Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

The aetiology of community-acquired pneumonia and implications for patient management

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ABSTRACT

Purpose: Understanding which pathogens are associated with clinical manifestation of community-acquired pneumonia (CAP) is important to optimise treatment. We performed a study on the aetiology of CAP and assessed possible implications for patient management in the Netherlands.

Methods: **P**atients with CAP attending the emergency department of a general hospital were invited to participate in the study. We used an extensive combination of microbiological techniques to determine recent infection with respiratory pathogens. Furthermore, we collected data on clinical parameters and potential risk factors.

Results: From November 2007 through January 2010, 339 patients were included. Single bacterial infection was found in 39% of these patients, single viral infection in 12%, and mixed bacterial-viral infection in 11%. *Streptococcus pneumoniae* was the most frequently identified pathogen (22%; n=74). Infection with atypical bacteria was detected in 69 (20%) of the patients.

Conclusion: Initial empirical antibiotics should be effective against *S. pneumoniae*, the most common pathogen identified in CAP patients. The large proportion of patients with infection with atypical bacteria points to the need for improved diagnostic algorithms including atypical bacteria, especially since these atypical bacteria are not covered by the first-choice antibiotic treatment according to the recently revised Dutch guidelines on the management of CAP.

KEYWORDS

Antibiotic treatment, clinical diagnosis, community-acquired pneumonia

INTRODUCTION

Pneumonia is a common and potentially serious respiratory disease, mainly in children and the elderly. It is the third most common cause of death worldwide.[1-5] Community-acquired pneumonia (CAP) is defined as an acute symptomatic infection of the lower respiratory tract which develops outside the hospital or nursing home, and whereby a new infiltrate is demonstrated on chest X-ray.[6]

Several studies show that *Streptococcus pneumoniae* is the predominant aetiological agent of CAP, followed by other bacteria such as *Haemophilus influenzae, Staphylococcus aureus, Chlamydia pneumoniae, Legionella* spp, and *Mycoplasma pneumoniae*.[1,7-12]

Recent developments in molecular diagnostics have resulted in increased detection of respiratory viruses, including influenza virus, para-influenza virus and respiratory syncytial virus (RSV), in patients with pneumonia.[2,13-17] Reported studies on the aetiology of CAP diverge with respect to the proportions of detected pathogens as well as the diagnostic deficit, i.e. the proportion of patients for which no pathogen could be detected. These discrepancies are attributable to differences in the epidemiology of pathogens, study population, available patient specimens and the diagnostic methods used.

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Changes in the aetiology of CAP and in bacterial antibiotic-resistance patterns over time might have important implications for patient management. However, in clinical practice, causative pathogens remain unknown in the majority of CAP patients, since microbiological tests are not used or are limited to blood and sputum cultures for bacterial causes. Most patients are therefore treated empirically.[1,13,18,19] Until recently, initial therapy with amoxicillin or doxycycline was recommended for patients with mild CAP in the Netherlands.[20] Due to the increasing resistance of *S. pneumoniae* to doxycycline, amoxicillin is now assigned as first-choice treatment.[6,21]

To optimise future treatment choices, clear understanding of respiratory pathogens in relation to the clinical manifestation of CAP is important. We therefore performed a prospective observational study on the aetiology of CAP in the Netherlands, using an extensive combination of microbiological and molecular techniques. Furthermore, we studied potential relations between the type of identified pathogen and specific host factors, comorbidity, and known risk factors for CAP.

MATERIAL AND METHODS

Study population

Patients attending the emergency department of the Jeroen Bosch Hospital (JBH) in 's-Hertogenbosch, serving a rural area in the Netherlands covering about 2% of the Dutch population, with CAP were invited to participate in the study during the time period November 2007 through January 2010. CAP is defined as an acute lower respiratory tract infection with at least two of the following clinical symptoms: new onset of cough, sputum production or change in colour of respiratory secretions in patients with chronic cough, fever or hypothermia or physical examination consistent with pneumonia, whereby a new infiltrate is demonstrated on chest X-ray. Exclusion criteria were age <18 years, being transferred from another hospital and being a nursing home resident. Ethical approval for the study was obtained from the Medical Ethical Review Committee (Tilburg, the Netherlands) and informed consent was obtained from all included patients.

At hospital admission, the attending physician or triage nurse used Case Report Forms (CRFs) to report data on: 1) patient characteristics, 2) use of antibiotics in the past two weeks, 3) influenza vaccination status, 4) comorbidity: COPD, congestive heart failure, malignancy, immune deficiency, renal disease, liver failure and cerebrovascular disease, (5) potential risk factors for CAP, such as influenza-like illness (ILI) in the past four weeks and smoking status, 6) specific medical history and physical examination, and (7) Pneumonia Severity Index (PSI) and CURB-65 score.[22]

Laboratory analyses

Blood, sputum and urine specimens, as well as combined nose and throat swabs, were obtained at hospital admission. Sera for paired serology were collected at acute (day of hospital admission) and convalescence phases (>28 days after hospital admission). Microbiological testing (*table 1*) was performed both at the JBH and the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands). All collected specimens were sent immediately to the JBH laboratory. Subsequently, EDTA blood specimens for *S. pneumoniae* PCR were sent to the RIVM within 48 hours after sampling. Following routine microbiological testing at JBH, the remaining serum and sputum specimens were stored at -20°C and -80°C, respectively. Immediately after sampling, combined nose and throat swabs were added to a virus transport medium (VTM). The swab was thoroughly vortexed in the VTM, and the VTM was distributed to three ampoules. One of these was used for diagnostic investigation at JBH and the other two were stored at -80°C. Urine specimens for detection of *S. pneumoniae* capsular polysaccharide were stored at -80°C. Periodically, the serum, sputum and urine samples as well as the VTM from the combined nose and throat swabs were transported to the RIVM for additional study-specific microbiological testing. Before performing additional tests at the RIVM, the sputum specimens were pre-treated using the MagNA Lyser (Roche) instrument to disrupt the sputum before nucleid acid extraction.

Statistical analyses

The data were entered in a Microsoft Access database and analysed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

Descriptive statistics were calculated, results of categorical variables are presented as percentages and continuous variables as median with range. Patients were grouped into four categories based on the results of blood culture, sputum culture, PCR, rapid antigen detection test, and rise in titre in paired sera. In case of infection with *S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis*, *S. aureus*, Viridans streptococci, *Escherichia coli*, *Enterococcus* spp, *Haemophilus parainfluenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, group A beta-haemolytic streptococci or group B beta-haemolytic streptococci, patients were assigned to the group 'typical bacteria'(1). In case of infection with *Coxiella burnetii*, *M. pneumoniae*, *Legionella pneumophila*, *Chlamydia psittaci* or *C. pneumoniae*, but without infection with the earlier mentioned (typical) bacteria, patients were assigned to the group 'atypical bacteria'(2). Patients with only viral infection were classified as 'only viral pathogens'(3) and the remaining patients were assigned to the group 'no pathogens'(4). Furthermore, we distinguished between patients with simultaneous bacterial and viral infection

Table 1. *Microbiological techniques performed on different samples at Jeroen Bosch Hospital ('s-Hertogenbosch, the Netherlands- JBH) and the National Institute for Public Health and the Environment (Bilthoven, the Netherlands – RIVM)*

Sample	Microbiological technique	Micro-organism	Laboratory
Whole blood	Aerobic and anaerobic blood culture	Among others *Streptococcus pneumonia*, group A beta-haemolytic streptococci, group B beta-haemolytic streptococci, *Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus* spp, anaerobic bacteria	JBH
EDTA blood	PCR	*S. pneumoniae*	RIVM
Serum	Serology	*Mycoplasma pneumoniae, Chlamydia psittaci, Legionella pneumophila 1-6, Coxiella burnetii*	JBH
	PCR	*C. burnetii, L. pneumophila*	JBH
Sputum	Bacterial culture a gram preparation	Among others *S. pneumoniae*, group A beta-haemolytic streptococci, group B beta-haemolytic streptococci, *H. influenzae, Haemophilus parainfluenzae, Moraxella catarrhalis, S.aureus, P.aeruginosa, Enterococcus* spp, anaerobic bacteria	JBH
	PCR	*L. pneumophila*	JBH
	PCR	*M. pneumoniae, Chlamydia pneumoniae*, influenza viruses, parainfluenza viruses, respiratory syncytial virus, coronaviruses[a], rhinovirus, adenovirus, enterovirus, human metapneumovirus	RIVM
Combined nose and throat swab	PCR	*L. pneumophila*	JBH
	PCR	*M. pneumoniae, Chlamydia pneumoniae*, influenza viruses, parainfluenza viruses, respiratory syncytial virus, coronaviruses[a], rhinovirus, adenovirus, enterovirus, human metapneumovirus	RIVM
Urine	Rapid antigen detection	*L. pneumophila* serogroup 1, *S. pneumoniae*	JBH
	Capsular polysaccharide identification	*S. pneumoniae*	RIVM

[a]**coronavirus types OC43, NL63 and 229E.**

(mixed bacterial-viral infections) and patients with only bacterial or only viral infection (single infections).

We used multivariate logistic regression analysis to examine whether gender, age ≥70 years, comorbidity, use of antibiotics in the past two weeks, ILI in the past four weeks, and smoking behaviour were associated with the type of infection detected (dependent variable). Variables with a p value ≤0.20 in the univariate model were included in the multivariate model. Backward selection was used to identify covariates that were independently associated with type of infection. Odds ratios (OR) were presented with 95% confidence intervals (CI).

RESULTS

Aetiology

In the period November 2007 through January 2010, a total of 339 patients meeting the study criteria were included. We were able to detect infection with one or more bacterial pathogens for 168 (50%) of the 339 patients (*table 2*). Furthermore, we assessed viral infection for 77 (23%) of the 339 patients (*table 3*). For 37 (11%) of the 339 patients both bacterial and viral infection was assessed. Despite the use of an extensive combination of microbiological and molecular techniques, no infection could be determined for 131 (39%) of the 339 patients.

The most frequently identified bacterial pathogen was *S. pneumoniae* (22%), followed by *C. burnetii* (14%), *M. pneumoniae* (6%) and *H. influenzae* (4%). Mixed bacterial-viral infection was found for 25 (34%) of the 74 patients with *S. pneumoniae* infection, for seven (35%) of the 20 patients with *M. pneumonia* infection and for five (33%) of the patients with *H. influenzae* infection, compared with only two (4%) of the 48 patients with *C. burnetii* infection. Rhinovirus was the most commonly identified viral pathogen (9%), followed by influenza virus type A (5%) and human metapneumovirus (hMPV; 4%). For 19 (66%) of the 29 patients with rhinovirus infection simultaneous bacterial infections were detected. For hMPV and influenza virus type A these numbers were six (46%) of the 13 and three (33%) of the 13, respectively.

Patient characteristics

Based on the identified infections, patients were grouped into the categories: 1) typical bacteria (n=99); 2) atypical bacteria (n=69); 3) only viral pathogens (n=40); and 4) no pathogens (n=131). Characteristics of these categories

Table 2. *Prevalence of bacterial infections identified in patients with community acquired pneumonia (CAP) attending the emergency room of a general hospital, November 2007-January 2010*

	All CAP patients n=339		CAP patients with only bacterial infection n=131		CAP patients with mixed bacterial-viral infection n=37	
Bacterial pathogen	**n**	**%**	**n**	**%**	**n**	**%**
Typical bacteria						
Streptococcus pneumoniae	74	21.8	49	37.4	25	67.6
Haemophilus influenzae	15	4.4	10	7.6	5	13.5
Moraxella catarrhalis	5	1.5	2	1.5	3	8.1
Staphylococcus aureus	3	0.9	3	2.3	0	-
Viridans streptococci	3	0.9	3	2.3	0	-
Escherichia coli	2	0.6	2	1.5	0	-
Enterococcus spp	2	0.6	2	1.5	0	-
Haemophilus parainfluenzae	1	0.3	1	0.8	0	-
Klebsiella pneumoniae	1	0.3	0	-	1	2.7
Pseudomonas aeruginosa	1	0.3	1	0.8	0	-
Group A beta-haemolytic streptococci	1	0.3	0	-	1	2.7
Group B beta-haemolytic streptococci	1	0.3	1	0.8	0	-
Atypical bacteria						
Coxiella burnetii[a]	48	14.2	46	36.8	2	5.4
Mycoplasma pneumoniae	20	5.9	13	9.9	7	18.9
Legionella pneumophila	7	2.1	6	4.6	1	2.7
Chlamydia psittaci	3	0.9	3	2.3	0	-
Chlamydia pneumoniae	2	0.6	2	1.5	0	-
Any bacterial pathogen[b]	168	49.6	NA[c]		NA	

[a]36 (75%) of the 48 infections with *C. burnetii* were found during the peak of the Q fever outbreak in 2009;[52] [b]One bacterial pathogen was detected for 147 patients, while two and three pathogens were detected for 19 and 2 patients, respectively; [c]not applicable.

Table 3. *Prevalence of viral infections identified in patients with community acquired pneumonia (CAP) attending the emergency room of a general hospital, November 2007-January 2010*

	All CAP patients n=339		CAP patients with only viral infection n=40		CAP patients with mixed bacterial-viral infection n=37	
Viral pathogen	**n**	**%**	**n**	**%**	**n**	**%**
Rhinovirus	29	8.6	10	25.0	19	51.4
Human metapneumovirus	13	3.8	7	17.5	6	16.2
Influenza virus type A[a]	13	3.8	10	25.0	3	8.1
Para-influenza virus	10	2.9	5	12.5	5	13.5
Coronavirus	6	1.8	2	5.0	4	10.8
Respiratory syncytial virus	5	1.5	4	10.0	1	2.7
Influenza virus type B	3	0.9	3	7.5	0	-
Adenovirus	1	0.3	0	-	1	2.7
Enterovirus	1	0.3	1	2.5	0	-
Any virus[b]	77	22.7	NA[c]		NA	

[a]10 (77%) of the 13 infections with influenza virus type A were found during the influenza pandemic in 2009;[53] [b]One viral pathogen was identified in 73 patients and two viral pathogens were identified in 4 patients; [c]not applicable.

are summarised in *table 4*. The category of atypical bacteria stands out with the relatively low median age, high proportion of males, low proportion of comorbidity, and high prior antibiotic use. The median age of the 69 patients with infection with atypical bacteria was 59 years (range: 18-96 years) and 77% were male, compared with 67 years (range: 20-96 years) and 59% male in the 268 remaining patients. *Figure 1* shows the age distribution of the study population by the type of identified pathogen. Comorbidity was reported for 42% of the patients, with COPD as most frequently reported underlying disorder (32%). For patients with infection with atypical bacteria, comorbidity was reported for 13 (19%) of the 69 patients, compared with 130 (49%) of the 268 remaining patients. Antibiotic use prior to hospitalisation was reported for 48% of the patients. The most commonly used types of antibiotics were penicillins (56%), followed by tetracyclines (30%). Prior use of penicillins, which are considered not effective against some of the atypical bacteria such as *C. burnetii*, was remarkably often reported in patients with infection with atypical bacteria (74%). Severe pneumonia, i.e. PSI risk class IV-V and/or CURB-score ≥2, was reported for 20 (32%) of the 62 patients with infection with atypical bacteria, compared with 146 (61%) of the 238 other patients.

These observations were confirmed in multivariate logistic regression analyses that showed age ≥70 years (OR 1.8; 95% CI 1.1-3.3) and prior ILI (OR 3.5; 95% CI 1.2-9.8) to be significantly more common among CAP patients with viral infection compared with patients with bacterial infection. Comparing CAP patients with infection with atypical bacteria to patients with infection with typical bacteria showed that patients with 'atypical pneumonia' had used antibiotics significantly more often before hospitalisation (OR 2.4; 95% CI 1.1-5.2) and had less comorbidity (OR 0.2; 95% CI 0.07-0.4). When infection with atypical bacteria was limited to *M. pneumoniae, L. pneumophila, C. psittaci* and *C. pneumoniae* we found that patients with 'atypical

Table 4. *Baseline characteristics of the patients with community acquired pneumonia (CAP) attending the emergency room of a general hospital grouped by the type of detected pathogen, November 2007-January 2010*

Characteristics	Typical bacteria n=99 No. (%) of patients[a]		Atypical bacteria n=69 No. (%) of patients[a]		Viral pathogens n=40 No. (%) of patients[a]		No pathogens n=131 No. (%) of patients[a]		All patients n=339 No. (%) of patients[a]	
Median age [range]	66 years [24-96]		59 years [18-96]		68 years [20-86]		67 years [26-89]		66 years [18-96]	
Distribution by age group										
<50 years	20	(20.2)	25	(36.2)	4	(10.0)	32	(24.4)	81	(23.9)
>50 and <70 years	43	(43.4)	27	(39.1)	19	(47.5)	42	(32.1)	131	(38.6)
>70 years	36	(36.4)	17	(24.6)	17	(42.5)	57	(43.5)	127	(37.5)
Male gender	59	(59.6)	53	(76.8)	23	(57.5)	77	(58.8)	212	(62.5)
Comorbidity[b]										
COPD[c]	41	(51.3)	5	(8.3)	13	(37.1)	33	(28.2)	92	(31.5)
Malignancy	10	(11.6)	4	(6.7)	3	(8.3)	8	(7.0)	25	(8.4)
	9	(10.6)	2	(3.2)	3	(7.7)	10	(8.4)	24	(7.9)
Renal disease	7	(8.1)	4	(6.3)	7	(17.9)	14	(11.8)	32	(10.4)
Cerebrovascular disease	2	(2.4)	2	(3.3)	1	(2.8)	6	(5.1)	11	(3.7)
Immune deficiency	-		-		-		2	(1.7)	2	(0.6)
Congenital heart defect	1	(1.1)	2	(3.2)	1	(2.6)	0	-	4	(1.3)
Liver failure	54	(55.7)	13	(18.8)	20	(50.0)	56	(42.8)	134	(42.4)
Any comorbidity										
Prior antibiotic use[b,d]	29	(40.3)	34	(57.6)	16	(48.5)	52	(47.3)	131	(47.8)
Type of antibiotic[b,e]										
Penicillins	12	(41.4)	25	(73.5)	8	(50.0)	27	(54.0)	72	(55.8)
Tetracyclines	7	(24.1)	8	(23.5)	6	(37.5)	17	(34.0)	38	(29.5)
Quinolones	7	(24.1)	2	(5.9)	-		2	(4.0)	11	(8.5)
Other	8	(27.6)	3	(8.8)	3	(18.8)	7	(14.0)	21	(16.3)
Prior influenza-like illness[f]	7	(11.1)	5	(10.4)	8	(28.6)	16	(18.2)	36	(15.9)
Smoking	31	(39.7)	24	(44.4)	9	(27.3)	29	(28.2)	93	(34.7)
Distribution according to PSI score[b,g]										
I-III	55	(64.7)	48	(77.4)	19	(54.3)	69	(59.5)	191	(64.1)
IV	22	(25.9)	8	(12.9)	11	(31.4)	39	(33.6)	80	(26.8)
V	8	(9.4)	6	(9.7)	5	(14.3)	8	(6.9)	27	(9.1)
median score [range]	82 [23-184]		62 [23-160]		90 [26-176]		88 [21-179]		81 [21-184]	
Distribution according to CURB-65 score[b,h]										
0-1	36	(41.9)	43	(70.5)	17	(48.6)	51	(44.0)	174	(49.3)
2	32	(37.2)	10	(16.4)	10	(28.6)	40	(34.5)	92	(30.9)
>2	18	(20.9)	8	(13.1)	8	(22.9)	25	(21.5)	59	(19.8)
Severe pneumonia[i]	55	(64.0)	20	(32.0)	21	(60.0)	70	(59.8)	166	(55.3)

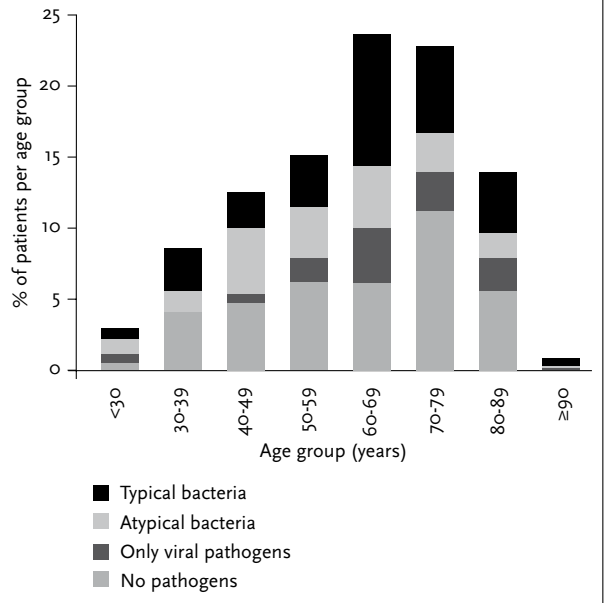
[a]Except where otherwise indicated; [b]Percentages are based on the number of patients for which information was available. These numbers can vary between variables; [c]COPD = chronic obstructive pulmonary disease; [d]Use of antibiotics in the past two weeks before attending the emergency department: 13 patients used two different types of antibiotics prior to hospitalisation; [f]influenza-like illness in the past four weeks before attending the emergency department; [g]Pneumonia Severity Score (PSI), risk class: I-III=low, IV=moderate, V=severe; [h]CURB-65 score: 0-1=mild pneumonia; 2=moderate pneumonia; 3-5=severe pneumonia; [i]severe pneumonia is defined as PSI risk class IV-V and/or CURB-score ≥2.

pneumonia' had less comorbidity (OR 0.3; 95% CI 0.1-0.9) compared with patients with 'typical pneumonia'. Age ≥70 years was statistically significantly more common in the 37 patients with mixed bacterial-viral infection, compared with the 171 patients with a single infection (OR 2.5, 95%CI 1.2-5.1). Comparing patients for whom no pathogens could be identified with those for whom one or more potential pathogens were identified showed no statistically significant differences between the two groups with respect to age ≥70 years, gender, comorbidity, prior use of antibiotics, prior ILI and smoking behaviour.

DISCUSSION

While *S. pneumoniae* remains the most common pathogen identified in adult CAP patients, we also detected infection with atypical bacteria in a large proportion of patients.[1,9,12,13,16,17] Initial empirical antibiotics should be effective against *S. pneumoniae,* which is the main reason that amoxicillin is recommended as first-choice treatment in the recently revised Dutch guidelines on the management of CAP.[6,21] We identified *C. burnetii* as the second most common pathogen. This is not surprising, as our study coincided in time and place with a large Q fever outbreak in the Netherlands.[23,24] However, in

Figure 1. *Age distribution of patients included in the study on the aetiology of community-acquired pneumonia in the period from November 2007 through January 2010, by age group and type of identified pathogen*



Spain, in a non-outbreak setting, 72/390 (18.5%) of CAP proved to be caused by *C. burnetii*, illustrating that the importance of *C. burnetii* may be underestimated in some aetiological studies.[12] We identified *M. pneumoniae* as third most common pathogen. Infection with atypical bacteria, including *C. burnetii* and *M. pneumoniae*, was found in 69 (20%) of the 339 patients included in our study, mainly in younger persons (median age 59 years) with relatively low pneumonia severity scores and little comorbidity. This is in line with other studies investigating the role of atypical pathogens in the aetiology of CAP.[18,25-29] Due to the recent changes in the Dutch guidelines on the management of CAP, atypical bacteria are no longer covered by the first-choice antibiotic treatment, because they are generally not susceptible to amoxicillin.[6] In the initial stage of the Q fever epidemic in the Netherlands, a quarter of the Q fever patients who were initially treated with penicillins, such as amoxicillin, had a less favourable outcome than those treated with tetracyclines, such as doxycycline.[30] In the present study, 74% of the patients with infection with atypical bacteria received penicillins, mainly amoxicillin, prior to hospitalisation.

The Dutch Q fever outbreak as well as recent outbreaks of *M. pneumoniae* in several European countries show that atypical bacteria are likely to remain an important cause of CAP.[18,23,24,31-36] This underlines the importance for physicians to be alert for atypical bacteria. Especially in younger and previously healthy patients, they could consider the use of empiric antibiotics (e.g. doxycycline) covering these bacteria. British and North American guidelines on the treatment of CAP recommend initial therapy with combinations of penicillins and macrolides, or monotherapy with quinolones for patients hospitalised with CAP.[37-39] Although atypical bacteria are covered by the recommended therapies, implementation of these guidelines in the Netherlands would result in a considerable increase in antibiotic use with possible adverse consequences.[40] A more preferable option is to improve diagnostic algorithms including typical bacteria, atypical bacteria and viruses in the first-line diagnostics of CAP, taking into account the differences in sensitivity and specificity of the various microbiological and molecular assays.

The increased use of molecular diagnostics has improved our knowledge about the aetiology of CAP.[13,14,17,41,42] However, the exact role of viruses in the pathogenesis of pneumonia remains unclear.[15,16] Rhinovirus and RSV are known to be capable of invading and replicating in the lower respiratory tract mucosa,[43,44] but it is unclear whether such a virus infection is a primary cause of pneumonia or paves the path for a secondary bacterial infection. Mixed bacterial-viral infections are increasingly diagnosed in CAP patients with rates varying between 6-35%, corresponding to the 11% we found.[2,15,16,45,46] Furthermore, our study confirmed that the commensal pathogens *S. pneumoniae* and *H. influenzae* are commonly identified in patients with mixed bacterial-viral infections.[15,16,41,45,47] On the contrary, *C. burnetii* was rarely involved in these mixed infections.

Although *S. pneumoniae* is the most commonly detected pathogen in CAP in many studies worldwide, the proportion of other detected micro-organisms, particularly atypical pathogens, varies by region as well as over of time.[3] Since our study by chance coincided in time and place with a large Q fever outbreak, the results cannot be extrapolated directly to other years and other countries. To improve the insight in occurrence, fluctuations and seasonality of pathogens associated with CAP, continuous microbiological surveillance is therefore preferred over aetiological studies. Since flora detected in respiratory specimens are not necessarily causative pathogens, but can also indicate the presence of commensal micro-organisms or asymptomatic infections, it is important to include information on clinical presentation in microbiological surveillance.[48]

One of the strengths of our study is the well-defined study population: various data on both patient characteristics and clinical disease were available, and CAP was confirmed by

chest X-ray for all patients. An additional strength is the use of an extensive combination of microbiological and molecular techniques, including molecular diagnostics on both bacterial and viral pathogens. Nevertheless, no pathogens could be detected in 40% of the patients. This is in line with several recent studies on the aetiology of CAP, reporting a failure of pathogen detection for 24-44% of the patients.[12,13,15,16,49,50] Because of this diagnostic deficit, which might partly be explained by the difficulty to collect paired serum samples as well as high quality sputum samples, we undoubtedly underestimated the prevalence of several pathogens. Another limitation of our study was the absence of a control group enabling the investigation of a causal link between clinical disease and detected pathogens. In a previous case-control study, we detected respiratory pathogens, mainly viruses, in ~30% of the persons without respiratory symptoms at the moment of specimen collection.[51] To optimise the comparability, controls should be matched to case patients by sample collection method. However, this is impossible for sputum samples since asymptomatic persons do not produce sputum.

In conclusion, our study showed that infection with atypical bacteria is commonly found in patients hospitalised with CAP. Especially in relatively young patients without underlying medical conditions and with relatively low pneumonia severity scores, physicians should be alert for atypical bacteria, which are not covered by the current first-choice antibiotic treatment according to the Dutch guidelines on the management of CAP. Improved diagnostic algorithms including both typical bacteria, atypical bacteria and viruses in the first-line diagnostics of CAP might be helpful for the dilemma whether to use empirical amoxicillin as most effective against *S. pneumoniae* or doxycycline as effective against most atypical bacterial pathogens.

ACKNOWLEDGEMENTS

We thank Gert Broekhaar (RIVM/Department of Statistics, Mathematical Modelling and Data Logistics) for the development of the database; Kees van Elst (Jeroen Bosch Hospital) and Loek Timmermans (RIVM/Centre for Infectious Disease Control) for providing data; Shireen Jenny, Pieter Overduin, Astrid Glas, Maaike van den Beld, Cornelis Blauwendraat, Janneke Bloem, Airien Harpal, Dieneke Hoeve, Iselle Koopmans and Olaf Nijst (RIVM/Centre for Infectious Disease Control) for technical laboratory assistance; Merel Wassenaar, Danielle Roorda, Janneke van der Ven, Janko van Beek, Yolanda van Weert, for their cooperation in the data collection.

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