempts to collect adequate respiratory samples in a timely fashion, we were successful in only 51% of hospitalizations, also leading to underestimates of bacterial infection. Conversely, it is also possible that bacterial infection based on results of sputum cultures overestimates the incidence of infection, as it is often not possible to distinguish chronic colonization from infection even with adequate samples. Last, the presence of viral RNA may not always be causally associated with the illness leading to hospitalization. Prolonged viral detection following respiratory tract infection due to rhinovirus and other viruses can been seen, particularly when using sensitive molecular diagnostic tests.

In conclusion, bacterial coinfection is associated with approximately 40% of serious respiratory viral infections in adults requiring hospitalization. Patients with positive results of viral tests should be carefully evaluated for concomitant bacterial infection, and it is prudent to initiate early empirical antibiotic therapy for patients with severe viral illness or definitive radiographic pneumonia. At the present time, establishing a specific bacterial diagnosis on the basis of traditional methods remains difficult, and better bacterial diagnostic tests are needed. Future studies are needed to develop treatment algorithms that use combinations of biomarkers and clinical parameters to accurately predict patients at low risk for bacterial infection in whom antibiotics may be safely discontinued.

Notes

Acknowledgments. We thank John-Peter Kokkinos, for assistance in performing the PCT assays; and the Rochester General Hospital Clinical Laboratories, for assistance with sample retrieval.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases (1R01AI079446-01).

Potential conflicts of interest. Dr Falsey has received research grants from Medimmune/AstraZeneca and sanofipasteur and consulting fees from Medimmune/AstraZeneca, sanofipasteur, GlaxoSmithKline, and Novavax. Dr Walsh has received consulting fees from Medimmune/AstraZeneca, GlaxoSmithKline, Novartis, Microdose, Alios Pharmaceuticals, Alnylam, and ClearPath. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med **2005**; 352:1749–59.
2. Templeton KE, Scheltinga SA, van den Eeden WC, Graffelman AW, van den Broek PJ, Claas EC. Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction. Clin Infect Dis **2005**; 41:345–51.
3. Widmer K, Zhu Y, Williams JV, Griffin MR, Edwards KM, Talbot HK. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. J Infect Dis **2012**; 206:56–62.
4. Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. J Clin Microbiol **2011**; 49:2449–53.
5. Shiley KT, Lautenbach E, Lee I. The use of antimicrobial agents after diagnosis of viral respiratory tract infections in hospitalized adults: antibiotics or anxiolytics? Infect Control Hosp Epidemiol **2010**; 31:1177–83.
6. Falsey AR, Murata Y, Walsh EE. Impact of rapid diagnosis on management of adults hospitalized with influenza. Arch Intern Med **2007**; 95:1029–37.
7. Louria DE, Blumenfeld HL, Ellis JT, Kilbourne ED, Rogers DE. Studies on influenza in the pandemic of 1957–1958. II. Pulmonary complications of influenza. J Clin Invest **1959**; 38:213–65.
8. Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. Lancet Infect Dis **2006**; 6:303–12.
9. McCullers JA, McAuley JL, Browall S, Iverson AR, Boyd KL, Henriques Normark B. Influenza enhances susceptibility to natural acquisition of and disease due to Streptococcus pneumoniae in ferrets. J Infect Dis **2010**; 202:1287–95.
10. de Roux A, Ewig S, Garcia E, et al. Mixed community-acquired pneumonia in hospitalised patients. Eur Respir J **2006**; 27:795–800.
11. Talbot TR, Poehling KA, Hartert TV, et al. Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. Am J Med **2005**; 118:285–91.
12. Bartlett JG. Diagnostic tests for agents of community-acquired pneumonia. Clin Infect Dis **2011**; 52 (Suppl 4):S296–304.
13. Muller B, Harbarth S, Stolz D, et al. Diagnostic and prognostic accuracy of clinical and laboratory parameters in community-acquired pneumonia. BMC Infect Dis **2007**; 7:10.
14. Becker KL, Snider R, Nylen ES. Procalcitonin assay in systemic inflammation, infection, and sepsis: clinical utility and limitations. Crit Care Med **2008**; 36:941–52.
15. Schuetz P, Chiappa V, Briel M, Greenwald JL. Procalcitonin algorithms for antibiotic therapy decisions: a systematic review of randomized controlled trials and recommendations for clinical algorithms. Arch Intern Med **2011**; 171:1322–31.
16. Bacterial Respiratory Pathogen Reference Laboratory. http://www.vaccine.uab.edu/. Accessed 30 January 2013.
17. Becker KL, Snider R, Nylen ES. Procalcitonin in sepsis and systemic inflammation: a harmful biomarker and a therapeutic target. Br J Pharmacol **2010**; 159:253–64.
18. Falsey AR, Treanor JJ, Betts RF, Walsh EE. Viral respiratory infections in the institutionalized elderly: clinical and epidemiologic findings. J Am Geriatr Soc **1992**; 40:115–9.
19. Walsh EE, Peterson DR, Falsey AR. Human metapneumovirus infections in adults: another piece of the puzzle. Arch Intern Med **2008**; 168:2489–96.
20. Falsey AR, Formica MA, Treanor JJ, Walsh EE. Comparison of quantitative reverse transcription-PCR to viral culture for assessment of respiratory syncytial virus shedding. J Clin Microbiol **2003**; 41:4160–5.
21. Gullsby K, Storm M, Bondeson K. Simultaneous detection of Chlamydophila pneumoniae and Mycoplasma pneumoniae by use of molecular beacons in a duplex real-time PCR. J Clin Microbiol **2008**; 46:727–31.
22. Lu X, Holloway B, Dare RK, et al. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. J Clin Microbiol **2008**; 46:533–9.
23. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of Royal Statistical Society **1995**; 57:289.
24. Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 InfluenzaBautista E, Chotpitayasunondh T, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med **2010**; 362:1708–19.

25. Gill JR, Sheng ZM, Ely SF, et al. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Arch Pathol Lab Med **2010**; 134:235–43.
26. Miggins M, Hasan A, Hohmann S, et al. The potential influence of common viral infections diagnosed during hospitalization among critically ill patients in the United States. PLoS One **2011**; 6:e18890.
27. Dhanoa A, Fang NC, Hassan SS, Kaniappan P, Rajasekaram G. Epidemiology and clinical characteristics of hospitalized patients with pandemic influenza A (H1N1) 2009 infections: the effects of bacterial coinfection. Virol J **2011**; 8:501.
28. Rice TW, Rubinson L, Uyeki TM, et al. Critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. Crit Care Med **2012**; 40:1487–98.
29. Muto CA, Pokrywka M, Shutt K, et al. A large outbreak of *Clostridium difficile*–associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol **2005**; 26:273–80.
30. Bafadhel M, Clark TW, Reid C, et al. Procalcitonin and C-reactive protein in hospitalized adult patients with community-acquired pneumonia or exacerbation of asthma or COPD. Chest **2011**; 139:1410–8.
31. Falsey AR, Becker KL, Swinburne AJ, et al. Utility of serum procalcitonin values in patients with acute exacerbations of chronic obstructive pulmonary disease: a cautionary note. Int J Chron Obstruct Pulmon Dis **2012**; 7:127–35.
32. Kherad O, Kaiser L, Bridevaux PO, et al. Upper-respiratory viral infection, biomarkers, and COPD exacerbations. Chest **2010**; 138:896–904.