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Viral and bacterial aetiology of community-acquired pneumonia in adults

Elisabeth G. W. Huijskens, Adriana J. M. van Erkel, Fernand M. H. Palmen, Anton G. M. Buiting, Jan A. J. W. Kluymans, John W. A. Rossen

Laboratory of Medical Microbiology and Immunology, St Elisabeth Hospital, Tilburg, The Netherlands. Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, The Netherlands. Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands.

Correspondence: Elisabeth G. W. Huijskens, Laboratory of Medical Microbiology and Immunology, St Elisabeth Hospital, PO Box 747, 5000 AS Tilburg, The Netherlands. E-mail: i.huijskens@gmail.com

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Background Modern molecular techniques reveal new information on the role of respiratory viruses in community-acquired pneumonia. In this study, we tried to determine the prevalence of respiratory viruses and bacteria in patients with community-acquired pneumonia who were admitted to the hospital.

Methods Between April 2008 and April 2009, 408 adult patients (aged between 20 and 94 years) with community-acquired pneumonia were tested for the presence of respiratory pathogens using bacterial cultures, real-time PCR for viruses and bacteria, urinary antigen testing for Legionella and Pneumococci and serology for the presence of viral and bacterial pathogens.

Results Pathogens were identified in 263 (64.5%) of the 408 patients. The most common single organisms in these 263 patients were Streptococcus pneumoniae (22.8%), Coxiella burnetii (6.8%) and influenza A virus (3.8%). Of the 263 patients detected with pathogens, 117 (44.5%) patients were positive for one or more viral pathogens. Of these 117 patients, 52 (44.4%) had no bacterial pathogen. Multiple virus infections (≥2) were found in 16 patients.

Conclusion In conclusion, respiratory viruses are frequently found in patients with CAP and may therefore play an important role in the aetiology of this disease.

Keywords Adults, aetiology, community-acquired pneumonia, respiratory virus infection, viral.

Introduction Community-acquired pneumonia (CAP) is a common disorder and a major medical problem. In 30–50% of the patients with CAP, no specific organism is identified, despite the extensive use of diagnostic tests.1–3 The most common causative pathogen of bacterial CAP is Streptococcus pneumoniae.4 Mycoplasma pneumoniae and Chlamydia pneumoniae are among the most common ‘atypical’ pathogens.

Potential viral causes of CAP are often not explored because of the lack of antiviral agents and the relative unfamiliarity with viral pneumonia. However, it is well known that viral infections of the respiratory tract are the cause for significant mortality and morbidity all over the world, particularly in children and elderly adults.

Viral respiratory pathogens that are commonly found include rhinoviruses, coronaviruses, influenza viruses, respiratory syncytial viruses, parainfluenza viruses and adenoviruses. Over the past decade, analysis of clinical specimens of the respiratory tract through different diagnostic methods have led to the discovery of new viruses, such as human metapneumovirus,9 human severe acute respiratory syndrome coronavirus,10 coronaviruses NL6311 and HKU1,12 human bocavirus13 and the recently described polyomaviruses KIPyV14 and WUPyV.15

In this study, we tried to reveal the aetiology of community-acquired pneumonia in patients admitted to the hospital with community-acquired pneumonia using extensive molecular testing for viral and bacterial pathogens.

Materials and methods From April 2008 through March 2009, we analysed all the patients aged 18 years and older who presented at the emergency ward of the St Elisabeth Hospital, Tilburg, or the TweeSteden Hospital, Tilburg, with the suspicion of community-acquired pneumonia.
Community-acquired pneumonia was defined as suspicion of acute respiratory tract infection with a new or progressive infiltrate on a chest radiograph and one of the following criteria: fever (temperature ≥38°C) or hypothermia (temperature <35°C), new cough with or without sputum production, abnormal percussion and altered breath sounds on auscultation, dyspnoea or tachypnea or hypoxia, and leucocytosis or leucopenia.

We excluded patients with recent hospitalization (<2 weeks) and those residing in long-term care facilities, patients with known bronchial obstruction or a history of post-obstructive pneumonia other than COPD, patients with known or suspected active tuberculosis. The study was approved by the local medical ethics committee. Written informed consent was obtained from all participants in the study.

Samples
At the emergency ward a throat swab was taken, and two sets of blood samples were obtained and cultured according to standard microbiological procedures. If available, a sputum sample was evaluated by use of Gram staining and culture.

Urinary antigen detection tests for Streptococcus pneumoniae and Legionella pneumophila were performed with the BinaxNOW pneumococcal urinary antigen test and the BinaxNOW Legionella urinary antigen test (both from Binax, ME, USA). Paired serum samples were obtained during the acute and convalescent phases of infection (separated by at least 2 weeks) for serological studies. A case report form was obtained for every patient.

Molecular detection of respiratory pathogens
All samples were tested using real-time PCR for the presence of respiratory viruses and bacteria including adenovirus (AdV), human bocavirus (hBoV), KI- and WU polyomaviruses (KIPyV and WUPyV), human metapneumovirus (hMPV), human rhinovirus (HRV), human coronaviruses (HCoV) (OC43, NL63, HKU and 229E), parainfluenza viruses (PIV), influenza viruses A and B (InfA, InfB), respiratory syncytial virus (RSV), Legionella pneumophila, Mycoplasma pneumoniae, Chlamydophila psittaci, Chlamydophila pneumoniae, Coxiella burnetii and Streptococcus pneumoniae. Real-time PCR procedures were performed as described in reference 16–22. Briefly, nucleic acids were extracted from the throat swabs with the MagNa pure LC using the total nucleic acid isolation kit system according to the manufacturer’s protocol (Roche Diagnostics, Basel, Switzerland). Each sample was eluted in 1000 μl of buffer sufficient to perform all the real-time PCRs. cDNA was synthesized by using MultiScribe reverse transcriptase (RT) and random hexamers (both from Applied Biosystems, Carlsbad, CA, USA). The reaction was performed in 100 μl of reaction mixture consisting of 10 μl RT-buffer (10×), 22 μl MgCl₂ (25 mm), 20 μl dNTP-mix (10 mm), 5 μl random hexamer (50 μm), 2.5 μl MultiScribe RT (50 U/μl), 2 μl Rnase inhibitor (20 U/μl) (EZ RT-PCR kit; Applied Biosystems) and 40 μl of the isolated sample. After incubation for 10 minutes at 25°C, RT was carried out for 30 minutes at 48°C, followed by RT inactivation for 5 minutes at 95°C.

Classification of aetiology
An aetiological agent for CAP was considered present, if any of the following criteria were met: a pathogenic microorganism was cultured from blood samples; the urinary antigen test was positive for S. pneumoniae or L. pneumophila; PCR of the throat swab or sputum samples yielded a positive result; sputum samples (presence of >25 polymorphonuclear leucocytes and <10 squamous cells per field) with a predominant organism and compatible results from Gram stain; the presence of IgM antibodies for M. pneumoniae, or a fourfold increase in IgG antibody titres for M. pneumoniae, L. pneumophila, Chlamydophila psittaci and Coxiella burnetii.

Statistical analysis
Groups were compared by a chi-squared test with a significance level of P < 0.05. Analyses were conducted using PASW Statistics 18 (IBM Company, Chicago, IL, USA).

Results
From April 2008 through March 2009, 408 patients were included. The demographic characteristics of the patients are presented in Table 1. Patients ranged in age from 20 to 94 years (mean 65 years; median 68 years), 61-3% of the patients were men and 38-7% were women.

Of the 408 patients, we collected 408 throat swabs, 408 pneumococcal urinary antigen tests, 405 legionella urinary antigen tests, 203 sputum samples for bacterial culture, 163 sputum samples for molecular detection, 329 blood cultures, 90 serum samples for M. pneumoniae, 66 serum samples for L. pneumophila, 44 serum samples for Chlamydophila psittaci and 104 serum samples for Coxiella burnetii. All samples were taken from unique patients, that is, none of the patients had duplicate samples taken.

Aetiology
Aetiology was identified in 263 (64.5%) of the 408 patients, and more than one pathogen was isolated in 106 patients (26.0%). Results are shown in Table 2. Of the 263 patients identified with a pathogen, 117 (28.7%) patients
were positive for one or more viral pathogens. A bacterial pathogen was detected in 65 (35.5%) of these 117 patients, whereas in 52 (44.4%) of these patients, only respiratory viruses were detected. *S. pneumoniae*, *Coxiella burnetii* and Infa were identified as the only micro-organism in 22-8%, 6-8% and 3-8% of the 263 patients, respectively.

In 139 patients with CAP, *S. pneumoniae* was detected. In 60 (43.2%) patients, *S. pneumoniae* was the only identifiable pathogen. Of those 60 patients, 17 were diagnosed by the urinary antigen assay alone, 14 were only detected by culture or molecular detection of *S. pneumoniae* in sputum samples and six had only positive blood cultures. In addition, in five patients, both blood culture and the urinary antigen test were positive, in three patients both blood culture and sputum samples were positive, in seven patients sputum samples and the urinary antigen test were positive and in eight patient all three tests were positive. Of the remaining 79 *S. pneumoniae* patients having other pathogens as well, 11 patients had only a positive urinary antigen assay, and 53 patients had a positive PCR from sputum, two patients had only a positive blood culture and urinary antigen test, three patients had a positive blood culture and a positive PCR from sputum samples and nine patients had a positive PCR from the sputum sample and a positive urinary antigen test. In 53 patients diagnosed with pneumococcal CAP, the following viruses were found: HRV 19 times, Infa 11 times, HCoV OC43 eight times, PIV1 seven times. RSV, HCoV 229E three times, hMPV, HCoV NL63, and Infb all two times and KIPyV, WUPyV and hBoV were each detected one time.

*Haemophilus influenzae* was found in 21 of the 203 sputum samples and in one blood culture. In 11 of these patients, one or more respiratory viruses were detected as well. HRV was detected three times, PIV1, Infa, HCoV OC43 and RSV were each detected two times, and hBoV, KIPyV and WUPyV were each detected one time.

Statistical analysis showed a significant association between patients with *H. influenzae* and viral pathogens (*P* = 0.023) and a significant association between patients with *S. pneumoniae* and viral pathogens (*P* = 0.003).

Legionella was found in 15 patients. Of two patients the *legionella* urinary antigen tests, serum samples and respiratory samples were positive for *L. pneumophila*. Three patients had a positive legionella urinary antigen test and positive respiratory sample, in four patients only the urinary antigen test was positive, in three patients only the respiratory sample and in one patient only the serum samples for *L. pneumophila* were positive.

*Mycoplasma pneumoniae*-specific IgM was found in only one of the 90 patients tested, and in two respiratory samples, *M. pneumoniae* DNA was detected.

*Coxiella burnetii*, causing Q fever, was found in 37 patients (in 12 serum samples and in 25 respiratory samples). *Chlamydophila psittaci* was found in seven patients and *Chlamydomphila pneumoniae* was found in two patients.

In total, 117 (28.7%; 69 male and 48 female) of the 408 patients were positive for one or more viral pathogens. There was no significant difference between viruses infecting men and women. All common respiratory viruses were detected. HRV was detected at the highest frequency, in 34 (8.3%) of the 408 patients. The detection rates for the other viruses were 5-6% for Infa, 4-4% for PIV1, 2-9% for HCoV OC43, 2-2% for Infb, 2-0% for RSV, 1-5% for HCoV NL63, 1-2% for PIV3 and HCoV 229E. For hMPV, PIV 2, HCoV HKU, hBoV, KIPyV, WUPyV and AdV, the detection rates were <1%. PIV4 was not detected.

Co-detection of respiratory viruses

In the 117 virus-positive patients, a total of 136 viruses were detected. Multiple virus infections (≥2) were found in 16 patients (Table 3). Thirteen (3-2%) of the 408 patients had two respiratory viruses and 3 (0-7%) patients had three viruses present in their respiratory samples. PIVs were the most frequently found viral agents in the multiple infected patients and were found in eight patients [PIV 1 (six times), PIV 2 (one time), PIV 3 (three times)], followed by coronavirus in seven patients [HKU (one time), OC43 (two times), 229E (one time), NL63 (four times)] and Infb in four patients, Infa in three patients. HRVs, hBoV, RSV, KIPyV and WUPyV were co-detected in two patients.
Seasonality and frequency of respiratory viruses
The period of the year in which viruses were detected varied per virus as presented in Figure 1. Whereas InfA had one major peak lasting from January to February 2009, InfB was detected throughout the whole study period but at a low rate. Also HRVs were found during the entire year, although a small peak was observed in May/June 2008. RSV was detected only from November 2008 to January 2009 and hMPV from January 2009 to March 2009. HCoV OC43 the most detected coronavirus peaked in December 2008.

Discussion
This study revealed the viral and bacterial aetiology in 263 (64.5%) of 408 patients with community-acquired pneumonia. In spite of using sputum samples, blood cultures, urine for antigen assays, throat swabs and serum samples, no causative agent was found in ~35% of the subjects. This is in line with the literature, where levels of identification of the causative micro-organisms in CAP vary from 46% to 83%. This variation is attributable to differences in detection techniques and in selection of patients who are included. We improved the yield by testing sputum samples for *S. pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci* and *Legionella pneumophila*.

We identified *S. pneumoniae* (22.8%) as the most common single organism in 263 patients, followed by *Coxiella burnetii* (6.8%) and influenza A virus (3.8%). This was similar to other studies, which also reported *S. pneumoniae* as a predominant causative agent. In the Netherlands, CAP affects about 5–10 persons per 1000 inhabitants, and only 5–20% of these
cases are admitted to hospitals. Furthermore, the distribution of pathogens causing CAP may vary by country, owing to geographic differences.

In our study, *Chlamydia pneumoniae* was detected in throat samples of two patients only. Some studies indicated *C. pneumoniae* as one of the most common ‘atypical’ pathogens. However, a recent study by Wellinghausen *et al.*,29 found a low prevalence (<1%) of *C. pneumoniae* which is in accordance with our findings. It is unclear what the reasons are for these different detection rates.

The most important limitation in our study was that we did not include a control group to determine the prevalence of viral respiratory pathogens and bacterial pathogens. In a case–control study by van Gageldonk-Lafeber *et al.*,30 the incidence and aetiology of acute respiratory tract infections in patients visiting their general practitioners was studied and the researchers detected pathogens, mostly viruses, in approximately 30% of the subjects with no respiratory complaints. Another limitation was the incomplete sputum sample collection. The reason was the inability of patients to produce sputum. Therefore, throat swabs were taken of all patients. As the sensitivity of throat swabs may be lower than the sensitivity of sputum, nasopharyngeal sampling or washings, it is possible that we underestimated the prevalence of viruses in our population.

Respiratory viruses were found as the only detectable pathogen in 52 patients who had been included throughout the year, covering all the seasons. Year-round inclusion is important to cover the complete spectrum of respiratory virus infections, because several viruses are known to be found only in particular months of the year.

InflA has been found as the second most frequent pathogen in CAP patients and the most common viral pathogen in all the age groups. In adults, InflA, RSV, rhinoviruses and adenoviruses are recognized as important causes for CAP.32 However, viruses that cause community-acquired pneumonia are often overlooked by clinicians. It still remains unclear whether some respiratory virus can cause pneumonia by itself or whether it needs the help of other respiratory pathogens. In our study, viral and bacterial pathogens were found in 65 (16%) patients. Co-infection rates have been described in 5.7%–22.5% of CAP in other studies. The most common bacterial co-pathogens were *H. influenzae* and *S. pneumoniae*. In agreement with other studies, we found an association between *S. pneumoniae* and viruses. We also found an association between *H. influenzae* and viruses.

In general, blood samples for bacterial culture are relatively easily obtainable, and if positive, they provide a microbiological diagnosis. In our study, 13.1% of the blood cultures revealed a pathogen, which is similar to the results of other studies.

In the literature, *S. pneumoniae* PCR on sputum samples as a diagnostic tool for pneumococcal disease has had mixed results because distinguishing colonization from infection using *S. pneumoniae* PCR is difficult even by quantifying the load.35–38 Culture has important limitations as well. Prior antibiotic therapy is of great influence on the growth of *S. pneumoniae* in sputum samples and blood cultures. Several studies found that during antibiotic treatment sputum samples became rapidly negative for *S. pneumoniae* in contrast to the *S. pneumoniae* PCR that remained positive. In our study, *S. pneumoniae* was detected in all culture-positive sputum samples and in many culture-negative sputum samples by PCR, presumably reflecting the increased sensitivity of molecular technique above traditional culture methods. Of the 60 patients with *S. pneumoniae* as causative organism, the pneumococcal antigen assay was positive in 62%. The results of the pneumococcal antigen assay showed a lower sensitivity compared with data reported by others.42 The reason for this difference in

### Table 3. Positive patients with multiple viruses

| Patients |
|----------|
| 1 | Influenza B virus + WU polyomavirus + Human Coronavirus NL63 |
| 2 | Parainfluenza virus 1 + Human rhinovirus + Human Coronavirus NL63 |
| 3 | Parainfluenza virus 1 + Human bocavirus + Human Coronavirus NL63 |
| 4 | Parainfluenza virus 1 + Human Coronavirus NL63 |
| 5 | Parainfluenza virus 3 + Human rhinovirus |
| 6 | Parainfluenza virus 3 + Parainfluenza virus 1 |
| 7 | Parainfluenza virus 3 + Parainfluenza virus 2 |
| 8 | Human Coronavirus HKU + Parainfluenza virus 1 |
| 9 | Human Coronavirus OC43 + Human coronavirus 229E |
| 10 | Human Coronavirus OC43 + Human bocavirus |
| 11 | Respiratory syncytial virus + KI polyomavirus |
| 12 | Respiratory syncytial virus + Influenza B virus |
| 13 | Influenza A virus + Influenza B virus |
| 14 | Influenza A virus + WU polyomavirus |
| 15 | Influenza A virus + Parainfluenza virus 1 |
| 16 | Influenza B virus + KI polyomavirus |
sensitivity is unclear but could be explained by the influence of prior antibiotic therapy, not concentrating the urine before executing the assay, and the fact that the pneumococcal antigen assay is more sensitive in patients who are bacteremic than in patients without a bacteremia.

*Legionella pneumophila* was diagnosed in 15 cases (3.8%), which is in agreement with results obtained by previous studies.44

Finally, in our study population, a relatively large number of CAP cases were caused by *Coxiella burnetii*. This was owing to a Q fever outbreak in our area with over 4000 notified cases in the Netherlands between 2007 and 2010.45

**Conclusions**

In 408 adult patients presenting at the hospital with CAP, a pathogen was demonstrated in 64.5%. *S. pneumoniae*, influenza A virus and *Coxiella burnetii* were the three most frequent pathogens. Mixed viral and bacterial infections were frequently observed, and in 29% of the patients with CAP, a virus was detected, including 13% of the patients in which only viruses were detected. Further investigations are warranted to elucidate the importance of viruses as causative agents in the pathogenesis of CAP in adult patients.

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**Conflict of interest**

The authors declare no conflict of interest.

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