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Aetiological role of viral and bacterial infections in acute adult lower respiratory tract infection (LRTI) in primary care.

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ABSTRACT

Background Lower respiratory tract infections (LRTI) are a common reason for consulting General Practitioners (GP). The aetiology is unknown in the majority of cases, yet the majority result in antibiotic prescription. We investigated the aetiology of LRTI in a prospective, controlled study.
Methods Adults (n=80) presenting to GP's with acute LRTI were recruited with controls (n=49) over 12 months. Throat swabs, nasal aspirates (patients and controls) and sputum (patients) were obtained and PCR and RT-PCR assays used to detect *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila*, influenza viruses (AH1, AH3 and B), parainfluenza viruses 1-3, coronaviruses, RSV, adenoviruses, rhinoviruses and enteroviruses . Standard sputum bacteriology was also performed. Outcome was recorded at a follow up visit.
Results Potential pathogens were identified in 55 LRTI patients (69%) and 7 controls (14%) ($p<0.0001$). The identification rate was 63% (viruses) and 26% (bacteria) for patients and 12% ($p<0.0001$) and 6% ($p=0.013$) for controls. The most common organisms identified were rhinoviruses (33%), influenza viruses (24%) and *Streptococcus pneumoniae* (19%) compared with 2% ($p<0.001$), 6% ($p=0.013$) and 4% ($p=0.034$) in controls. Multiple pathogens were identified in 18/80 (22.5%) LRTI patients and 2/49 (4%) controls ($p=0.011$). Atypical organisms were rarely identified. Cases with bacterial aetiology were clinically indistinguishable from those with viral aetiology
Conclusion Acute adult LRTI presenting to GP's is a predominantly viral illness most commonly caused by rhinoviruses and influenza viruses.

KEY WORDS
Lower respiratory tract infection, polymerase chain reaction, rhinovirus, influenza virus, *Streptococcus pneumoniae*.

INTRODUCTION

Respiratory tract infections (RTI) are the most common reason for primary care consultations (1). One-third of all RTI's are lower respiratory tract infections(LRTI) (2) with an incidence of 44-50/1000 (3;4). Most LRTI in primary care in Europe including the UK are treated with antibiotics (2) despite the limited evidence of clinical benefit and the fact overuse of antibiotics is associated with an increasing rate of antibiotic resistance (5).

The aetiology of acute LRTI presenting to primary care physicians is also poorly established due to inadequate patient definition and limited pathogen detection (16-55%; 3,4, 6-8), resulting from a combination of inadequate clinical sampling and pathogen detection methodology, particularly for respiratory viruses. For example, a study using serology for respiratory viruses and atypical bacteria found evidence of infection in only 16% of adults with acute bronchitis (8). Yields of 29% and 40% were achieved when viral culture of nasopharyngeal washings and sputum culture for bacteria was added (6,7). A diagnostic yield of 44% was achieved but required a range of samples including induced sputum, urine, saliva, throat swab and blood for serology) to identify pathogens (4).

Thus, little is known about the aetiology of ambulatory patients with mild or moderate LRTI without evidence of pneumonia. Moreover, none of the previous studies investigating the aetiology of LRTI in primary care have included organism detection in a control population. A comparison of the detection rates of potential viral and bacterial pathogens between patients and matched controls is essential in attributing a pathologic role for the organisms detected. We present the results of a 12 month observational study of the aetiology of LRTI using both culture and nucleic acid amplification techniques for the detection of potential pathogens.

METHODS

The study was approved by the Royal Free NHS Trust local ethics committee. Written consent was obtained from all patients and controls prior to inclusion into the study.

Subjects

Adult patients (≥ 18 years) were recruited from two general practices with a multi-ethnic patient population of 15 000 from social classes one to five. All patients were surgery attendees, no recruitment was undertaken out of hours or on home visits. Acute LRTI were defined as a new or worsening cough and at least one other lower respiratory tract symptom for which there was no other explanation, present for ≤21 days (3). Patients were excluded if they had underlying chronic suppurative lung disease (defined as bronchiectasis, lung abscess or empyema), tuberculosis, immunodeficiency or previous study participation (3 weeks). Antibiotic prescription, investigation and follow up were at the discretion of the GP. Age, gender and season matched controls were recruited from general practice patients attending for non- respiratory and non-infective illnesses and other healthy volunteers with no history of respiratory tract symptoms for two months prior to recruitment using the same exclusion criteria as patients. Recruitment occurred between May 2000 and April 2001.

Study design

All patients were seen by the study research fellow (DDC) at the initial GP consultation and at a planned visit four weeks later. Demographic and clinical data (duration and nature of symptoms, past medical history, pulse rate, respiratory rate and temperature) and details of the patient's management (antibiotic therapy) were recorded.

Specimen collection and processing

Throat swabs, nasal aspirates and blood for C-reactive protein (CRP, normal range <5mg/dl) (from patients and controls) and sputum (from patients when possible) were collected at the first visit. In those patients who were prescribed antibiotics specimens were obtained before antibiotics were taken.

Samples to be analysed by the viral and atypical bacterial Reverse Transcription-PCR (RT-PCR) panel

Nasal aspirates were collected, mixed with 4ml of viral transport medium (VTM), aliquotted and stored at -70°C within 1 hour of sampling (9). When rhinorrhoea was absent or minimal it was induced using a single 100µl nasal spray to each nostril of sterile 8mg/ml histamine (Nova Laboratories Ltd, Leicester, UK) (9). A sputum plug was mixed in 4ml VTM, aliquotted into four sterile RNAase free microtubes (Bioquote Ltd) and stored at -70°C within 1 hour of sampling.

Samples to be analysed by the bacterial PCR panel and by standard sputum culture

Sputum was processed on the day of collection and standard Gram's staining and bacterial culture performed. Heat-killed sputum aliquots were stored for PCR at -70ºC. Throat swabs were transported to the laboratory within 24 hours of collection, stored at 4ºC for up to 48 hours and then stored at -70ºC.

Sample processing for PCR

DNA extraction for PCR

Throat swabs were expressed in 1ml DNAse free sterile water. For DNA extraction either 250 µl of throat swab diluent or 250 µl sputum aliquots were mixed with an equal volume of 10% Chelex (Sigma, UK) and the supernatant removed after centrifugation and used as

the sample template for PCR. DNA was extracted from positive controls (*S. pneumonaie*, *S. aureus*, *H. influenzae*, *M. pneumonaie*, *L. pneumophila*, *M. tuberculosis*, *E. coli*, *P. aeruginosa*, group C streptococcus, viridans type streptococci, coagulase negative staphylococcus and candida species) by proteinase K and 10% sarkosyl digestion followed by a standard phenol-chloroform extraction method. The positive control for *C. pneumonaie* was a plasmid containing the *C. pneumoniae* outer membrane protein gene.

RNA extraction for RT-PCR

RNA extraction from 140µl of sputum and nasal aspirate aliquots and positive controls (influenza viruses AH1, AH3 and B, parainfluenza viruses 1, 2 and 3, rhinovirus 16, coronaviruses 229E and OC43, RSV, adenovirus, *M. pneumonaie* and *C. pneumonaie)* was performed using QIAamp viral RNA mini spin columns following the manufacturers instructions (QIAGEN Ltd, West Sussex, UK).

Reverse transcription

RNA was reverse transcribed using both random hexamers (Promega, Southampton, UK) and a specific picornavirus (rhinovirus and enterovirus) primers, OL27 (9) (Oswel, Southampton, UK) to produce cDNA representative of all RNA species in the original clinical sample and picornavirus specific cDNA respectively.

PCR Protocols

The RT-PCR panel was used to identify *M. pneumonaie* (10), *C. pneumonaie* (11), influenza viruses AH1, AH3 and B (12), parainfluenza viruses 1-3 (13), coronaviruses 229E and OC43 (14), RSV A & B (15), adenoviruses (16) and rhinoviruses and enteroviruses (17). The PCR panel was used to identify *L. pneumophila* (18), *M. pneumonaie* (10), *C. pneumonaie* (19) and *S. pneumonaie* using a novel PCR. This protocol was designed and optimised to target the pneumolysin gene using TGTTGAGACTAAGGTTACAGCT (PNL1) and ACCTGAGGATAGAGAGTTGTTC (PNL2) primers. Positive and negative controls were included in each series of up to 20 samples.

Statistical analysis

Statistical significance was assessed using the χ^2 test for categorical variables and the Mann-Whitney U test and Student's *t* test for non-parametric and parametric continuous variables respectively using GB-STAT™ statistics software (Dynamic Microsystems, Inc, Silver Spring, USA). A p-value of <0.05 was considered to be statistically significant. Data are presented as mean and standard deviations unless otherwise stated.

RESULTS

A total of 80 patients with acute LRTI and 49 controls were recruited during the 12 month study period. Recruited LRTI patients were representative of the adult LRTI population (Table 1), as both groups had similar age, percent female, re-consultation rates and GP antibiotic prescription rates. Smoking history and a history of obstructive lung disease were less common in the total LRTI population compared to the recruited LRTI patients. A comparison found (Table 1) no significant differences between age, percent female and smoking histories ($p>0.05$), however a history of obstructive lung disease was less common amongst controls ($p=0.02$). Throat swabs were collected from all patients and controls and nasal aspirates from 77 (96%) and 48 (98%) respectively. Sputum was collected from 60 (75%) patients. No patient entered the study on a second occasion.

Table 1: Demographic details of the general LRTI population, LRTI patients and controls

	Total LRTI Population (n=368)	Recruited LRTI Patients (n=80)	Controls (n=49)	P (recruited LRTI vs controls)
Percent female	63	63.8	63.3	0.894
Age(mean,(SD))	52.1 (19.8)	49.9 (19.7)	49.7 (17.3)	0.91
Age(range	18-97	18-90	22-83	Not applicable
Smokers	30%	53.2%	46.9%	0.516
History of asthma/COPD	21%	44.9%	22.4%	0.0178
Antibiotic Prescription	64%	64%	Not applicable	Not applicable
Re-consultation rate	30%	33%	Not applicable	Not applicable

Clinical features of recruited acute adult community-acquired LRTI and controls
There were no significant differences between recruited LRTI patients and controls in temperature ($p=0.01$), but respiratory rates, pulse rates and CRP levels were significantly higher in patients ($p<0.001$) (Table 2). At presentation patients had been ill (duration of illness) for 8.7 (SD; 5.5) days. Symptoms were recorded in decreasing frequency as follows: cough (100%), discoloured sputum (83%), upper respiratory tract symptoms (81%), breathlessness (77%), wheeze (71%), sweating (69%), headache (69%), fever (60%), chest pain (56%), myalgia (50%) and haemoptysis (9%).

Table 2: Clinical features of LRTi patients and controls

	LRTi mean (SD) n = 80	Controls mean (SD) n = 49	Mean Difference mean, Upper / Lower CI	P value
Respiratory rate	20 (4.99)	14.7 (3.32)	-5.24 -6.84 to -3.65	<0.0001
Pulse rate	81.1 (11.6)	73.1 (11.2)	-8.09 -12.21 to -3.97	<0.001
Temperature °C	36.4 (0.52)	36.2 (0.47)	-0.23 -0.41 to -0.05	0.01
CRP	19.3 (27.8)	3.2 (2.8)	-16.1 -24.63 to -7.55	<0.001

Antibiotic prescription for LRTI

Antibiotics were prescribed to 64% of the recruited patients (Table 1). A second course of antibiotics was prescribed to 40% of the recruited LRTI patients who re-consulted within the 4 weeks prior to the second study visit.

Aetiology of acute adult community-acquired LRTI

At least one organism was identified in 55 LRTI patients (69%) compared to 7 controls (14%) ($p<0.0001$). A total of 79 organisms (57 viruses, 22 bacteria) were identified in LRTI patients compared to a total of 9 organisms (6 viruses, 3 bacteria) in controls ($p<0.0001$) (Table 3). The overall identification rates for the patient population were 63% for viruses and 26% for bacteria and 12% ($p<0.0001$) and 6% ($p=0.013$) respectively for the control population, demonstrating the underlying carriage rates for these groups of organisms.

The most common viruses identified in LRTI were rhinoviruses in 33% and influenza viruses in 24% compared with 2% ($p<0.001$) and 6% ($p=0.013$) in controls respectively. The pneumolysin gene PCR detected *S. pneumonaie* in 15 LRTI patients (including the one patient who was *S. pneumonaie* sputum culture positive) and 2 controls ($p=0.03$). Sputum bacterial culture detected 7 organisms (5 *H. influenzae*, 1 *M. catarrhalis* and 1 *S. pneumonaie*).

Multiple organisms were identified in 18/80 (22.5%) LRTI patients and 2/49 (4%) controls ($p=0.011$). Influenza viruses were the most common virus type associated with mixed viral and bacterial infection (10/15) followed by rhinoviruses (8/15). Mixed viruses were detected in 8 patients, most commonly rhinovirus and influenza virus (7/8). Mixed bacterial infection was found in one patient with an associated mixed viral infection (*H. influenzae*, *S. pneumonaie*, coronavirus and rhinovirus). Four patients had 3 organisms identified (3 with *S. pneumonaie*, influenza virus and rhinovirus, 1 with *M. pneumonaie*, influenza virus and rhinovirus). Only 6/21 LRTI patients with bacteria detected had isolated bacterial infection (3 *S. pneumonaie*, 2 *H. influenzae* and 1 *M. catarrhalis*).

Table 3 Organisms detected in LRTi study patients and controls

Organism	Patients N=80	Control N=49	p-value
Overall detection rate	**55 (69%)**	**7 (14%)**	**<0.001**
No. of Organisms detected	79	9	<0.001
Viral detection rate	**49(63%)**	**6 (12%)**	**<0.001**
No. of viruses detected	57	6	<0.001
Rhinoviruses	26 (33%)	1	<0.001
Influenza viruses	19 (24%)	3	0.019
Coronaviruses	5	2	0.873
Parainfluenza viruses	3	0	0.441
RSV 2 0 0.691	2	0	0.691
Enteroviruses	2	0	0.691
Bacterial detection rate	**21 (26%)**	3 (6%)	0.013
No. of bacteria detected	22	3	0.006
S.pneumoniae	15 (19%)	2 (4%)	0.034
H.influenzae	5	0	0.189
M.catarrhalis	1	0	0.804
M.pneumoniae	1	0	0.804
C.pneumoniae	1	0	0.804
L.pneumophila	0	0	-

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DISCUSSION

In this 12 month observational study we employed nucliec acid amplification assays to detect both bacteria and viruses in acute adult LRTI in primary care, we demonstrate that the majority of mild LRTI's have a viral aetiology, that mixed viral/bacterial aetiology is also common and that those with a bacterial aetiology alone are not clinically distinguishable from viral LRTI's. A low rate of recruitment led to a small study size, this was limited by the requirement to meet with a second physician (the Study Clinical Fellow) after the initial consultation with their GP. However, the study population was representative of the LRTI population presenting to the recruiting GPs. In particular there were no significant difference in age, the predominance of female consultations, prescription rate or re-consultation rate observed (Table 1). The characteristics of the patients in this study are also similar to recent studies performed in the UK which have reported the a higher proportion of females, high prescription and re-consultation rates (3). Lower rates of smoking and obstructive lung disease in the entire LRTI population compared with the study population were noted and may be the result of under reporting of smoking and obstructive lung disease in the GP records. The LRTI incidence of 37/1000 adult population per year in this study is also comparable to the rate of 44/1000 recently reported (3), with the slightly lower rate coinciding with the reported low consultation levels for acute bronchitis and acute respiratory illness for England during the 2000/2001 season (20).

In this study an organism was detected in 69% of acute adult moderate LRTI's and was. achieved by incorporation of PCR assays for the most common respiratory viruses and bacteria, and also by collection of specimens from throat swabs, nasal aspirates as well as sputum. At the time of the study PCR assays were not available for *H. influenzae* or *M. catarrhalis* in our laboratory and addition of these may have further improved the rate of detection of pathogens. Evenso, this detection rate is considerably higher than previous studies (16% (8) and 55%(4)). In the present study respiratory viruses were the most common cause of acute adult LRTI, occurring in 63% of patients, bacteria were detected in 26% .

Previous studies have reported rates of virus detection from 9-31% (4,6,7,8) in acute adult LRTI using standard. The studies with higher detection rates relied heavily on serology for virus detection and these data could be interpreted as suggesting that viruses are not important causes of acute adult LRTI either. However serological detection for rhinoviruses and coronaviruses is not widely used and viral culture is very insensitive. In this study viruses were detected in 63% of patients using combined nasal aspiration and sputum sampling where available. The most common viruses were rhinoviruses (33% of patients) followed by influenza viruses (24%) and coronaviruses (6%). In contrast a previous study used throat swabs alone (3) acheiving a total virus detection rate of 19%, the 3 most common viruses being influenza, coronaviruses and rhinoviruses. Both the current study and the study by Macfarlane (3) used PCR assays for virus detection suggesting the differences in viral detection rates may be due to the addition of nasal aspirates and sputum for virus PCR in our study. This interpretation is supported by previous studies in which nasal aspirates and sputum were used successfully with detection rates of 76-85% (21,22).

Bacteria were detected in 26% of patients and the distribution of organisms corroborates data from previous studies in which bacterial detection varied between 6-26% (3,6,7), indicating that direct detection of bacterial infection in acute adult LRTI in primary care is relatively uncommon. A bacterial yield of 44% was obtained in a primary care study of patients with LRTI treated with antibiotics using a combination of sputum culture (induced if necessary) and serology (4), *H. influenzae* (8%) was cultured from sputum more commonly than *S. pneumonaie* (6%) as it was in our study. However, the majority of bacterial infections and particularly *S. pneumonaie* (30%) were identified by serology and not by direct detection (4). The present study directly detected only a single case of *M. pneumonaie*, in comparison, the study by MacFarlane et al (3) which reported the highest yield of infectious agents in adult LRTI in primary care so far (55%), reported that infection with atypical pathogens (predominantly *C. pneumonaie*) was inferred by serology in 25% of patients. PCR did not yield any positive samples in the study by MacFarlane et al.. This difference may be due to recruitment occurring over different seasons or that the discrepancy between the serology and PCR results in MacFarlane et al study might result from use of throat swabs for the detection of these organisms by PCR. In our study PCR in throat swabs, nasal aspirates and sputum were used with concordant results and *M. pneumonaie* was detected in a single patient. Other organisms were identified suggesting there were no significant inhibitors of PCR present in the assays. These data suggest that in contrast to the previous study employing serology (3), in the season studied, *M. pneumonaie* and *C. pneumonaie* were an uncommon cause of acute LRTI in adults.

Co-infection with more than one organism was found in 22.5% (18/80) of LRTI patients, and the majority of these were mixed bacteria/viral infections (15/18, 83%). Co-infection rates of 25% (3) and 32% (4) have been reported in previous LRTI studies. The clinical features and outcome of those with isolated bacterial infection was not significantly different to those with isolated viral infection. Hence, there is no clinically useful way to identify those patients with bacterial infection from those with viral infection and thus no clinical way to guide antibiotic treatment. Nevertheless the prescription rate in the present study was 64%, the same as the prescription rate for adult LRTI patients not recruited into the study and similar to the rate reported elsewhere for LRTI in primary care recently (3).

This is the first study of LRTI in primary care to compare patients with a matched control population, permitting aetiological relationships to be inferred. This is particularly important in studies using sensitive methods of detection such as PCR and inclusion of controls would have aided interpretation of the previous studies that relied heavily on serology for diagnosis. In the present study controls were well matched for age, percent female and smoking history. A history of obstructive lung disease was significantly more common amongst recruited LRTI patients compared to controls. The significantly higher respiratory rate and CRP levels in patients compared to controls suggests the likelihood of patients having an infective respiratory illness ($p<0.001$). The significantly higher identification rates in the patient population (69%) compared to the controls (14%,

p<0.001) and the similarity of our detection rate in matched controls to previous reports (23), provides confirmation of the causative role of the vast majority of the pathogens detected in this study.

In summary we have demonstrated that LRTI in primary care is predominantly a viral illness with a high rate of antibiotic prescription that is unrelated to bacterial aetiology. Infection with atypical bacterial pathogens was negligible.

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