THE IMPACT OF THE PNEUMOCOCCAL CONJUGATE VACCINE ON THE EPIDEMIOLOGY AND AETIOLOGY OF CHILDHOOD PNEUMONIA

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To,

My beloved parents, sister, wife, all family, and children: Ahmed, Abdulrahman, Nosaiba and Tasneem for their endless support, encouragement and prayers

"It is the Almighty Allah Who bestows success, and guides to the straight path"

Declaration

I hereby declare that the data presented have not previously been submitted by me for a qualification to this or any other institutions. The work contained is my own work except where due recognition has been given to the contribution of others.

Specifically, the study was conceived by Dr Julia Clark and with Dr Andy Gennery and myself planned the field work and arranged the logistic support. For the purpose of this study, I led the process of development and application of the multiplex pneumococcal serotype-specific PCR assay at the Health Protection Agency (HPA) Public Health Laboratory Newcastle. I received one-to-one educational and technical training on this test, and supervision at all stages by Dr Andrew Sails.

Together with the research nurses Kerry Pollard and Pauline Singleton, I collected and validated the data. Data were entered into the study databases either by me or Kerry Pollard. Data analysis was performed by me under the supervision of Prof Stephen Rushton. I designed and managed the study databases for the epidemiology and aetiology parts under the guidance of Mark Shirley.

I also declare that according to the code of good practice on conducting a research, I maintained confidentiality, good ethics and respect toward my research team, contributors, and enrolled children and their parents. This research was ethically approved by the regional Newcastle and North Tyneside Research Ethics Committee, and Caldicott approvals were granted from all collaborating sites.

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Abbreviations

ASOT	antistreptolysin O titre
BTS	British Thoracic Society
CAP	community-acquired pneumonia
CDC	Centres for Disease Control and Prevention
CF	complement fixation
CI	confidence interval
CIE	counter-current immunoelectrophoresis
CRP	C-reactive protein
Ct	cycle threshold
ELISA	enzyme-linked immunosorbent assay
GAS	group A *Streptococcus*
HES	hospital episodes statistics
hMPV	human metapneumovirus
HIV	human immunodeficiency virus
HPA	health protection agency
IFAT	immunofluorescence antibody testing
IMD	index of multiple deprivation
IPD	invasive pneumococcal disease
IQR	interquartile range
IR	incidence rate
IV	intravenous
k	kappa
LHR	likelihood ratio
LSOAs	lower super out areas
NPA/S	nasopharyngeal aspirate/swab

ONS	Office for National Statistics
OR	odds ratio
PACS	Picture Archiving and Communications System
PCR	polymerase chain reaction
PCV*n*	*n*-valent pneumococcal conjugate vaccine
ply	pneumolysin
ROC	receiver operating characteristic
RSV	respiratory syncytial virus
SD	standard deviation
STGG	skim milk-tryptone-glucose-glycerol
VE	vaccine efficacy
WCC	white cell count
WHO	World Health Organization

Abstract

Background

The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced routinely in the UK in September 2006 and replaced by PCV13 from April 2010.

Aims

To evaluate the impact of PCV7 on the incidence of all-cause community-acquired pneumonia (CAP) in children. Also to investigate the aetiology of CAP before and after the introduction of PCV as well as serotype the pneumococcal infections.

Methods

Enrolled children were from North East England (excluding Cumbria) who were aged 0–16 years and presented with clinical and radiological features suggestive of pneumonia. Epidemiology survey was prospectively undertaken in 2008–2009 at 11 hospitals in North East England. Data were compared to those from a similar survey undertaken in the same hospitals in 2001–2002. Aetiology studies were prospectively conducted in 2001–2002 (pre-vaccine) and 2009–2011 (post-vaccine) in Newcastle and Middlesbrough. Investigations included culture, serology, immunofluorescence antibody, urinary pneumococcal antigen and PCR assays.

Epidemiology Results

A total of 542 children were enrolled, of which 74% were aged <5 years. PCV7 uptake was 90.7%. The annual incidence of pneumonia was 11.8/10 000 (95% CI 10.9–12.9), and the hospitalisation rate was 9.9/10 000 (95% CI 9.0–10.9). Compared to 2001, there was a 19% (95% CI 8–29) reduction in the annual rate of CAP in those aged <5 years, and in those <2 years a 33.1% (95% CI 20–45) reduction in the annual incidence of

CAP and 38.1% (95% CI 24–50) reduction in hospitalisation rates. However, for those unvaccinated aged ≥5 years, there was no difference in the annual incidence of CAP and hospitalisation rate between both surveys. Since 2001, the overall reduction in annual incidence was 17.7% (95% CI 8–26) and for hospitalisation 18.5% (95% CI 8–28).

Aetiology Results

A total of 401 children were enrolled; 241 and 160 respectively in the pre- and post-vaccine studies (73% aged <5 years), for whom at least one diagnostic investigation had been performed. Identification of a definite pathogen was higher post-vaccine (61%) than pre-vaccine (48.5%) [p=0.019]. Rates of bacterial infections were not different between post- and pre-vaccine (17.5% versus 24%, p=0.258). Viral (31%) and mixed infections (12.5%) found more often post-vaccine than pre-vaccine (19.5% [p=0.021] and 5% [p=0.015] respectively). Pneumococcal detection post-vaccine was substantially improved when PCR assays were used compared to culture (21.6% versus 6%, p=0.0004). A serotype was identified in 75% (18/24) post-vaccine including serotypes 1 (44.4%), 3 (27.8%), 19A (22.2%) and 7A/F (5.6%).

Conclusions

PCV7 has reduced both the annual incidence and rate of hospitalisation of pneumonia in children, particularly those aged <2 years. Pneumococcal serotypes which are included in PCV13 but not PCV7 predominated. This suggests that the replacement with PCV13 likely to be associated with a reduction in the incidence of pneumococcal-related pneumonia. Continued surveillance is required to monitor for emerging serotypes.

Chapter 1 Introduction

1.1 Outline of the thesis

The thesis contains eight chapters that cover three themes on community-acquired pneumonia in children following the introduction of the national pneumococcal conjugate vaccination programme with PCV7 in 2006 which was replaced by PCV13 in 2010. Data were compared with findings from similar study undertaken in the North East of England in 2001–2002. Themes include the epidemiology and aetiology of pneumonia and the issues surrounding the radiological diagnosis of pneumonia. Appendices provide information on the ethical approvals, standard study questionnaire used for data collection including all variables and outcomes, study information sheets for parents and age-appropriate for children with consent and assent forms, and list and copies of publications that were generated from this research as well as presentations of data at scientific meetings.

With the use of literature review in chapter one and study methods in chapters two, all chapters were written for publications and manuscripts are either published, being in press or under peer review as outlined below:

1. Chapter one covers the literature review on the epidemiology and aetiology of pneumonia in children during the era of pneumococcal conjugate vaccination programme and also brings in the discussion the implementation of national management recommendations of childhood pneumonia from the British Thoracic Society which were published during the period between the two studies in 2002. It also discusses the increasing role of PCR-based assays in establishing the causes of pneumonia. Another important section discusses the application and limitations of WHO criteria to diagnose pneumonia in children and the recognised continuing inter-observer variability in the interpretation of chest radiographs. It includes the study hypotheses and aims.

2. Chapter two outlines the study methods for the epidemiology survey, aetiology and radiological studies. It covers the study designs and populations, processes of data collection and validation, laboratory procedures including the steps I followed to develop a sequential multiplex pneumococcal serotype-specific PCR locally for the purpose of the aetiology study.
3. Chapter three presents and discusses the results of the epidemiology survey which were compared with data from 2001–2002 survey in terms of annual disease incidence rates, hospitalisation rates and risk factors for the development of severe pneumonia. The findings suggest that PCV7 from this prospective survey outside the trial settings was effective in reducing both the annual incidence of childhood pneumonia seen in hospital and annual rates of hospitalisation in one population within the UK when compared with randomised trials evaluated the impact of pneumococcal conjugate vaccination on the incidence of pneumonia.
    - Published in Epidemiology and Infection
4. Chapter four presents and discusses data on the clinical presenting features and management of pneumonia in children seen in hospital. This survey completes the audit cycle started with a similar prior to the publication of the national management guidelines of childhood pneumonia. The findings showed that there has been a positive change in the management practices of childhood pneumonia reflected by reduced number of overall investigations performed and an increased preference for oral antibiotic use.
    - In peer review with Journal of Evaluation in Clinical Practice
5. Chapter five presents and discusses the results of the first study to describe the aetiology of pneumonia in UK children prior to and following the introduction of the pneumococcal conjugate vaccination programme. The findings showed

that although viruses are the most common cause of pneumonia, around one fifth of children had bacterial infections. The combined use of culture, serology and PCR-based diagnostic tests significantly improved the identification of causative pathogens in childhood pneumonia.

- Published in European Respiratory Journal

6. Chapter six presents and discusses the results of diagnostic approaches to pneumococcal infections and provides the first information on serotype distribution of pneumococcal CAP in UK children after the introduction of the pneumococcal conjugate vaccination programme. The findings showed that non-PCV7 but PCV13 serotypes were the major contributor to the aetiology of pneumococcal pneumonia in UK children. Therefore continued surveillance is required to monitor for the emergence of serotype replacement.

    - Published in Diagnostic Microbiology and Infectious Disease

7. Chapter seven presents and discusses the substantially observed inter-observer variability in the interpretation of chest radiographs for the diagnosis of paediatric pneumonia. These findings add to the recognized variability in the literature demonstrating that there may be a need for evaluation of the WHO categorization of radiological pneumonia in children to improve the validity and encourage widespread adoption of the criteria.

    - In peer review with Pediatric Pulmonology

8. Chapter eight summarises the overall study outcomes, strengths and limitations and conclusions. It also highlights the impacts of this research and areas for future studies.

1.2 Epidemiology of childhood pneumonia

Community-acquired pneumonia (CAP) is a common childhood infection and an important cause for hospital admission.[1-3] The course of illness has a variable severity ranging from mild to severe, and can be complicated by systemic disease and death.[4-6] It is a major public health problem and causes approximately 20% (two million) of annual global childhood morbidity and mortality mostly among those aged under five years, of which 70% occurs in resource limited countries.[7-10] The range of implicated pathogens is wide and includes viruses, bacteria or co-infection with both.[11-15] Different laboratory diagnostic techniques with an increasing application of PCR-based assays over the last decade are used to establish the aetiology of CAP in children.[16-18] Laboratory diagnostic approaches carry variable sensitivity and specificity.[19, 20] Causative pathogens in young children are predominately viruses or co-infection with bacteria compared to older children over five years where bacterial pathogens are more common.[16, 21] All of these factors could relate to potential causative pathogens for different age groups, hence variable disease incidence rates of CAP.[22-26]

Streptococcus pneumoniae is thought to be the leading bacterial cause of pneumonia among young children.[27] In the UK, pneumococcal infection was identified in nearly 10% of CAP in children,[28, 29] compared to 15.5% from a previous local aetiological study in the North East of England. Studies in the USA and Finland suggested that among children aged under two years, *S. pneumoniae* causes up to 45% of pneumonia seen in hospital.[11, 12, 30] This is likely to be an underestimation of the true burden of pneumococcal disease, given the relative imprecision of microbiological diagnosis of pneumonia in children.[1] It has been estimated that the annual incidence of childhood CAP in Europe is 2.5 million.[31] Studies of incidence and mortality of CAP before the

era of the 7-valent pneumococcal conjugate vaccine (PCV7) estimated a variable annual incidence of approximately 40/1000 for children under five years of age, and 15/1000 for those aged 5–14 years.[5, 32, 33] This variation in incidence rates of all-cause pneumonia has been related to age, causative pathogens including viruses and bacteria, severity assessment, admission criteria and referral pathways, clinical and radiological definitions of pneumonia, and seasonal and geographical changes.[1, 2, 11, 34-44] The observed variable annual incidence rates of overall IPD across the world likely reflect poor case ascertainment and may be underestimated.[1, 2, 4, 35, 45-47] This is because the diagnosis is usually made by culturing of clinical biological samples, which needs the presence of viable pathogens.[48-50]

Pneumococcal serotype surveillance is monitored by serological capsular identification of clinical isolates after culture.[51, 52] The recovery rate of *S. pneumoniae* from culturing of blood and pleural fluids is approximately 10%.[53, 54] Sensitive and specific serotype monitoring require a wide range of sera to cover the prevailing types.[46] This technique has cost implications and can be limited by inconclusive results due to the occurrence of auto-agglutination.[55] Therefore in developed countries usually typing is limited to the serotypes contained in the 23-valent pneumococcal polysaccharide vaccine.[56] Vergison and colleagues actively surveyed 98.5% of paediatric units in Belgium to prospectively establish the annual incidence of IPD in children aged under five years to investigate the problem of underestimation of the disease burden.[46] They showed a twofold increase in the incidence of IPD (59.5 cases per 10 000 children per year) between 2002 and 2003 when compared with previous passive epidemiologic surveillance. Variations in the adapted epidemiologic surveillance methods in terms of logistic and laboratory approaches as well as lack of

use culture-negative techniques in resource limited countries contribute considerably in the differences in annual incidence rates of overall IPD.[4, 35, 46, 50]

Identifying the aetiology of CAP in children is challenging with a large number of potential pathogens, some of which may also be carried as commensal organisms, which can complicate the interpretation of the results of testing nasopharyngeal samples.[57] Conventional methods such as blood culture and serology often have limited sensitivity due to inadequate sample volume or lack of convalescent sera.[1] Molecular diagnostics are now routinely used in the assessment of viral respiratory infections and similar techniques have been developed for the detection of bacterial respiratory infections.[17, 20] Resti and colleagues demonstrated a significant improvement in the identification of pneumococcal pneumonia in children by PCR on blood samples (15.4%) when applied simultaneously with blood culture (3.8%).[18] In a recent study of Italian children aged under five years, overall bacteremic pneumococcal pneumonia was identified in 14.3%, which was established by PCR in 92%, blood culture 1% and both in 7%.[58]

The annual incidence of childhood CAP seen in hospitals in the North East of England was evaluated prospectively in 2001–2002.[59] At that time the annual incidence of childhood pneumonia was 14.4/10 000 (95% CI 13.4–15.4) and 33.8/10 000 (95% CI 31.1–36.7) for those aged under five years.[59] The annual incidence of pneumonia was higher in boys and children aged under five years with a ratio of 1.3 and 4 respectively. There was a positive association between the severity of illness and young age (under five years old) and prematurity (24–28 weeks gestation). Although there was variation in the incidence by county of residence,[59] it was not possible to ascertain the actual reasons behind this.[11, 34] An extensive work by the WHO with accumulating evidence over the years from different continents showed that the risk factors for

development of acute lower respiratory infections in children particularly those aged under five years either related to host or environment.[4, 8, 60-64] These include a likely risk factors such as poor nutritional status, low birth weight (<2.5 kg), suboptimal breast feeding during the first half year of life, inadequate immunisation, household crowding, air pollution and parental smoking, low socioeconomic status, zinc deficiency, young mother's age, and presence of comorbidity and other infections.[4, 65-75] Other potential factors include attendance at day-care centres, nasopharyngeal carriage of viruses and bacteria, low maternal education, vitamin A deficiency, and cold and humid weather as well as environmental pollution.[4, 76]

In the USA, PCV7 was licensed for use in February 2000, and in February 2001 in the European Union for active immunisation of children for prevention of invasive pneumococcal disease (IPD).[77] The introduction of PCV7 against *S. pneumoniae*, which is highly effective against invasive bacteraemia and meningitis in young children, was found to decrease the annual incidence of lobar pneumonia by up to 35% in the USA and Canada.[78, 79] It has been associated with a considerable reduction in IPD,[80-86] and vaccine serotype pneumococcal nasopharyngeal carriage in children.[87] Among young children aged under two years, IPD caused by vaccine and non-vaccine-related serotypes significantly reduced by approximately 80% and 50% respectively.[80] There has been also significant reduction of IPD in the non-vaccinated population resulting from herd immunity (accurately herd effect).[88-91] The term 'herd immunity' means the proportion of immunised individuals in a given population, whereas indirect protection 'herd effect' refers to the reduction in the incidence of infection in the unimmunised individuals due to the presence of immunised part of the community.[92-94]

However, there has been a substantial global increase in childhood empyema thoracis over the last 15 years particularly between 2000 and 2005 compared with the previous data.[95-101] Pneumococcal, staphylococcal and group A streptococcal infections are being the common bacterial causative pathogens of empyema in children.[102-107] A worldwide rising trend in empyema thoracis due to pneumococcal pneumonia, typically following infection with *S. pneumoniae* serotype 1 has been reported.[102, 108, 109] PCV7 does not contain antigen against this serotype, and there is some evidence from the USA that the introduction of this vaccine into the routine immunisation schedule was associated with emergence of empyema related to other non-vaccine pneumococcal serotypes, particularly 1, 19A, 3, 6A and 7F.[86, 96, 108]

There is much interest in exploring the increase in empyema thoracis which has been observed in the recent years.[110] The incidence of empyema has increased rapidly in the UK over the last decade, and it is known from PCR and serotype-specific ELISA studies that this mostly related to infection with *S. pneumoniae* serotype 1.[102, 111] Rees and colleagues first reported a sevenfold increase in the number of cases of empyema managed at the regional respiratory unit for the West Midlands in the UK between 1995/1996 compared with the previous three years.[112] In England, annual admission rates for empyema in children aged under 15 years from 14 per million population in 1995/1996 to 26 per million in 2002/2003 ($p=0.003$), and 14 per million to 46 per million ($p<0.001$) among those aged under five years over the same period.[113] Similarly, study of Scottish children aged under 15 years showed that annual admission rates of empyema increased from <10 per million population in 1998 to 37 per million in 2005.[114] This increase was more among those aged under five years, from 6.5 per million annually between 1981 and 1998 to 66 per million in 2005. Instead overall annual admission rates for pneumonia remained unchanged among those

aged under five years where gradually rose by an average of 50 per million annually between 1981 and 2005.[114] Using HES data, Koshy and colleagues recently reported a 22% decrease between 2006 and 2008 in admissions of empyema in children after the routine introduction of PCV7 in England.[115] Whereas in England and Wales,[116] IPD caused by PCV7 serotypes was reduced by 98% in children aged under two years between 2000–2006 period and 2009/2010. On the other hand non-PCV7 serotypes IPD increased by 68%, giving an overall reduction in IPD of 56% in this age group.[116]

Most invasive pneumococcal infection is thought to arise as a consequence of previous colonisation of the nasopharynx.[117-119] *S. pneumoniae* serotype 1 does not normally colonise the nasopharynx, and the mechanism by which the organism is transmitted between individuals and the subsequent pathogenesis of invasive infection is uncertain.[118, 120, 121] It is unclear whether the reported increase in the annual incidence of empyema is a reflection of an increase in the percentage of cases of pneumonia which are due to pneumococcal serotype 1, or whether there has been an increase in the relative number of patients with pneumonia progressing to empyema.[110] In a recent small school outbreak of pneumococcal serotype 1 pneumonia, one adult was found to have a positive nasopharyngeal culture with this organism raising the possible theoretical hypothesis that adult to child transmission may be an important factor.[122]

In September 2006, PCV7 including antigen for serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F was added routinely to the UK immunisation programme.[123, 124] The vaccine schedule is three doses administered at 2, 4 and 13 months of age. When introduced, those over and under one year of age received one and two doses respectively as part of a catch up programme for children aged under two years. Subsequently this was

replaced by PCV13 from April 2010, which also includes antigen for the additional serotypes 1, 3, 5, 6A, 7F, and 19A.[125] Previous studies in the UK anticipated that the vaccine coverage for the included serotypes to be approximately 76%.[126, 127] Based on estimates of serotype coverage, and vaccine efficacy and uptake, the potential reduction in IPD among children aged under five years is approximately 67% with a predicted vaccine uptake to reach 90% in those aged one year.[127] Vaccine coverage is a complex process and requires combined strategies such as improvement in socioeconomic and nutritional status and education of people in poor communities to achieve better availability and affordability with sustainable delivery and uptake of the immunisation programme.[128-132] These measures in return facilitate making it cost-effective intervention.[133-135]

Following the introduction of PCV7 routinely in most of European countries, there was a substantial reduction in the incidence of overall IPD caused by PCV7 serotypes,[136] but associated with counter increase of IPD related to serotypes included in PCV13; 1, 19A, 3, 6A, and 7F.[86, 137] In England and Wales, non-PCV7 serotypes are now associated with IPD [116] and an increase in pneumococcal serotype 19A is associated with complicated pneumonia with empyema.[138] The observed increase in detection of pneumococci in this study is presumably related to both improved molecular techniques and continued pneumococcal disease due to replacement with non-PCV7 serotypes. Similar findings were reported from the USA on children with empyema, where 98% were non-PCV7 serotypes.[108] Gorton and colleagues showed a 90% (95% CI 61–99) reduction of IPD caused by PCV7 serotypes in children aged under five years in the North East of England between 2006/2007 and 2009/2010.[139] They also showed a non-significant increase in IPD caused by non-PCV7 serotypes in this age group of 88% (95% CI -10 to 312) which was mainly caused by serotypes 7F, 19A and 22F.[139] This

suggests that PCV13 could substantially reduce IPD.[125, 140] Recent data following the introduction of PCV13 in the USA showed that there were marginal increase in IPD caused by non-PCV13 serotypes; 33F, 22F, 12, 15B, 15C, 23A and 11.[141]

The efficacy of pneumococcal conjugate vaccines in preventing radiological pneumonia has been shown to be up to 37% in randomized controlled trials.[142-146] Koshy and colleagues reported a 19% reduction in both annual incidence of childhood pneumonia and hospitalisation rate in England between 2006 and 2008 by using hospital episodes statistics (HES) data.[115] There was a 13% reduction in the annual rate of hospitalisation in the under five age group following the introduction of PCV7 in Canada.[79] Estimates from other studies of PCV11 in the Philippines [147] and PCV7 in the USA [144] reported decreases in the annual incidence of all-cause pneumonia by approximately 22%. This is compared to 25% reduction against radiological pneumonia reported in a randomized controlled trial of PCV9 in South Africa [142] and 30% in the USA [148]. This may be a reflection of the differences in pneumococcal disease between populations.[50, 149] Furthermore, variable vaccine efficacies between these trials might be related to different valency of the vaccines used, variations in disease incidence rates between countries and pneumococcal serotype distribution, missed to follow up of some enrolled cases, definitions of radiological pneumonia and primary outcomes, presence of other chronic diseases and concurrent acute infections such as human immunodeficiency virus (HIV), tuberculosis, measles and gastroenteritis, and poor socioeconomic and nutritional status.[4, 35, 145, 146]

Studies and randomised-controlled trials of the pneumococcal conjugate vaccine used the same WHO criteria for radiological classification of pneumonia in children in epidemiological studies which are applied in the present research.[150, 151] These trials

are individually summarised here. Immunisation with PCV7 of American Indian children aged under two years in a setting with high rates of IPD in the Navajo and White Mountain Apache Indian reservations, the vaccine efficacy was 76.8% in reducing the disease incidence.[152] In the USA, PCV7 was given to infants at 2, 4, 6 and 12 to 15 months of age.[144] It was found to be more effective in reducing pneumonia in children aged under a year (32.2%) than that of 23.4% and 9.1% among those aged under or over two years respectively.[144] Three doses of PCV9 were given to infants aged 6–51 weeks at an interval of 25 days between doses in the Gambian trial.[143] The vaccine demonstrated an efficacy of 37% and 7% against first episode of radiological and clinical pneumonia respectively. It also had 77% VE against IPD caused by PCV7 serotypes.[143] Infants in South Africa were immunised in the PCV9 trial with doses given at 6, 10, and 14 weeks of age.[142] It resulted in VE of 83% in reducing first episode IPD among those without HIV infection compared to 65% among those with HIV infection. Among children without HIV infection, the VE was 20% against the incidence of first episodes of radiological pneumonia.[142] In a trial of PCV11 in The Philippines, three doses were given at four weeks apart for infants aged between six weeks and six months.[147] It had 22.9% (p=0.06) VE against radiological pneumonia for children aged 3 to 23 months old, whereas subgroup analysis showed VE of 34% (p=0.02) and 2.7% (p=0.88) for those aged 3 to 11 months and 12 to 23 months old respectively. There was no significant VE against clinical pneumonia (p=0.99).[147]

Interestingly, the major reduction in pneumonia admissions was observed in those aged under two years by approximately 40% in the USA,[153, 154] but lesser (15%) during the PCV9 trial in the Gambia.[143] A decrease in the admission rate among those aged 2–4 years was observed in 17%.[153] There was marked reduction in the disease incidence in the under two age group of up to 37% from the pneumococcal conjugate

vaccination trials and pooled review data.[143, 145, 146] However, prospective epidemiological studies are valuable in establishing the impact of vaccines in populations' outside trial settings.[155, 156] Furthermore, childhood CAP is a frequent cause of admission to hospital.[1, 6] Clinical features of pneumonia are often non-specific in young children.[157, 158] Management decisions are generally based on a combination of clinical signs, symptoms and radiological changes.[157, 159] The clinical features and management outcomes of pneumonia in children were previously described in a survey conducted in hospitals in the North East of England in 2001–2002.[157] National UK clinical guidelines for childhood CAP were published in 2002 [160] and updated in 2011 [1]. They synthesized evidence and expert opinion to produce best practice national standards, which included statements on investigations and antibiotics use as outlined below:[160]

1. Blood cultures should be performed in all children suspected of having bacterial pneumonia.
2. Nasopharyngeal aspirates from all children under the age of 18 months should be sent for viral antigen detection with or without viral culture.
3. Acute phase reactants should not be measured routinely.
4. Amoxicillin is first choice for oral antibiotic therapy in children under the age of five years and macrolide antibiotics may be used as first line empirical treatment in children aged five and above.
5. Antibiotics administered orally are safe and effective for children presenting with CAP.
6. Intravenous antibiotics should be used in the treatment of pneumonia in children when the child is unable to absorb oral antibiotics (for example, because of vomiting) or presents with severe signs and symptoms.

7. Appropriate intravenous antibiotics for severe pneumonia include Co-amoxiclav, Cefuroxime, and Cefotaxime.
8. If clinical or microbiological data suggest that *S. pneumoniae* is the causative organism, Amoxicillin, Ampicillin, or Penicillin alone may be used.

Evidence for the safety and efficacy of oral antibiotics even in severe pneumonia in children accumulated over the six years period between surveys, including a Cochrane review in 2006 [161] and the PIVOT trial in 2007 [162]. Recent review of data sets from four studies of the management outcomes of severe pneumonia in children aged under three years in the community was associated with few complications, supporting the management of such cases with oral antibiotics in primary care settings.[163] Antibiotic stewardship programs and management guidelines have been shown to improve the selection of appropriate investigations and antibiotics for management of infections in children.[164-167] These measures allow better use of health resources and reduction of antibiotic drug resistance which are becoming global challenges.[168-173]

Therefore the initiatives of pneumococcal conjugate vaccination programme and BTS management guidelines of pneumonia mean that now is an ideal time to review the incidence, epidemiology and management of pneumonia presenting to hospital using the only population in the UK where this has previously been established, in order to explore the impact of PCV7 on the prevention of all-cause pneumonia the influence of the national guidelines on management of CAP in children. Such data are essential for plans for future public health preventative strategies and newer vaccine generation.

1.3 Aetiology of childhood pneumonia

The range of implicated pathogens is wide and includes viruses, bacteria or co-infection with both.[6, 174] *S. pneumoniae* is the leading bacterial cause of this infection particularly in resource limited countries.[4] Pathogens can be difficult to identify in children with pneumonia.[1, 2] Studies of pneumonia frequently report low levels of pathogen identification although improved knowledge of pneumonia aetiology is essential for development of targeted management and effective public health strategies and assessment of interventions such as vaccination.[1, 175] Depending on the diagnostic methods adopted, causative pathogens can be identified in 40–85% of childhood CAP.[11-13, 15, 176-178] Bacterial pathogens are identified in approximately 50%, where as viral and mixed viral-bacterial infections are implicated respectively in up to two-thirds and a third of CAP in children.[11, 13, 15]

The three previous UK studies investigating the aetiology of pneumonia in children prior to the introduction of the conjugate pneumococcal vaccination were able to identify the aetiology of pneumonia in between 24% and 54% of cases.[28, 29, 176] One tested blood for *S. pneumoniae*, *Mycoplasma* and *Chlamydophila* using PCR and identified 8% of children with pneumococcal pneumonia.[28] Another study identified 6% pneumococcal infection using pneumolysin ELISA on blood.[29] However, none of these studies investigated bacterial aetiology or evaluated the serotypes involved in pneumococcal pneumonia in a comprehensive manner or evaluated the serotypes involved in pneumococcal pneumonia.

S. pneumonia causes a range of life-threatening diseases including pneumonia, septicaemia and meningitis which lead to a substantial global childhood morbidity and mortality.[7, 149, 179] It is a gram positive bacterium normally colonised in the

nasopharynx.[180] The polysaccharide capsule forms the main virulent antiphagocytic component, and is the target of serotype-specific vaccines for prevention of invasive pneumococcal disease.[181] Immunochemistry of the capsular serotype facilitated the identification of 91 serotypes and classification of immunologically similar serotypes into 46 serogroups.[51, 180, 182] Data before the era of PCV7 showed that most of IPD is caused by approximately 15 common serotypes with a relative large geographical variation worldwide.[110, 149, 183] In Europe the commonly prevalent serogroups among young children ranked in decreasing order include: 14, 6, 19, 18, 23, 9, 1, 7, 4, 5, 3, 24, 15, 33 and 10,[149] whereas in Britain particularly serotypes 14, 6 (B/A), 19 (F/A), 18 (C/B/A/F), 23 (F/A/B), 9 (V/N/A), 7 (F/A), 4, 5 and 3 are responsible for the majority of IPD.[126, 149, 184] Since the introduction of PCV7 in Europe, there has been a decrease in annual incidence of IPD, antibiotics resistance and vaccine serotypes.[86] But worryingly a replacement with new prevailing non-vaccine serotypes has been observed including 1, 19A, 3, 6A, and 7F.[86, 116, 138]

The observed variable annual incidence of IPD across the world likely reflects poor ascertainment and may be underestimated.[45] This is because the diagnosis is usually made by culturing of clinical biological samples, which needs the presence of viable pathogens.[48] Serotype surveillance is monitored by serological capsular identification of clinical isolates after culture.[51, 52] The recovery rate of *S. pneumoniae* from culturing of blood and pleural fluids is approximately 10%.[53, 54] Sensitive and specific serotype monitoring require a wide range of sera to cover the prevailing types. This technique has cost implications and sometimes constrained by inconclusive results due to the occurrence of auto-agglutination.[55] Therefore, in developed countries usually typing is limited to the serotypes contained in the 23-valent pneumococcal polysaccharide vaccine.[56]

Identifying the aetiology of CAP in children is challenging with a large number of potential pathogens, some of which may also be carried as commensal organisms, which can complicate the interpretation of the results of testing nasopharyngeal samples.[57] Conventional laboratory methods such as blood culture and serology often have limited sensitivity due to inadequate sample volume, minimal presence of bacteremia and lack of convalescent sera.[1, 13, 185-187] Children are not usually able to produce sputum, and direct sampling of the lung using percutaneous aspiration or bronchoalveolar lavage is not a routine practice in the UK.[160] Pathogens are difficult to identify in children with pneumonia, with blood culture and serological testing often negative due to minimal presence of bacteremia.[186, 187] This paucity of *S. pneumoniae* isolation makes examining pneumococcal serotype distribution in childhood CAP difficult, with no UK, and little worldwide, data. Using non-culture techniques including pneumococcal detection by PCR in blood [18] and pleural fluid [188], pneumococcal antigen detection in urine [189] and pneumococcal serotype detection by PCR [18, 58], there was an improved insight into the contribution of *S. pneumoniae* and specific serotypes to the aetiology of CAP in children.

Many patients have received antibiotics prior to hospitalisation which can affect the isolation of bacterial pathogens.[190] The rate of positive blood culture in children with pneumonia is about 5%.[13] In order to address these issues various antigenic assays have been developed.[191, 192] However, the significance of a positive pneumococcal antigen test in urine is rendered difficult to interpret because of the high frequency of nasopharyngeal pneumococcal carriage in normal children which generates a false positive antigen signal.[193-197] As *S. pneumoniae* serotype 1 does not normally colonise the nasopharynx,[118, 120, 121] it would not be expected that a healthy

uninfected children would have positive urinary antigen test for this specific serotype. Although urinary pneumococcal antigen detection by Binax Now has limited specificity (56%), it has good sensitivity (100%) in children with suspected IPD,[189] but is confounded as a diagnostic test by nasopharyngeal carriage where up to 21% may be positive.[196] Urinary antigen may also be positive in 4% healthy nasopharyngeal culture-negative children,[196] although given the increased sensitivity of PCR compared to culture, it is likely that nasopharyngeal culture-negative children would have pneumococcal carriage if tested by PCR.[198] Thus, a positive urinary antigen in a child with pneumonia and no pneumococcal nasopharyngeal carriage by PCR is likely to reflect invasive pneumococcal infections.

Molecular diagnostics are now routinely used in the assessment of viral respiratory infections and similar techniques have been developed for the detection of bacterial respiratory infections.[17, 20, 199] Resti and colleagues demonstrated a significant improvement in the identification of pneumococcal pneumonia in children by PCR assay on blood samples (15.4%) when applied simultaneously with blood culture (3.8%).[18] In a recent study of Italian children aged under five years, overall bacteremic pneumococcal pneumonia was identified in 14.3%, which was established by PCR assay in 92%, blood culture 1% and both in 7%.[58] A molecular-based method using sequential multiplex PCR assay to specifically identify the capsular serotype-specific sequences provides a practical and cost-effective tool for the surveillance of IPD.[200, 201] The test has been developed at the Centres for Disease Control and Prevention (CDC) in the USA.[200] Molecular pneumococcal serotyping has been found effective when performed either on culture-positive samples,[200, 202] or directly on clinical biological samples.[48] Pai and colleagues studied 29 primer pairs to target the prevalent pneumococcal serotypes in the USA.[200] The primers were

grouped into seven multiplex reactions. Of the 29 primers, 18 were fully specific for the targeted serotypes; 1, 3, 4, 5, 8, 10A, 14, 15A, 15B/C, 16F, 17F, 19A, 19F, 20, 23F, 31, 34, and 35B. Serotyping was established in 95% of isolates (54% fully specific and 41% cross-reactive with minor serotypes). The same 29 primers were studied by an Italian group.[48] The molecular diagnosis was carried out directly on clinical biological samples by real-time PCR and confirmed by sequential multiplex PCR. These PCR assays confirmed the serotypes in 86% of those with *S. pneumoniae* infection.

The changes in the prevailing serotypes of *S. pneumoniae* in children with pneumonia and empyema are important to monitor with the introduction of conjugate pneumococcal vaccination.[86, 203, 204] *S. pneumoniae* is rarely identified in these children, and the development of a molecular model for serotype specific detection informs essential epidemiological and aetiological surveillance required for evaluation of the effectiveness of currently available pneumococcal vaccines and development of new vaccines.[86, 205, 206] The national Respiratory and Systemic Infection Laboratory at the Health Protection Agency (HPA) in London have developed a multiplexed immunoassay using xMAP beads for detection of serotype-specific *S. pneumoniae* antigens.[207] This assay can identify all of the serotypes included in PCV13 plus serotype 8. It is suitable for use on body fluids for the determination of the commonest serogroups and serotypes of *S. pneumoniae* prevalent in the UK.[192] This test therefore has considerable potential for the epidemiological assessment of pneumococcal infections including serotype distribution and replacement in children. A national surveillance of IPD has been extended to include pleural fluid from empyema thoracis and a national prospective reporting system is being established in order to monitor the incidence of pleural empyema. With the expected decrease in incidence,

new data on the aetiology of those presenting with pneumonia and pneumococcal contribution are important and nothing is yet known about this in the UK.

The timing of the study towards the end of three years of PCV7 and during the first year of PCV13 gives a unique opportunity for future evaluation of the aetiology of pneumonia in the same setting. It also aimed to investigate the contribution of *S. pneumoniae* in the aetiology of CAP in hospitalised children and identifies the pneumococcal serotypes responsible within a population routinely offered PCV.

1.4 Radiological diagnosis of pneumonia

Chest radiograph is frequently performed when managing pneumonia in children,[208] but usually does not affect the clinical outcome.[209] In epidemiological studies, the chest radiograph remains a major criterion in classification of pneumonia.[150, 151] Variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children is a recognised problem.[38] This problem is well-known since radiology reporting was initiated in the middle of last century.[210, 211] It has been suggested that if all radiologists followed the standardised WHO radiological criteria for classifying pneumonia,[150] this would allow more accurate comparative data in epidemiological studies for assessment of the impact of pneumococcal vaccination.[151] Broadly four categories are defined: "End-point consolidation", "Other (non-end-point) infiltrate", "Pleural effusion" and "No pneumonia".

The WHO criteria of radiological pneumonia are summarised as following:[150, 151]

1. "End-point consolidation": a dense opacity that may be a fluffy consolidation of a portion or whole of a lobe or of the entire lung, often containing air bronchogram and sometimes associated with pleural effusion.
2. "Other (non-end-point) infiltrate": a linear and patchy densities (interstitial infiltrate) in a lacy pattern involving both lungs, featuring peribronchial thickening and multiple areas of atelectasis with lung inflation is being normal to increased. It also includes minor patchy infiltrates that are not of sufficient magnitude to constitute primary end-point consolidation, and small areas of atelectasis which in children can be difficult to distinguish from consolidation.
3. "Pleural effusion": this refers to the presence of fluid in the pleural space between the lung and chest wall. Mostly this will be seen at the costo-phrenic angle or as a layer of fluid adjacent to the lateral chest wall. This does not

include fluid seen in the horizontal or oblique fissures. Pleural effusion is considered as primary end-point if it is in the lateral pleural space (and not just in the minor or oblique fissure) and is spatially associated with a pulmonary parenchymal infiltrate (including other infiltrate), or if the effusion obliterates enough the hemithorax to obscure an opacity.

4. "No pneumonia": if there is no evidence of consolidation, infiltrate, or pleural effusion.

The diagnosis of pneumonia in children based on a combination of clinical and radiological features is important for prompt management.[159] Yet, subtle radiographic changes can be difficult to recognise or interpret and failure to diagnose pneumonia may result in inappropriate management.[212, 213] The initial interpretation of chest radiographs is usually performed by clinicians with the radiologists' reports following later, often after the patient has been discharged from hospital.[212] Interpretation by clinicians could be biased by inadequate training in radiology and lack of clinical information may limit the accuracy of reporting by the radiologists.[214] For research purposes blinded interpretation of the chest radiograph may improve detection of subtle changes and differentiating normal biological variants.[215] Making clinical information available may reduce inter-observer variability but does not result in marked improvement in the overall accuracy.[216]

Usually inter-observer variability is related to the interpretation of patchy and perihilar changes, which need careful viewing and the availability of clinical information during interpretation.[217] It is well recognised that abnormal chest radiographs may be interpreted as normal.[217] A recently reported chest radiographs according to the WHO radiological classification of Pakistani children aged 2–59 months diagnosed with

non-severe pneumonia showed normal films in 82% (1519/1848) and lobar consolidation in 26 children.[218] Variation in reporting of chest radiographs mostly occur in those aged under five years which represents particular challenge of making a radiological diagnosis of pneumonia in this age group.[213, 219] It is widely accepted in the literature that chest radiographs cannot reliably differentiate viral from bacterial aetiology of pneumonia.[1, 2] Therefore these variations on the interpretation of chest radiographs do not significantly affect the clinical outcomes and management decisions of pneumonia in children.[1, 2, 209, 220, 221]

It is interesting that irrespective of the level of experience there continues to be significant variability in interpretation between reporters, particularly senior radiologists.[213, 222] A previous study showed that qualified radiologists had less inter-observer variability on reporting of chest radiographs compared to radiology trainees and physicians.[223] Despite the specialized training in paediatric radiology and advanced technology, human error remains a likely factor.[211] The level of variability between the senior radiologists could be a reflection of inconsistency in the application of the WHO criteria, as this has been shown to decrease inter-observer variability.[224] However, false negative reports between the two interpretations of chest radiographs is a well recognised problem [217] which may jeopardize the results of epidemiological studies by underestimating the true burden of pneumococcal pneumonia.[225] In previous pneumococcal vaccine efficacy studies the radiographic evidence of pneumonia was observed in up to 34% of the enrolled children.[226] It has been suggested that using the WHO criteria would make any differences in the results reflect geographical variations in disease epidemiology or vaccine effects rather than methodological factors.[151] Despite the application of this classification, the concordance rate between two trained reviewers was only 48% (250/521).[227]

Radiological findings were part of the entry criteria to the epidemiology and radiology studies in this research. Therefore using data from the aetiology study this analysis aimed to characterise inter-observer variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children according to the WHO radiological classification.[150]

1.5 Hypotheses and aims

1.5.1 Hypotheses

The study hypotheses were as following:

1. The introduction of the pneumococcal conjugate vaccination programme in 2006 was associated with a reduction in the incidence of radiologically-confirmed pneumonia and rates of hospitalisation in children.
2. The management practices of CAP in children have changed since the implementation of national management guidelines from the British Thoracic Society (BTS) in 2002 [160].
3. The application of more PCR-based assays and expanded microbiological screening would improve the detection rates of causative pathogens.
4. The non-PCV7 serotypes are an important cause of pneumococcal infections in childhood CAP.
5. Inter-observer variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children is continuing despite the acceptance of the recommended WHO criteria [150, 151] for reporting radiological changes in childhood pneumonia.

1.5.2 Aims

There were two parts of this research that involved children aged 0–16 years with clinical and radiological features suggestive of pneumonia. The aims were as following:

1.5.2.1 Epidemiology survey

1. To investigate the annual incidence of all-cause community-acquired pneumonia (CAP) in children seen in hospital in the North East of England.
2. To evaluate the impact of PCV7 on the incidence of childhood CAP by comparing the established data with those from a similar survey undertaken in the same hospitals in 2001–2002 [59].
3. To identify the risk factors for the development of severe disease in children presented to hospital with pneumonia based on demographic and social data.
4. To compare the clinical features and management of childhood CAP following the publication of the national management guidelines from the BTS in 2002 [160] with data from a similar survey at the same hospitals in 2001–2002 (pre-guidelines) [157].

1.5.2.2 Aetiology study

1. To investigate the aetiology of CAP in children seen in hospital before the introduction of the pneumococcal conjugate vaccine by analysing a previously collected prospective data in 2001–2002.
2. To investigate the aetiology of CAP after the introduction of the pneumococcal conjugate vaccine.
3. To determine the contribution of *S. pneumoniae* in the aetiology of CAP in hospitalised children and identify the pneumococcal serotypes responsible within a population routinely offered pneumococcal conjugate vaccine using culture and molecular identification methods.

4. To develop, validate and apply a molecular test using a sequential multiplex PCR assay to identify the pneumococcal capsular serotype-specific sequences.
5. To identify the serotypes of *S. pneumoniae* causing pneumonia using sequential multiplex PCR assay on clinical biological samples.

1.5.2.3 Radiology study

1. Among children enrolled in the aetiology study (2009–2011), to characterise inter-observer variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children according to the WHO radiological classification [150, 151].

Chapter 2 Materials and Methods

2.1 Epidemiology survey

2.1.1 Study design and participants

This was a prospective survey involving 11 hospital sites in the North East of England (excluding Cumbria). It was conducted from August 2008 to July 2009, covering the same months as the survey in 2001–2002. The hospital configuration changed slightly from the previous survey in 2001, with a reduction in the number of units treating children from 13 to 11. However, the geographical area and population served by these hospitals were the same as in 2001 as were the methods of enrolment criteria and case ascertainment.[59] The participating hospitals were: Queen Elizabeth Gateshead, James Cook Middlesbrough, North Tyneside, South Tyneside, Sunderland Royal, North Tees, North Durham, Darlington Memorial, Freeman, Newcastle General and Royal Victoria Infirmary. A family doctor/General Practitioner or medical staff at the accident and emergency departments saw children before referral for further assessment by the hospital-based paediatric team if secondary care was required. The survey included only children who attended the paediatric services but not the accident and emergency departments.

Eligibility criteria were children aged 0–16 year, who presented with clinical and radiological features of pneumonia and were seen in hospital by a paediatrician. Data on chest radiographs were collated from local radiologists' reports and findings were grouped according to a modified version of the WHO criteria.[150, 151] Radiological reports were grouped into five categories of lobar, patchy consolidation, perihilar infiltrates, other infiltrates/abnormalities and normal. The other infiltrates/abnormalities included reports of increased bronchovascular markings, peribronchial thickening, bronchial wall thickening, or peribronchial cuffing and were analysed as pneumonia.

The modification of including a further category of non-end-point pneumonia (other infiltrates/abnormalities) is in line with the extended definitions of pneumonia used by Enwere and colleagues [228] in their study of the epidemiology of pneumonia in The Gambian PCV9 trial. This was used because WHO did not include specific criteria for 'other infiltrates/abnormalities'. Exclusion criteria included being resident outside the North East of England, clinically-diagnosed bronchiolitis, hospitalization for any reason in the preceding three weeks, or a chest radiograph reported as normal.

A favourable ethical opinion was obtained from the regional Newcastle and North Tyneside Research Ethics Committee (Appendix 1). Caldicott approvals were granted from all collaborating sites.

2.1.2 Case ascertainment and data management

Children were identified prospectively by local paediatric teams who completed a questionnaire containing data on demographics (including date of birth, sex, date of admission and discharge, parents age and occupation and postcode of residence), preadmission use of antibiotics, potential risk factors (including gestational age, immunodeficiency, chronic lung disease, cystic fibrosis, sickle cell disease, bronchiectasis, use of steroids, attendance of nursery school, and child and parental smoking), clinical examination findings, treatment given and management outcomes as well as any complications occurred. Also data were gathered of the results of any performed laboratory investigations as part of routine clinical care (Appendix 2). This questionnaire was approved by the Ethics Committee when the present survey was approved and is the same used one that was validated and approved by the Ethics Committee in 2001–2002 survey.[59] No data were collected neither on the number of referrals from primary care nor children seen in accident and emergency departments.

A recently pooled review data showed that parental and household smoking is a significant risk factor of lower respiratory tract infections and asthma in children.[73, 74, 229] Self-reporting of parental smoking is subject to underestimation.[230-232] In the present survey collected information on parental smoking was based on self-reporting. Hence this might introduce bias when to the analysis of smoking as a predictor of severe disease. In contrast the use of women recall of gestational age of their children from maternal interviews is valid for use in epidemiological studies.[233-236]

Enrolment data were cross-checked to assure complete ascertainment by reviewing ward admission diaries for children admitted with respiratory symptoms (eight sites), or by obtaining hospital coding data on pneumonia where admissions are carried out electronically (three sites). Case notes and electronic records were reviewed to confirm the diagnosis, and to collect any missing data. Pneumococcal immunisation history was obtained for each child from parents, and where available it was cross-checked with the child's health records. If there was uncertainty about the immunisation history, general practice surgeries/primary care providers were contacted and practice records of vaccines given checked.

The data sets were manually entered into a Microsoft Office Access Database. Data cleaning was carried out manually and electronically for systematic errors; extreme values or random samples were cross-checked against the hard copy of original questionnaire where it was felt necessary. Duplicates and those who did not fulfil the inclusion criteria were removed after the completion of validation process. The master data file and subfolders used for statistical analyses were encrypted to secure patients' data protection and only the study team members have access to these data.

2.1.3 Classification of disease severity and social class

Disease severity was determined using modified criteria from the British Thoracic Society (BTS) management guidelines for pneumonia.[160] The symptom of dyspnoea was excluded as the definition was deemed subjective, particularly in preschool-age children.[237] Any of the following led to the classification of "severe disease": respiratory rate >70 or >50 for ≤1- or >1-year-olds respectively; oxygen saturation <93%; oxygen therapy; nasogastric feeds; intravenous fluid infusion; septicaemia; empyema; high dependency or intensive care admission. "Mild disease" included immediate discharge home or hospital stay <3 days and no oxygen; no nasogastric feeds and no intravenous fluid infusion. Children with none of the above were classified as "moderate disease".

Deprivation has multiple dimensions such as financial, health, education, services or crime.[238-240] Townsend score [238] which includes census-derived deprivation indices does not adequately correlate with health in rural areas of the UK.[241] Therefore the index of multiple deprivation (IMD) has been developed and used in the UK to identify small areas of deprivation,[242, 243] and is based on methodology developed at the University of Oxford Social Disadvantage Research Centre.[244] It was used in childhood health study in the North East of England and was found to be valid to identify inequalities in accessing primary dental care for children.[245]

Parental occupation information was incomplete, therefore socioeconomic class and the measure of deprivation were derived for each child based on the IMD score for the parental postcode of residence (The English Index of Deprivation 2007, Office for National Statistics (ONS)).[242, 243] It is measured at the Lower Super Output Areas (LSOAs) and includes domains which are related to income deprivation, employment

deprivation, health deprivation and disability, education skills and training deprivation, barriers to housing and services, living environment deprivation, and crime.[242] It was used in this in the present survey to determine residential area-level deprivation of rural and urban areas.[242, 246]

2.1.4 Sample size and statistical analysis

The sample size calculation was based on a previous local regional data before the introduction of PCV7.[59] These data suggested that approximately 750 children could be seen with clinical diagnosis and radiologically-confirmed pneumonia over a year period. Pooled review data concluded that the pneumococcal conjugate vaccines were associated with 27% to 32% reduction in the rates of radiological pneumonia.[145, 146] Therefore, it was estimated that a sample size of 530 children with pneumonia confirmed by chest radiograph would be sufficient to identify the true changes in disease incidence rates with 80% power.

2.1.4.1 Incidence of pneumonia

Annual incidence rates were established by age and sex using the population estimates for the North East Strategic Health Authority area from the UK Office of National Statistics for 2009, and compared with those from the 2001 survey.[59] There were 458 500 children aged under 16 years, of which 146 200 were aged under five years.[247] Confidence intervals (CIs) of annual incidence rates were calculated assuming a Poisson distribution and using the EpiTools package in R statistical software version 2.14.0 (The R Foundation for Statistical Computing, Austria). Univariate and multivariate logistic regression was used to establish risk factors for severe compared to mild/moderate CAP. Fisher's exact test with calculated odds ratios (ORs) and 95% CIs was used to compare differences in count data between 2001 and 2009 for disease severity.

Although the geographical area and population served by the reduced number hospitals from 13 to 11 were the same as in 2001 as were the methods of enrolment criteria and case ascertainment,[59] calculation of annual incidence rates by county of residence of lobar finding in chest radiographs and compare them between hospitals to relate any

geographical differences to the potential variation in reporting of chest radiographs was deemed to be a source of bias to data. This is because the referral pathways from primary care to secondary care have changed overtime. Therefore comparisons of overall figures for both the annual disease incidence and lobar findings would be more accurate than the geographical sub-analysis of data. Hence geographical analysis either by county or hospital of recruitment was not performed to avoid inaccuracies of the conclusions. However, variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children is presented and discussed at greater length in (Chapter 7) of the radiology study on the application of WHO radiological classification of end-point pneumonia. This can give an idea on the inter-observer variability of radiological reporting.

2.1.4.2 Outline of the steps for the calculation of incidence of pneumonia

The following steps were undertaken to establish the denominators for the calculation of annual disease incidence and hospitalisation rates between the two surveys as well as incidence of pneumonia in PCV7-vaccinated versus unvaccinated groups. A meeting with epidemiologists: Prof Stephen Rushton and Dr Russell Gorton and discussion with supervisors: Drs Julia Clark and Andrew Gennery were carried out about the calculation of rates and agreed the steps. It was also agreed that because of sample size limitation the calculation of vaccine efficacy would be subject to bias and inaccuracy.

- **2001–2002 survey [59]**

1. Of 711, 530 aged <5 years, 181 ≥5 years.
2. But 750 is value the incidence was calculated on (include 39 where data incomplete but known to have pneumonia).
3. Therefore predict the age distribution of those that are missing based on pre-existing data.

4. Assuming that those missing are equally distributed between different age groups:
    a. Therefore 530/711 = 74.5%
    b. Therefore 30/39 presumed to be aged <5 years
    c. 9/39 presumed to be aged ≥5 years
    d. Therefore cases are: 190 aged ≥5 and 560 aged <5 years
5. These numbers are the basis for the incidence rates quoted in the paper at the moment.
6. However, there is an issue with the population estimates as if use the official ONS population estimates these no longer match the published 2001 data – likely a revision of numbers post 2001 census. This means there is an issue with using the official ONS published data.
7. So calculated from published rate of 33.8/10000 for those aged <5 years and total cases of 560 (as above):
    a. Estimate of the <5 years old population is therefore:
        i. (560/33.8)*10000 = 165680.47 people aged <5 years
    b. Therefore children aged 5-16 years = Total population – <5 years population = 522158 – 165680.47 = 356478
    c. Therefore rate in 5-16 = (190/356478)*10000
8. Now hospitalisations were 636 children according to the paper [59] (i.e. none of 39 missing having been admitted).
    a. Therefore using hospitalisation rate of those aged <5 years and population estimate to calculate distribution of hospitalisations:
    b. Therefore hospitalisations of those aged <5 years = population * rate = 165680.47 *28.7 = 476 of 636 aged <5 years hospitalised
    c. Therefore children aged ≥5 years = 636 – 476 = 160

- **2008–2009 survey**

1. Calculating vaccine efficacy given numbers with known vaccination status:
    a. Aged <5 years = 400
    b. Cases with known vaccine status = 392
    c. Vaccinated cases = 321
    d. Unvaccinated cases = 71
    e. Aged <5 years population = 146200
2. Primary PCV7 coverage varied between 87–94.2%
3. Catch up coverage of PCV7 in Scotland 86%
4. Catch up coverage of PCV7 in North East 70.3%
5. So calculate on best/worst case scenarios:
    a. Best scenario = 94.2%
    b. Worst scenario =70.3%
6. Best scenario for VE:
    a. Vaccinated population = 146200 * 0.942 = 137720.4
    b. Unvaccinated population = 146200 – 137720.4 = 8479.6
    c. Therefore vaccine efficacy (VE) = 1 – ((321/137720.4)/(71/8479.6))
    d. With CI calculated:
        i. VE = 0.7216291 (95% CI = 0.6422732 to 0.7857344)
7. Worst scenario for VE:
    a. Vaccinated population = 146200 * 0.703 = 102778.6
    b. Unvaccinated population = 146200 – 102778.6 = 43421.4
    c. Therefore VE = 1 – ((321/102778.6)/(71/43421.4))
    d. With CI calculated:
        i. VE = -0.9100635 (95% CI = -0.4702005 to -1.454571)

8. Fisher's exact test comparing counts of vaccinated versus unvaccinated cases and population:
    a. Note: still dependent on knowing the correct vaccinated/unvaccinated distribution in population.
    b. Cases of pneumonia: vaccinated = 321, unvaccinated = 71
    c. Non-cases (population): vaccinated = 127143, unvaccinated = 19057
    d. P-value = 0.004216

2.1.4.3 Audit of the national management guidelines of pneumonia

Descriptive statistical analysis was performed using Epi Info™ 7. Fisher's exact test with ORs and 95% CIs was used to compare counts of individual cases as classified using categorical variables between groups and with those from the pre-guidelines survey.[157] A comparison of treatment approaches, clinical and radiological features for severe versus mild/moderate CAP was performed using logistic regression. Logistic regression analyses were undertaken in the Predictive Analytics SoftWare program (PASW Statistics 17).

Cox-proportional hazards models [248] were used to investigate the impact of different covariates including disease severity (categorical), hospital site (categorical), use of antibiotics and their route of admission (categorical) on the length of stay (continuous). The discharge from hospital was assumed an event and that these parameters influenced this event. It was hypothesised that the severity and absence of antibiotic treatment would decrease the risk of early discharge from hospital. Models were fitted in the statistical software R-2.14.0 using the survival package of Therneau and Grambsch (2001).[249] Length of stay is a variable constrained by the time of zero, with many children diagnosed to have pneumonia being discharged early from hospital with few children staying longer. Such data are effectively "life time" of stay in hospital which

means that these covariates are most appropriately analysed using a survival analysis approach. Analyses were undertaken with leaving date from hospital as the response and disease severity, hospital site and use of antibiotics and their route of admission as covariates of risk of factors for prolonged hospital stay whilst adjusting for age. For each covariate, likelihood ratio (LHR) test p-value was calculated to show the overall association between a covariate and length of stay. This is different to the Wald test p-values that just show whether each level of a covariate is significantly different to the reference level or not.

The baseline hazard function in the Cox proportional hazard model is modified multiplicatively by the above mentioned covariates. This makes the interest is in the cumulative hazards which will be a proportional factors rather than the baseline hazard. Then conditional on the event of discharge from hospital, the probability does not depend on the baseline hazard of each covariate. The hazard ratio yields an estimate of the ratio between the baseline excess hazards of longer hospital stay attributable to children admitted with pneumonia and the population hazard for each child.[250] The association between the covariates and length of stay and discharge from hospital as outcomes was investigated using event analysis while adjusting for child's age.[248] Initially fitted full models with all variables and identified the best model by a stepwise removal of non-significant variables. For validity of significant models' modelling, the assumptions of proportionality of hazard were assessed using the Schoenfeld residuals according to the methodology of Therneau and Grambsch.[249] Then the best models were used for each covariate to predict the time of discharge from hospital.

2.2 Aetiology study

2.2.1 Study design and participants

Two prospective studies were undertaken from August 2001 to July 2002 and October 2009 to March 2011 of children aged 0–16 years with clinical and radiological features suggestive of pneumonia. They were from the North East England (excluding Cumbria) who presented or transferred to the paediatric services at the Great North Children's Hospital (formerly Newcastle General and Royal Victoria Infirmary), the regional cardiothoracic centre at Freeman Hospital Newcastle where empyema is managed or the James Cook University Hospital in Middlesbrough. The cohort of 2001–2002 study was a proportion of children with pneumonia seen at these recruitment sites as part of a previously published epidemiological survey.[59, 157] They were consented and enrolled in the aetiological study with an extended panel of investigations.

Recruitment methods and enrolment criteria were consistent across the two studies and included children with any history, signs or symptoms suggestive of lower respiratory tract infection and chest radiographic findings consistent with infection as determined by the local paediatrician and subsequently approached by a member from the research team. No recruitment was carried out in accident and emergency departments. Exclusion criteria included resident outside of North East England; clinical diagnosis of bronchiolitis; hospitalisation in the preceding three weeks or normal chest radiograph after formal reporting by a radiologist. All chest radiographs were reviewed by a second consultant radiologists (Drs R Lee and M Muller in the pre- and post-vaccine studies respectively) at the regional centre in Newcastle who were blinded to both clinical data and the first reports. Chest radiographic findings were categorised into lobar, patchy or perihilar according to the WHO criteria.[150, 151] Research teams of doctors and

nurses led and ascertained the standardised diagnosis of pneumonia and the recruitment procedures. Pneumococcal conjugate immunisation history including the vaccine valency was obtained from parents and cross-checked with the child's parent held health records. General practice surgeries were contacted to clarify doses given if there was uncertainty. This immunisation history was not collected in the pre-vaccine study.

Ethical approvals were granted by the Newcastle and North Tyneside Research Ethics Committee for both studies and Research Approval Board at South Tees Hospitals NHS Trust, Middlesbrough (Appendix 1). Caldicott approvals were also obtained.

I led the recruitment procedures of 2009 study at the sites of Newcastle upon Tyne Hospitals NHS Trust, supported by Kerry Pollard (research nurse). At the JCUH, the recruitment was facilitated by Dr Fiona Hampton (consultant paediatrician) and Pauline Singleton (research nurse). I visited James Cook site to validate data on enrolled children. Written information on the pneumonia study consisted of a four page parent information sheet and a two page age appropriate information document for the child of either aged under or above 10 years. Written informed consent was obtained from child's parents as well as assent from older children using a generic consent forms (Appendix 3).

Data were collected on standard proforma for epidemiological, laboratory and clinical characteristics (Appendix 2), together with samples of nasopharyngeal secretions, urine and blood. If blood tests were performed as part of the child's routine care then a little more blood was taken at the time for the purposes of the study. If the children did not require blood tests as part of their routine care then blood sample was collected for the purpose of the study if convenient for both child and parents.

2.2.2 Laboratory procedures

Samples included blood, urine and respiratory secretions. Approximately four weeks later blood was collected for convalescent serology. Parents often declined returning for these convalescent samples (all in the post-vaccine study), contributing to the variability of investigations performed. Blood samples were collected for serum, blood culture (BacT/ALERT®, bioMérieux, France) and pneumococcal PCR testing. Nasopharyngeal secretions included aspirates (NPA) from infants as appropriate for age, and/or two swabs (NPS) from older children. The NPA sample was placed in 0.9% sodium chloride transport solution or swabs (Medical Wire & Equipment Co Ltd, UK). Tracheobronchial secretions (collected via endotracheal tube or bronchoalveolar lavage), non-induced sputum and pleural fluids were tested when obtained. The nature of collected samples were standardised across all ages and in both studies.

Where tests were not part of routine clinical care, samples were stored at –20°C for subsequent analysis. Investigations were performed in the Microbiology Laboratory, Newcastle upon Tyne Hospitals NHS Trust and the Health Protection Agency (HPA) Public Health Laboratory, Newcastle. Apart from locally performed routine diagnostic tests, samples from Middlesbrough were transported to Newcastle via daily transport services. Pneumococcal isolates from blood, urine (positive antigen testing) and respiratory secretions including pleural fluids were serotyped by multiplexed immunoassay using xMAP beads for detection of serotype-specific *S. pneumoniae* antigens at the national HPA Respiratory and Systemic Infection Laboratory in London.[207]

2.2.2.1 Viral laboratory diagnostic tests

In the pre-vaccine study, immunofluorescence antibody testing (IFAT) was applied to respiratory secretions using Chemicon SimuFluor FITC for respiratory syncytial virus (RSV), influenza A and B, parainfluenza 1-3, and adenovirus and human metapneumovirus (hMPV) was tested for using IFAT utilising an in-house pool of anti-hMPV monoclonal antibodies.[251] Viral screening was performed in the post-vaccine study using an in-house multiplex real-time PCR assay. The target panel was expanded to include pandemic influenza A subtype H1N1, parainfluenza virus 4, rhinovirus, coronavirus (229E, OC43 and NL63), and bocavirus plus the viruses previously tested for by IFAT. Viral serological tests included respiratory syncytial virus (RSV), influenza A and B virus, and adenovirus.

2.2.2.2 Bacterial laboratory diagnostic tests

An aliquot of NPA or bacterial NPS was inoculated onto plates of Columbia agar (Oxoid) supplemented with 5% horse blood (CBA). Samples were also inoculated into Oxoid brain-heart infusion enrichment broth with 10% serum (BO0129E; Oxoid, UK) and were incubated overnight at 37°C. Enrichment cultures were sub-cultured (10 µL) into CBA plates which were incubated at 37°C in atmospheric air supplemented with 5% carbon dioxide for 48 hr. Isolates of *S. pneumoniae* and group A *Streptococcus* (GAS) were identified by standard methods including latex agglutination and API 32 STREP (bioMérieux, France). These were stored in STGG medium (skim milk-tryptone-glucose-glycerol), prepared in-house as previously described.[252] In the post-vaccine study, bacterial screening of respiratory secretions was performed using an in-house real-time PCR assays which targeted *S. pneumoniae, Haemophilus influenzae, Bordetella pertussis, Mycoplasma pneumoniae,* and *Chlamydophila pneumoniae*.[253-

256] All of the assays used have been validated for the detection of the target organisms (Dr Andrew Sails, personal communication).

Total nucleic acid was extracted from blood samples from both studies and tested using a *S. pneumoniae* specific PCR assay targeting the pneumolysin gene.[255] Samples post-vaccine were also tested in a 16S rRNA PCR assay (Molzym GmbH and Co., Bremen, Germany). An acute complement fixation test (CF) antibody screen for 'atypical' bacteria and respiratory viruses was also performed which included *Mycoplasma* IgM antibody, *M. pneumoniae, C. pneumoniae and C. psittaci*, as well as, *Coxiella burnetii*, and *Legionella pneumophila* serogroup 1-6 in the post-vaccine study. Antistreptolysin O titre (ASOT) was assayed in both studies by Rheumajet ASO kit (Launch Diagnostics, UK). In the pre-vaccine study, urine samples were tested for pneumococcal antigen with an in-house counter-current immunoelectrophoresis (CIE) assay and in the post-vaccine study this was replaced by Binax NOW (Inverness Medical Innovations Ltd, Galway, Ireland).

Isolation of pathogenic bacteria from sputum samples was considered as possible infection due to the risk of contamination from nasopharyngeal secretions. Together with blood and pleural fluids, tracheobronchial secretions were considered as normally sterile sites, so any recovered bacteria from these sources suggested a definite infection as per diagnostic criteria outlined in Table 2-1.

2.2.2.3 Pneumococcal PCR assays and serotyping

Blood samples were subjected to molecular diagnostic investigations including 16S rRNA PCR (Molzym GmbH and Co., Bremen, Germany) and a *S. pneumoniae* specific PCR targeting the pneumolysin gene using an in-house assay.[255] Clinical pneumococcal isolates from respiratory secretions, pleural fluids and blood, plus

pneumococcal antigen positive urine samples were serotyped by a multiplexed immunoassay using xMAP beads for detection of serotype-specific S. *pneumoniae* antigens at the national HPA Respiratory and Systemic Infection Laboratory in London.[207] The pneumococcal serotyping on pleural fluids was part of the enhanced surveillance of pneumococcal empyema in UK children (UK-ESPE study).

For the 16S rRNA PCR assay, DNA was extracted from 1 mL aliquots of blood (following storage at -80^0C) using a MolYsis Complete 5 kit (Molzym GmbH and Co., Bremen, Germany) according to the manufacturer's instructions. The kit utilises a novel technology which facilitates the selective lysis of blood cells followed by the quantitative degradation the released DNA by a proprietary DNase (MolDNase). The bacteria are then enriched from the lysate by centrifugation, and DNA is extracted by column purification. The DNA extracts were used as template in a PCR assay targeting a conserved region of the 16S rDNA gene. Following PCR amplification PCR products were analysed by gel electrophoresis.

The pneumolysin PCR used in this study was established by Corless and colleagues in the UK.[255] It was developed on the ABI 7700 Sequence Detection System (TaqMan) for the detection of *S. pneumoniae* from clinical samples of cerebrospinal fluid, plasma, serum, and whole blood. Pneumolysin (*ply*) gene target specific for *S. pneumoniae* was selected. Sensitivity was evaluated using these clinical samples which were collected from culture-confirmed cases of pneumococcal infections. It gave an overall 91.8% sensitivity and 100% for the 36 samples tested. The *ply* primers amplified pneumococcal serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10A, 11A, 12, 14, 15B, 17F, 18C, 19, 20, 22, 23, 24, 31, and 33.[255] The in house pneumolysin PCR used in the present study has been validated and used previously for the detection and identification of *S.*

pneumoniae for many years in the Microbiology Laboratory, Newcastle Hospitals NHS Trust and the Newcastle HPA Laboratory (Dr Andrew Sails, personal communication).

The multiplexed immunoassay using xMAP beads for detection of serotype-specific *S. pneumoniae* antigens was developed at the HPA in London.[207] This assay can identify all of the serotypes included in PCV13 plus serotype 8. It is suitable for use on body fluids for the determination of the commonest serogroups and serotypes of *S. pneumoniae* prevalent in the UK.[192] It has good sensitivity (79.3%, 46/58) and specificity (99.3%, 145/146) on correctly identifying the pneumococcal serotypes when testing urine samples from patients with culture-confirmed pneumococcal or non-pneumococcal disease.[207] This test therefore has considerable potential for the epidemiological assessment of pneumococcal infections including serotype distribution and replacement in children. A national active surveillance of IPD has been extended to include pleural fluid from empyema thoracis and a national prospective reporting system is being established in order to monitor the incidence rates of pleural empyema (UK-ESPE study).

2.2.3 Development of pneumococcal serotype-specific PCR

A sequential multiplex pneumococcal serotype-specific PCR was developed at the HPA Public Health Laboratory Newcastle. Its technical development was adopted from the previously described study by Pai and colleagues [200] at the CDC in the USA. It targeted the prevalent 29 primer pairs of pneumococcal serotypes which were grouped into seven multiplex reactions. These serotypes included 1, 3, 4, 5, 6A/B, 7F, 7C, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B/C, 16F, 17F, 18, 19A, 19F, 20, 22F, 23F, 31, 33, 34, 35B, 35F, and 38.

For the purpose of this study, I led the process of development and application of this test under the supervision of Dr Andrew Sails. I performed the aliquoting of primers, prepared the master mix solution and extracted the DNA from the batched pneumococcal isolates from blood and nasopharyngeal secretions. All primers were synthesised at the Eurogentec. The control pneumococcal serotypes were provided by the Microbiology Laboratory at the Newcastle upon Tyne Hospitals NHS Trust and the Respiratory and Systemic Infection Laboratory, HPA in London. I attempted using this test to serotype the stored pneumococcal PCR-positive samples from blood obtained from previous aetiological study in the same setting in 2001–2002. But unfortunately this was unsuccessful because the long storage period of the samples resulted in DNA degradation, causing multiple non-specific PCR amplification.

This test was applied to the culture-negative, pneumolysin PCR-positive blood or respiratory secretion samples. The selected samples were those with a cycle threshold (Ct) of ≤30. This cut off was decided following testing couple of representative samples of different Ct levels of positive pneumolysin PCR assays to detect a good DNA signal for the capsular polysaccharide antigens (cps).

2.2.4 Case ascertainment and data management

Admitted children with pneumonia were identified prospectively by me and research nurses. Questionnaire containing data on demographics (including date of birth, sex, date of admission and discharge, parents age and occupation and postcode of residence), preadmission use of antibiotics, potential risk factors (including gestational age, immunodeficiency, chronic lung disease, cystic fibrosis, sickle cell disease, bronchiectasis, use of steroids, attendance of nursery school, and child and parental smoking), clinical examination findings, treatment given and management outcomes as well as any complications occurred (Appendix 2). This questionnaire was approved by the Ethics Committee when the present survey was approved and is the same used one that was validated and approved by the Ethics Committee in 2001–2002 survey.[59] With the approval from Ethics Committee, few questions were added on the use of Ibuprofen (anti-inflammatory drug). This was to investigate a separate secondary outcome of its potential association with the development of severe pneumonia.[95, 257] No data were collected neither on the number of referrals from primary care to paediatric assessment units nor children seen in accident and emergency departments.

The data sets were manually entered into a Microsoft Office Access Database. Data cleaning was carried out manually and electronically for systematic errors; extreme values or random samples were cross-checked against the hard copy of original questionnaire where it was felt necessary. Duplicates and those who did not fulfil the inclusion criteria were removed after the completion of validation process. The master data file and subfolders used for statistical analyses were encrypted to secure patients' data protection and only the study team members have access to these data. Data were analysed using Epi Info^{TM} 7.

2.2.5 Statistical analysis

2.2.5.1 Causes of pneumonia

Summary of the isolated pathogens was presented as frequencies and categorised as viral, bacterial or mixed viral-bacterial infections. Detection rates of pathogens are expressed as proportions of those tested, and results were compared in relation to age group. The age group classification was under/above five years as by the start of post-vaccine study, children in the under five age group should have been vaccinated with PCV. This would allow the investigation of the relative contribution of pneumococcal infection in causing pneumonia, as well as the role of other pathogens. Depending on the nature of samples and type of test applied, positive results were classified as definite or possible according to defined diagnostic criteria (Table 2-1), and only the definite results are presented and discussed. A Venn diagram was used to show different positive applied diagnostic approaches for pneumococcal and group A streptococcal infections.

Recovery of bacterial pathogens from NPAs, NPSs or sputum was not considered evidence of definite infection due to the risk of physiological colonisation, but bacteria from tracheobronchial secretions was.[1] Where there was a common methodology for diagnosis between studies, identification rates were compared using Fisher's exact test with ORs and 95% CIs.

2.2.5.2 Diagnosis and serotyping of pneumococcal infections

Detection rates of pathogens are expressed as proportions of those tested. According to the nature of samples and type of test applied, positive results were classified as definite or possible and only the definite results are presented and discussed. Isolation of pneumococci from NPAs, NPSs or sputum was not considered evidence of definite

infection whereas isolation from tracheobronchial secretions was.[1] Together with blood and pleural fluids, tracheobronchial secretions were considered as normally sterile sites, so any recovered *S. pneumoniae* from these sources suggested a definite infection as per the diagnostic criteria outlined in Table 2-1.

Fisher's exact test was used to compare infection status between different detection methods and ORs and 95% CIs calculated where possible. Logistic regression was used to investigate the extent to which selected clinical and laboratory variables were predictors of positive status by each diagnostic test with a view to providing clinical guidelines for use of different tests during assessment at presentation. The receiver operating characteristic (ROC) curve was used to determine the specificity and sensitivity of urinary pneumococcal antigen test to identify definite pneumococcal infection.

Table 2-1 Laboratory investigations and diagnostic criteria

		Tests[*]		Diagnostic criteria	
Sample	**Pathogen/antigen**	**2001–2002 study**	**2009–2011 study**	**Definite**	**Possible**
Serum	Respiratory viruses	Complement fixation	Complement fixation	Acute titre ≥1/128 or 4-fold rise between paired sera	NA[†]
	Atypical bacteria				
	Mycoplasma	IgM antibody	IgM antibody	Positive	NA
	Group A *Streptococcus*	ASOT (IU/mL)	ASOT (IU/mL)	Acute 2-fold rise or 4-fold rise between paired sera	NA
Blood	*S. pneumoniae*	Real-time PCR	Real-time PCR	Positive	NA
	16S rRNA gene	Not tested	PCR	Positive	NA
	Bacteria	Culture	Culture	Growth	NA
Nasopharyngeal secretions/sputum	Respiratory viruses	IFAT	Real-time PCR	Positive	NA
	Bacteria	Culture	Culture/real-time PCR	NA	Growth/Positive

		Tests*		Diagnostic criteria	
Sample	**Pathogen/antigen**	**2001–2002 study**	**2009–2011 study**	**Definite**	**Possible**
Tracheobronchial secretions (collected via bronchoalveolar lavage and/or endotracheal)	Respiratory viruses	IFAT	Real-time PCR	Positive	NA†
	Bacteria	Culture	Culture/real-time PCR	Growth/Positive	NA
Pleural fluids	Bacteria	Culture	Culture	Growth	NA
	Pneumococcal antigen	ELISA	ELISA	Positive	NA
	S. pneumoniae	Not tested	Real-time PCR	Positive	NA
Urine	*S. pneumoniae*	CIE	Binax NOW	NA	Positive

*ASOT, antistreptolysin O titre; IFAT, immunofluorescence antibody testing; ELISA, enzyme-linked immunosorbent assay; CIE, counter-current immunoelectrophoresis.

†NA, not applicable.

2.3 Radiology study

2.3.1 Study design and participants

A prospective study to investigate the aetiology of pneumonia in children was undertaken from October 2009 to March 2011 in two teaching hospitals in North of England as described in section 2.2. Caldicott approval was granted and the study was ethically approved by the Newcastle and North Tyneside Research Ethics Committee (No: 08/H0906/105), and the Research Approval Board at South Tees Hospitals NHS Trust (No: 2008075) (Appendix 1).

Children aged 0–16 years who presented to paediatric services with signs and symptoms suggestive of lower respiratory tract infection including any of fever, tachypnoea, dyspnoea, cough, respiratory distress and auscultatory chest crackles, with chest radiographic findings consistent with pneumonia as determined initially by the admitting paediatrician were enrolled. Paediatricians were not asked to give specific radiological interpretations which were provided by radiologists. As this study was on the CAP aetiology, exclusions included being resident outside of North East England, clinical bronchiolitis, or hospitalization in the preceding three weeks. Children with recent hospitalisation were excluded in order to eliminate the potential risk of having hospital rather than community-acquired pneumonia. Children with underlying chronic chest diseases (such as cystic fibrosis) were also excluded to avoid any ambiguity in the interpretation of acute and chronic changes on chest radiographs. Research teams of doctors and nurses led and ascertained the standardised diagnosis of pneumonia and the recruitment process across the two sites. All enrolled children irrespective of the chest radiographic findings received treatment for pneumonia according to the management guidelines from the BTS.[1]

2.3.2 Radiology

All chest radiographs were anteroposterior views and first reported by radiologists locally as per routine clinical care and viewed electronically via the Picture Archiving and Communications System (PACS). There were uniform and regular quality assessments performed on the system performance including display characteristics. All reporters used similar workstations of radiological standards when reporting the chest radiographs. Using the full text written first reports, each radiograph was categorised into lobar (end-point consolidation), patchy, perihilar (non-end-point consolidation/infiltrate) or normal (no pneumonia) according to the WHO criteria.[150, 151] Effusion with fluid in the pleural space between the lung and chest wall was considered as primary end-point and classified simply as either present or absent.[151] This does not include fluid in the horizontal or oblique fissures. First reports were generated with the benefit of clinical information, a standard institutional requirement for routine reporting. All radiographs were reviewed by a second consultant cardiothoracic radiologist (Dr Michelle Muller) at the regional centre (designated as the "gold standard") who was blinded to both the first report and specific clinical data. However, this radiologist knew the radiographs were from a child enrolled in the CAP study, thus clinically pneumonia had been suspected. Radiologists involved in performing the first and second reporting received the same training in radiology including the classification of radiological pneumonia. Those involved in first reporting included five radiology trainees, three consultants general, two paediatric and two cardiothoracic radiologists.

A workshop including me, Dr Michelle Muller, Dr David Spencer (consultant respiratory paediatrician) and Dr Julia Clark (chief investigator and consultant in paediatric infections disease) was carried out before the application of WHO criteria

[150] on the first reports and performing the second reading in order to discuss and refine the potential definitions which could be a source of disagreement such as interstitial infiltrates of patchy or perihilar changes. The study team agreed that if more than one radiographic change were reported, then in line with WHO recommendations the most significant one is reported.[150] The WHO criteria were prioritised according to the clinical significance, as follows: lobar (end-point consolidation) in favour of other changes (non-end-point infiltrates) if both were present.[150] When more than one radiographic change was reported then the radiograph was classified overall according to the most significant category. I carried out the grouping of the first reports and there was no ambiguity on the wording of first reports that might cause confusion on categorization. Inter-observer variability in the interpretation of chest radiographs was measured by the comparison of first reports with their second reading. The intra-observer variation was not calculated because all radiologists read the radiographs only once.

2.3.3 Statistical analysis

Data analysis was performed using the PASW Statistics 19 program. The significance of inter-observer variability was assessed using Fisher's exact test because there were small values <5 in the tables. Cohen's kappa index (*k*) was calculated to measure the agreement between the first and second readers above that which would be expected by chance.

Chapter 3 Incidence of Childhood Pneumonia

3.1 Results

A total of 582 children were initially identified; 40 were excluded (34 had a normal chest radiograph, six lived outside of the North East), leaving 542 eligible for inclusion (58% males). There were no deaths. Overall, 98% received antibiotics and 84% were admitted to hospital. Lobar consolidation was reported in 30%, and pleural effusion was present on 9.6% of the chest radiographs. Four hundred (74%) children aged under five years old were included. Of these, 320 were vaccinated with PCV7, 33 were eligible for this vaccine but had not received it, for nine their vaccination status was unknown and 38 were ineligible for the vaccine on age grounds. One child who was ineligible on age grounds had received the vaccine. Hence, the PCV7 uptake was 90.7% amongst the eligible children in the survey.

The annual incidence of pneumonia was 11.8/10 000 children aged ≤16 years (95% CI 10.9–12.9), with 27.4/10 000 (95% CI 24.8–30.2) in the under five age group. This compared to 14.4/10 000 (95% CI 13.4–15.4) and 33.8/10 000 (95% CI 31.1–36.7), respectively in 2001.[59] The annual hospitalisation rate was 9.9/10 000 (95% CI 9.0–10.9) for all, and 22.4/10 000 (95% CI 20.1–25.0) for the under fives. This was lower than the 2001 annual rates; 12.2/10 000 (95% CI 11.3–13.2) and 28.7 (95% CI 26.2–31.4), respectively. By calculation of the incidence rate ratio, the overall reduction in annual incidence between 2001 and 2009 was 17.7% (95% CI 8–26) and the reduction in annual hospitalisation rate 18.5% (95% CI 8–28). The reduction in annual incidence of pneumonia in the under fives was 19% (95% CI 8–29%). Table 3-1 compares data between the 2001 and 2009 surveys.

There was a significantly lower annual incidence of pneumonia among children aged under five vaccinated with PCV7 (25.2/10 000, 95% CI 22.6–28.2) compared with those

that were unvaccinated (37.4/10 000, 95% CI 29.2–47.1) (OR 4.5, 95% CI 3.5–5.9). However, there was no significant difference in the annual incidence of severe disease between the vaccinated children (13.0/10 000, 95% CI 11.1–15.1) and those unvaccinated in the under five age group (22.6/10 000, 95% CI 16.4–30.5) (OR 0.7, 95% CI 0.4–1.2). Amongst those aged under two years, there was a significant reduction by 33.1% (95% CI 20–45) in the annual incidence of pneumonia from 49.9/10 000 (95% CI 44.1–56.4) in 2001 to 33.5/10 000 (95% CI 28.9–38.4) in 2009, whereas the reduction in annual hospitalisation rate was 38.1% (95% CI 24–50) between 2001 and 2009 (Table 3-1). Reduction in both the annual incidence of pneumonia and hospitalisation rate between 2001 and 2009 was also observed among the 2.0–4.9 years age group by 23.1% (95% CI 7–36) and 29.8% (95% CI 14–43) respectively. In the over five age group, there was no difference in the annual incidence of pneumonia between 2001 and 2009 (incidence rate ratio 0.85, 95% CI 0.7–1.2), nor the annual hospitalisation rate between both studies (incidence rate ratio 0.9, 95% CI 0.7–1.2).

In common with the 2001 analysis [59], the differences in annual rates of pneumonia between girls and boys, and between different socioeconomic groups were not significant. Most cases (*n*=363, 67%) occurred during the winter and spring seasons. Table 3-2 summarises the annual incidence rates of CAP by age group for disease severity and chest radiographic findings. Overall, males had higher rates of CAP for different categories of disease severity. In both males and females the annual rates in children under five were six-times higher than those in the over fives. Patchy changes were the most common chest radiographic finding particularly in the under five age group. Lobar pneumonia was seen in a quarter of children aged under five years old, compared to approximately 15% in 2001. There was an overall significant increase in the annual incidence of lobar pneumonia from 2.8/10 000 (95% CI 2.3–3.3) in 2001 to

3.5/10 000 (95% CI 3.0–4.1) in 2009 (OR 1.3, 95% CI 1.01–1.6). No significant risk factors for severe pneumonia were identified with univariate or multivariate logistic regression. These included age, gender, socioeconomic status, prematurity, parental smoking and asthma (Table 3-3). Although parental smoking was not a significant risk factor for severe disease, where parents were smokers, 58% (84/146) of their children had severe disease. This is compared to 50% (123/247) of children with severe disease of non-smoking parents (OR 1.3, 95% CI 0.8–1.9, p=0.253).

Table 3-1 Comparison of annual incidence of pneumonia and hospitalisation between 2001 and 2008 data per 10 000 children

	2001–2002 survey	**2008–2009 survey**	**Reduction**
Variables	**IR (95% CI)***	**IR (95% CI)**	**% (95% CI)**
Overall			
Pneumonia	14.4 (13.4–15.4)	11.8 (10.9–12.9)	17.7 (8 to 26)
Hospitalisation	12.2 (11.3–13.2)	9.9 (9.0–10.9)	18.5 (8 to 28)
Under two years (0–1.9)			
Pneumonia	49.9 (44.1–56.4)	33.5 (28.9–38.4)	33.1 (20 to 45)
Hospitalisation	45.6 (40.1–51.8)	28.2 (24.1–32.8)	38.1 (24 to 50)
2.0–4.9 years			
Pneumonia	30.7 (27.1–34.6)	23.6 (20.4–27.1)	23.1 (7 to 36)
Hospitalisation	27.5 (24.1–31.3)	19.3 (16.5–22.5)	29.8 (14 to 43)
Under five years			
Pneumonia	33.8 (31.1–36.7)	27.4 (24.8–30.2)	19.1 (8 to 29)
Hospitalisation	28.7 (26.2–31.4)	22.4 (20.1–25.0)	21.9 (10 to 32)
Over five years			
Pneumonia	5.3 (4.6–6.1)	4.5 (3.8–5.4)	14.7 (-7 to 32)†
Hospitalisation	4.5 (3.8–5.2)	4.1 (3.4–4.8)	9.4 (-15 to 29)†

*IR, incidence rate; CI, confidence interval.

†Negative numbers denote an estimate of an increase in incidence.

Table 3-2 Annual incidence of pneumonia per 10 000 children (2008–2009)

	Under fives (*n*=400)		Over fives (*n*=142)		Overall (*n*=542)	
Variables	***n* (%)**	**IR (95% CI)***	***n* (%)**	**IR (95% CI)**	***n* (%)**	**IR (95% CI)**
Male	227 (56.8)	15.5 (13.6–17.7)	86 (60.6)	2.8 (2.2–3.4)	313 (57.7)	6.8 (6.1–7.6)
Female	173 (43.2)	11.8 (10.1–13.7)	56 (39.4)	1.8 (1.4–2.3)	229 (42.3)	5.0 (4.4–5.7)
Disease severity						
Mild	147 (36.8)	10.1 (8.5–11.8)	56 (39.4)	1.8 (1.4–2.3)	203 (37.5)	4.4 (3.9–5.1)
Moderate	40 (10.0)	2.7 (1.9–3.7)	16 (11.3)	0.5 (0.3–0.8)	56 (10.3)	1.2 (0.9–1.6)
Severe	213 (53.2)	14.6 (12.7–16.7)	70 (49.3)	2.2 (1.8–2.8)	283 (52.2)	6.2 (5.5–6.9)
Chest radiographic findings						
Patchy	227 (56.8)	15.5 (13.6–17.7)	69 (48.6)	2.2 (1.7–2.8)	296 (54.6)	6.5 (5.7–7.2)
Lobar	99 (24.8)	6.8 (5.5–8.2)	63 (44.4)	2.0 (1.6–2.6)	162 (29.9)	3.5 (3.0–4.1)
Perihilar	61 (15.2)	4.2 (3.2–5.4)	6 (4.2)	0.2 (0.1–0.4)	67 (12.4)	1.5 (1.1–1.9)
Other infiltrates	13 (3.2)	0.9 (0.5–1.5)	4 (2.8)	0.1 (0.03–0.3)	17 (3.1)	0.4 (0.2–0.6)
Social class (IMD score)†						
1st quantile (2.97–14.46)	101 (25.2)	6.9 (5.6–8.4)	29 (20.4)	0.9 (0.6–1.3)	130 (24.0)	2.8 (2.4–3.4)
2nd quantile (14.47–25.33)	92 (23.0)	6.3 (5.1–7.7)	36 (25.3)	1.2 (0.8–1.6)	128 (23.6)	2.8 (2.3–3.3)
3rd quantile (25.34–42.44)	102 (25.5)	6.9 (5.7–8.5)	43 (30.3)	1.4 (0.9–1.9)	145 (26.8)	3.2 (2.7–3.7)
4th quantile (42.45–78.53)	105 (26.3)	7.2 (5.9–8.7)	34 (23.9)	1.1 (0.8–1.5)	139 (25.6)	3.0 (2.6–3.6)

*IR, incidence rate; CI, confidence interval; †IMD, index of multiple deprivation.

Table 3-3 Univariate risk factors of severe versus mild/moderate pneumonia

		Disease severity, *n* (%)				
Characteristics	***n* (%)**	**Severe**	**M/M***	**OR†**	**95% CI**	***P***
Age group (years)						
Under fives	400 (73.8)	204 (51.0)	196 (49.0)	1.7	0.8–1.6	0.418
Over fives	142 (26.2)	79 (55.6)	63 (44.4)	1.0	–	
Sex						
Female	229 (42.3)	115 (50.2)	114 (49.8)	1.0	–	
Male	313 (57.7)	168 (53.7)	145 (46.3)	1.2	0.8–1.6	0.427
Social class (IMD score)						
1st quantile (2.97–14.46)	130 (24.0)	67 (51.5)	63 (48.5)	1.0	–	
2nd quantile (14.47–25.33)	128 (23.6)	70 (54.7)	58 (45.3)	1.1	0.7–1.9	0.612
3rd quantile (25.34–42.44)	145 (26.8)	72 (49.7)	73 (50.3)	0.9	0.6–1.5	0.755
4th quantile (42.45–78.53)	139 (25.6)	74 (53.2)	65 (46.8)	1.1	0.7–1.7	0.780
Gestation (weeks)						
24–28	7 (1.3)	3 (42.9)	4 (57.1)	5.8	0.7–48.4	0.105
29–32	13 (2.4)	7 (53.8)	6 (46.2)	1.5	0.5–4.8	0.452
33–36	25 (4.6)	13 (52.0)	12 (48.0)	1.7	0.7–3.9	0.206
≥37	497 (91.7)	260 (52.3)	237 (47.7)	1.0	–	
Parental smoking						
No	247 (62.8)	123 (49.8)	124 (50.2)	1.0	–	
Yes	146 (37.2)	84 (57.5)	62 (42.5)	1.3	0.8–1.9	0.253
Asthma						
No	501 (92.4)	261 (52.0)	240 (48.0)	1.0	–	
Yes	41 (7.6)	22 (53.7)	19 (46.3)	1.6	0.9–3.2	0.139

*M/M, mild/moderate.

†OR, odds ratio.

3.2 Discussion

This is the first prospective survey in the UK to evaluate the effect of PCV7 on the incidence of childhood CAP. It reports an 18% reduction in both the annual incidence of CAP presenting to hospital and annual hospitalisation rate between 2001 and 2009. There was a lower incidence of pneumonia among PCV7-vaccinated children under five years old than those unvaccinated. Rates of pneumonia and likelihood of hospital admission were highest among the under fives, consistent with previous studies.[33, 59] As in 2001, there were trends towards higher annual rates of pneumonia among male children living in deprived socioeconomic areas.[59, 258-260]

There were no significant risk factors for severe pneumonia in this survey, although extreme prematurity was a risk factor for severe disease in 2001.[59] This may reflect changes in neonatal care in the intervening period or could be related to small sample size. However, in a recent study our region had the highest rate of bronchopulmonary dysplasia rate in Europe, suggesting that the relationship may be complex.[261] It was surprising that parental smoking was not a risk factor for severe pneumonia, given that there was a relative excess of smoking in the studied cohort (37%) compared to the national average rate of 21% for adults.[1] Although parental smoking was not a significant risk factor for severe disease, where parents were smokers, 58% of their children had severe disease. A recently pooled review data showed that passive family smoking is a risk factor of respiratory diseases in children.[73, 74, 229] Self-reporting of parental smoking is usually underestimated.[230-232] It has been suggested to measure cotinine levels in blood and urine of children to overcome under reporting of passive smoking.[230, 232] Therefore the lack of smoking as predictor of severe disease in the present survey is potentially influenced by recall bias. Similarly there is variability on defining asthma in children which makes parents inaccurately report

it.[262, 263] A recent study in the North East of England showed that IPD is not associated with low socioeconomic status in children.[264] This supports the finding from the present survey of lack of deprivation as a risk factor for severe disease.

3.2.1 Comparison to other studies

The reduction in both annual incidence of pneumonia and hospitalisation rate in this survey is comparable with a previous study in England,[115] which reported a 19% decrease between 2006 and 2008 in childhood pneumonia using hospital episodes statistics (HES) data. The reduction in the annual rate of hospitalisation was more than that reported in Canada of 13% in the under five following the routine introduction of PCV7.[79] The annual disease incidence is also close to the estimates of other studies of PCV11 in the Philippines [147] and PCV7 in the USA [144] who reported decreases in all-cause pneumonia by approximately 22%. It is however, lower than the 25% reduction against radiological pneumonia reported in randomized controlled trial of PCV9 in South Africa [142] and 30% in the USA [148]. This may be a reflection of the differences in pneumococcal disease between populations or in adherence and vaccine usage in our population compared to the trial settings. As this survey used a standard and comparable radiological definition of pneumonia it should not reflect differences in disease ascertainment between the studies.

Interestingly, the major reduction in pneumonia admissions (38%) was observed in those aged under two years. This is similar to the finding from the USA of approximately 40%,[153] but higher than that observed (15%) during the PCV9 trial in The Gambia.[143] This variation with The Gambian trial could be related to the fact that many cases of pneumonia were missed as only 14% of all children were recruited at the sites where research teams were permanently onsite with the rest living in areas served by irregular mother-child-health clinics.[143] The marked reduction in the

annual disease incidence in the under two age group of 33%, is also comparable to the reported incidence reduction of up to 37% from the pneumococcal conjugate vaccination trials and pooled review data.[143, 145, 146] There was observed a greater decrease (30%) in the annual admission rate among those aged 2–4 years compared to the 17% found by Grijalva and colleagues.[153]

This survey has demonstrated a reduction in all-cause pneumonia. Previous estimates have suggested around 10% of childhood pneumonia is attributable to *S. pneumoniae* in the UK.[28, 29] Given the decline in pneumonia and assuming the absence of other changes in disease or admission procedures, it seems likely that 10% is a significant underestimation of the true burden of pneumococcal related childhood pneumonia in the UK.[1, 265] It is only recently that any studies have been able to describe the relative contributions of different pneumococcal serotypes in paediatric pneumonia,[18] and these have not yet been established in UK children. At the moment the pneumococcal serotype distribution in childhood pneumonia in the UK has only been inferred from surveillance of invasive pneumococcal disease by the Health Protection Agency and studies from other countries. Thus the potential reduction in pneumococcal childhood pneumonia in the UK provided by PCV7 is not known with certainty. This survey is a significant step towards reducing that uncertainty. Future studies are needed to carefully evaluate the epidemiological and health economic impacts of the new generation of conjugate vaccines.

3.2.2 Strengths and limitations

The strengths of this survey include the use of a multi-centre large scale approach, well-validated disease definition and previously studied population allowing accurate historical comparisons. Its significant limitation is that while the introduction of PCV7 is the major change between the two surveys, the ecological nature of the survey means

that the decrease in disease incidence cannot be causally attributed to PCV7 alone.[50, 266] Further potentially relevant factors include natural variations in disease incidence, other public health interventions such as anti-smoking campaigns,[267, 268] variation in national and local health policies, changes in admission criteria, referral pathways and threshold for radiological investigation, and the implementation of national guidelines for the management of CAP in children by the BTS in 2002.[160] The later factor might have resulted in more children being managed in primary care including accident and emergency departments. While cannot rule out these factors using the methodology in this survey, I feel it is unlikely that any of these factors would have reduced the incidence of pneumonia to the degree observed. Furthermore, it would be speculated that these factors would alter the overall incidence rate regardless of age group. The fact that no significant difference was found in the annual incidence of pneumonia in the over five age group, by definition non-vaccine recipients, therefore increases the likelihood that the observed changes were attributable to PCV7.

It could be speculated that changes in the incidence of viral disease or vaccination may have contributed to the observed differences in the annual rates of pneumonia, though this is unlikely given that the neither age group (and specifically the under two age group) are routinely vaccinated against respiratory viral disease. No specific data were collected on influenza vaccination status but it is most likely that the overwhelming majority of enrolled children were unvaccinated. It has also been hypothesised that a considerable proportion of viral pneumonia may in fact have co-infection with bacterial pathogens including *S. pneumoniae* as shown by Michelow and colleagues [11] which could potentially ameliorate the effect of variations in the incidence of seasonal influenza or other viral infections.

The inclusion of a further group of the other infiltrates/abnormalities chest radiographic feature to the WHO definition of radiological pneumonia could have overestimated the incidence of pneumonia within our population. However, the number of such individuals was low and represented only 3% of all cases of pneumonia within the studied cohort. It would be therefore suggested that this should not have significantly influenced these findings, given the magnitude of the changes reported in this survey. In contrast to the observed substantial reduction in the annual incidence of lobar pneumonia following the conjugate vaccination programme in Canada,[79] this survey reported increased lobar findings. This could be attributed to either the relative implication of non-PCV7 pneumococcal serotypes in the aetiology of pneumonia in children or due to the recognized variation in the interpretation of paediatric chest radiographs,[38] which in this survey were reported by local radiologists that differed between sites and from the original survey. Although the diagnosis of end-point pneumonia was dependent on reading non-standardised chest radiograph reports by local radiologists, the application of standardised criteria provided by the WHO on defining the radiological end-point pneumonia would allow more accurate comparative data in epidemiological studies for assessment of the impact of pneumococcal vaccination.[150, 151]

3.3 Conclusions

The findings suggest that PCV7 was effective in reducing by 18% both the annual incidence of childhood pneumonia seen in hospital and annual rates of hospitalisation in one population within the UK. In particular, these reductions were more marked, by nearly a third, in the under two age group. Cotinine levels in blood and urine of children should be measured in epidemiological studies of pneumonia to minimise inaccuracies of self-reporting passive household smoking. In addition, care should be taken to clearly define asthma when reporting it as a predictor of severe pneumonia in children.

Chapter 4 Management of Pneumonia in Children

4.1 Results

A total of 582 children with suspected pneumonia were identified initially; 40 were excluded (34 had a normal chest radiograph, 6 lived outside of the North East), leaving 542 eligible for inclusion (58% males; 74% under five years). Similar to the pre-guidelines survey (89%), 84% children were admitted. Of those who were discharged home after initial assessment, none returned to hospital within three weeks with clinical features suggestive of lower respiratory tract infection.

Four hundred children were under five, of these 320 were vaccinated with PCV7, 33 were eligible for this vaccine but had not received it, vaccination status was unknown in nine and 38 were ineligible for the vaccine on age grounds. One child who was ineligible on age grounds had received the vaccine. Hence, the PCV7 uptake was 90.7% amongst the eligible children in the survey, comparable to the national immunisation records for England.[269]

4.1.1 Presentation

Table 4-1 summarises the clinical features at presentation across both surveys. Comparing post- with pre-guidelines surveys; fewer children presented with severe disease (52% versus 59%) [OR 0.8, 95% CI 0.61–0.96, p=0.023], although there were no differences in the rates of hypoxia (p=0.204). There was no difference in disease severity between those aged under and over two years in both the post-guidelines (p=0.860) and pre-guidelines (p=0.615).

Lobar changes were reported more often in the pre-guidelines survey (p=0.0001), whilst patchy findings (p=0.019) and perihilar infiltrates (p=0.006) were less common. The rate of empyema complicating pneumonia increased between the survey periods to 5.4%

compared to 3%. The rates of pleural effusion were similar between the two surveys; 9.6% and 9% post- and pre-guidelines respectively [OR 1.1, 95% CI 0.72–1.55, p=0.845]. Among those with pleural effusion, reported lobar changes were present in 77% post-guidelines compared to 42% pre-guidelines [OR 0.2, 95% CI 0.09–0.48, p=0.0002]. Empyema was associated with lobar changes in 96.6% and 62.5% post- and pre-guidelines respectively [OR 0.06, 95% CI 0.007–0.52, p=0.003].

Logistic regression analysis of the post-guidelines data suggested that children over two not given preadmission antibiotics were more likely to develop severe disease [OR 2.01, 95% CI 1.17–3.45, p=0.010]. Hospitalisation was associated with disease severity [OR 6.9, 95% CI 3.83–12.37, p<0.001], but not with pyrexia (triage temperature >38^{o}C) (p=0.487) or chest radiographic changes (p=0.368). Disease severity was not associated with radiological findings (p=0.498).

Table 4-1 Clinical features at presentation

	All (0–16 y), *n* (%)		Infants (≤1 y)	0–4 y	5–16 y
Characteristics	***n*=711 [2001–2002]**	***n*=542 [2008–2009]**	***n*=86 (16%)**	***n*=400 (74%)**	***n*=142 (26%)**
Triage temperature >38°C	435 (61.0)	266 (49.1)	29 (34.5)	205 (52.2)	61 (44.2)
Oxygen saturation <93%	213 (30.0)	145 (26.7)	21 (25.0)	106 (27.1)	39 (28.3)
Disease severity					
Mild	155 (22.0)	203 (37.5)	27 (31.4)	147 (36.7)	56 (39.4)
Moderate	138 (19.0)	56 (10.3)	9 (10.5)	40 (10.0)	16 (11.3)
Severe	418 (59.0)	283 (52.2)	50 (58.1)	213 (53.3)	70 (49.3)
Chest radiographic findings					
Lobar	141 (20.0)	162 (29.9)	22 (25.6)	99 (24.8)	63 (44.4)
Patchy	435 (61.0)	296 (54.6)	55 (64.0)	227 (56.8)	69 (48.6)
Perihilar	127 (18.0)	67 (12.4)	7 (8.1)	61 (15.3)	6 (4.2)
Other infiltrates	–	17 (3.1)	2 (2.3)	13 (3.3)	4 (2.8)
Pleural effusion (including empyema)	65 (9.0)	52 (9.6)	1 (1.2)	21 (5.3)	31 (21.8)
Empyema	24 (3.0)	29 (5.4)	1 (1.2)	12 (3.0)	17 (12.0)

4.1.2 Investigations

There was an association between the collection of blood samples for investigation(s) and use of intravenous (IV) antibiotics pre-guidelines [OR 37.7, 95% CI 21.43–66.16, p<0.001], but not in the post-guideline period [OR 0.97, 95% CI 0.68–1.37, p=0.858]. There was a significant reduction in the number of all investigations performed (p<0.001) except C-reactive protein (CRP) (p=0.448) between the pre- and post-guidelines surveys. Full blood count (FBC) decreased from 76% to 61%; blood culture from 70% to 53%; testing respiratory secretions for viruses (24% to 12%) and bacteria (18% to 8%); and CRP from 62% to 59%. The yield of blood culture was the same in both surveys (4% and 4.9%) and not related to age (p=0.451). Post-guidelines, viral PCR assay (immunofluorescence test was instead used in pre-guidelines) was performed on respiratory secretions from 66 children with 26 (39%) positive. Obtaining a viral respiratory screen was age-dependent and more frequently performed in those aged under two (22%) than over two years, but less often when compared with pre-guidelines (34%) [OR 0.5, 95% CI 0.33–0.75, p=0.001].

CRP was obtained in 322 (59%). Of which 27% were >100 mg/L; 9% of infants, 58% of under five years old and 42% in the above five. Pleural effusion was associated with higher CRP greater than 100 mg/L (p<0.001). Lobar and patchy changes were associated with a CRP more than 150 mg/L (p<0.05). Mean values of CRP, total white cell count (WCC) and neutrophils were higher with lobar changes (p<0.001). There was no significant difference in the CRP and WCC values with disease severity.

4.1.3 Management

Table 4-2 summarises the clinical management. Preadmission prescription of antibiotics in the community was less frequent post-guidelines (22%) than pre-guidelines (30%) [OR 0.65, 95% CI 0.50–0.85, p=0.001]. The use of IV fluids and nasogastric feeds were related to severe disease in both surveys (p<0.001) and hence both were given less frequently post-guidelines. IV fluids were given in 13.7% versus 21% [OR 0.6, 95% CI 0.45–0.83, p=0.002] post- compared with pre-guidelines, and nasogastric feeds 4.1% versus 9% [OR 0.5, 95% CI 0.27–0.75, p=0.002] respectively.

Between the pre- and post-guidelines surveys, overall IV antibiotics as a proportion of the total prescribed antibiotics decreased from 47% (501/1065) to 36% (318/891) [OR 1.6, 95% CI 1.33–1.93, p<0.001], and oral antibiotics alone increased from 16% to 50% [OR 4.4, 95% CI 3.37–5.71, p<0.001]. There was also a reduction in the use of IV route only from 8% to 5% [OR 1.8, 95% CI 1.08–2.86, p=0.025] and the use of both oral and IV routes (p<0.001) between the pre- and post-guidelines surveys respectively. Despite the overall reduction in IV antibiotic use post guidelines there was a wide variation in IV use between hospitals, from as little as 30% of admissions to as many as 70% (Figure 4-1). Post-guidelines, Amoxicillin prescription both orally and intravenously increased (p<0.001) with a decrease in IV cephalosporins (Cefuroxime and Cefotaxime) [OR 4.7, 95% CI 3.47–6.49, p<0.001] and total oral macrolides (Erythromycin, Azithromycin and Clarithromycin) [OR 2.4, 95% CI 1.79–3.14, p<0.001]. However, the individual use of Azithromycin or Clarithromycin remained the same, whilst use of Erythromycin decreased (p<0.001). Comparison of individual antibiotics prescribed in both surveys is shown in Table 4-3.

Pre-guidelines, initial IV antibiotics were significantly associated with severe disease (p=0.003); lobar changes (p=0.002); pleural effusion (p=0.00003); or pyrexia of >38^{o}C (p=0.014); but not with low oxygen saturation of <93% (p=0.826). These associations were replicated in post-guidelines with the initial use of IV antibiotics being significantly associated with severe disease [OR 1.91, 95% CI 1.35–2.70, p=0.0003], lobar changes [OR 0.6, 95% CI 0.43–0.91, p=0.018], or pleural effusion [OR 1.9, 95% CI 1.03–3.37, p=0.041], but not with low oxygen saturation of <93% (p=0.324) or pyrexia of >38^{o}C (p=0.161). Comparing post- with pre-guidelines; IV antibiotics were more likely to be given to those with lobar chest radiographic findings (35% versus 25%) [OR 0.6, 95% CI 0.45–0.85, p=0.004], but less likely to be given to children presenting with low oxygen saturations (25% versus 34%) [OR 0.6, 95% CI 0.45–0.89, p=0.009]. There were no differences in the surveys between rates of IV antibiotic administration and disease severity (p=0.08), pleural effusion (p=0.908) or pyrexia (p=0.646). Table 4-4 summarises antibiotic treatment by disease severity and radiological findings in the post-guidelines survey.

Mean (± standard deviation (SD)) hospital stay decreased from the pre- to post-guidelines surveys (4.7 ± SD 7.16 versus 3.2 ± SD 3.02 days, p<0.001). Those with severe disease, lobar changes or pleural effusion had a longer hospitalisation (p<0.001). All children irrespective of their age group who received any IV antibiotics (alone or in combination with oral) had a longer average hospitalisation than those who had only oral (4.1 ± SD 3.4 versus 2.0 ± SD 1.9 days, p<0.001). Figure 4-2 shows the probability of discharge from hospital in relation to the duration of admission. Approximately 75% of children were likely to be discharged within two days of hospital admission, whilst hospital stay for up to five days was required for nearly 20% of children. Approximately

5% of children stayed for nearly three weeks because of complications and presence of pre-morbid medical illnesses.

In the survival analyses (Table 4-5), use of IV antibiotics alone or in combination with oral was a risk factor for an extended hospital stay by 66% and 58% respectively ($p<0.05$). Unsurprisingly, moderate and severe disease were associated with risk of longer hospitalisation in 83% and 79% respectively ($p<0.001$). Children admitted to site 'G' had a 70% chance of being discharged sooner ($p=0.024$) in relation to the reference site 'F'. Likelihood ratio (LHR) test showed that the overall association of these risk factors with longer hospital stay was only significant for disease severity (LHR=148, $p=0.001$).

Table 4-2 Clinical management outcomes

	All (0–16 y), *n* (%)		Infants (≤1 y)	0–4 y	5–16 y
Characteristics	***n*=711 [2001–2002]**	***n*=542 [2008–2009]**	***n*=86 (16%)**	***n*=400 (74%)**	***n*=142 (26%)**
Pre-admission antibiotics	214 (30.0)	119 (22.0)	21 (24.4)	90 (22.5)	29 (20.4)
Days given, median (IQR)* [range]	–	4 (2–7) [1–14]	4 (3–7) [2–14]	4 (2–7) [1–14]	4 (2–7) [1–14]
Oxygen therapy	276 (39.0)	197 (36.3)	46 (53.5)	148 (37.0)	49 (34.5)
Days given, median (IQR) [range]	2 (1–4) [1–57]	2 (1–3) [1–14]	2 (1–4) [1–11]	2 (1–3) [1–11]	2 (1–4) [1–14]
Nasogastric feeds	61 (9.0)	22 (4.1)	17 (19.8)	18 (4.5)	4 (2.8)
Days given, median (IQR) [range]	4 (2–10) [1–28]	3 (2–5) [1–9]	3 (1.5–4.5) [1–6]	3 (1.5–4.5) [1–6]	7 (5–9) [5–9]
Intravenous (IV) fluid infusion	147 (21.0)	74 (13.7)	21 (24.4)	52 (13.0)	22 (15.5)
Days given, median (IQR) [range]	2 (1–3) [1–21]	1 (1–2) [1–10]	1 (1–3.7) [1–6]	1 (1–2) [1–8]	2 (1–2.5) [1–10]
Antibiotics treatment	682 (96.0)	531 (98.0)	83 (96.5)	391 (97.8)	140 (98.6)
Oral route [days], median (IQR) [range]	6 (5–7) [1–90]	7 (5–7) [1–56]	7 (5–7) [1–28]	7 (5–7) [1–42]	7 (5–9.5) [2–56]
IV route [days], median (IQR) [range]	2 (2–4) [1–22]	2 (2–4) [1–20]	4 (2–6) [1–18]	2 (2–4) [1–18]	3 (2–6) [1–20]
Hospitalisation by disease severity					
Mild disease	102 (66.0)	131 (64.5)	18 (66.7)	89 (60.5)	42 (75.0)
Moderate disease	138 (100)	56 (100)	9 (100)	40 (100)	16 (100)
Severe disease	403 (94.0)	268 (94.7)	50 (100)	199 (93.4)	69 (98.6)
Days in hospital, median (IQR) [range]	3 (2–5) [1–122]	2.5 (1–4) [1–23]	–	–	–
Mild/moderate disease	3 (2.5–4.5) [1–14]	2.5 (1–2.5) [1–13]	2 (1.5–3) [1–6]	2 (1–2) [1–13]	2 (1.5–3) [1–5]
Severe disease	3 (2–6.5) [1–122]	3.5 (2.5–5.5) [1–23]	2 (1–5.5) [1–11]	3.5 (2.5–5.5) [1–23]	3.5 (2.5–5.5) [1–20]

*IQR, interquartile range.

Table 4-3 Comparison of prescribed antibiotics (2001 and 2008)

		2001 survey	**2008 survey**		
Route	**Antibiotics**	***n* (%)**	***n* (%)**	**OR (95% CI)**	***P***
Oral		[N=564]	[N=573]		
	Amoxicillin	134 (25)	253 (44)	0.4 (0.30–0.51)	<0.001
	Erythromycin	114 (20)	29 (5)	4.7 (3.07–7.55	<0.001
	Co-amoxiclav	103 (19)	124 (22)	0.8 (0.59–1.09)	0.159
	Azithromycin	80 (14)	66 (12)	1.3 (0.89–1.83)	0.185
	Cephalexin	73 (13)	14 (2)	5.9 (3.27–11.53)	<0.001
	Clarithromycin	6 (1)	13 (2)	0.5 (0.14–1.32)	0.164
Intravenous (IV)		[N=501]	[N=318]		
	Cefuroxime	304 (61)	92 (29)	3.8 (2.77–5.19)	<0.001
	Benzylpenicillin	57 (11)	6 (2)	6.7 (2.83–19.15)	<0.001
	Amoxicillin	56 (11)	120 (38)	0.2 (0.14–0.30)	<0.001
	Cefotaxime	50 (10)	15 (5)	2.2 (1.21–4.37)	0.008
	Co-amoxiclav	28 (6)	32 (10)	0.5 (0.30–0.93)	0.019

Table 4-4 Antibiotic treatment by disease severity and radiological findings

	Disease severity			**Chest radiographic findings**			
Antibiotics	**Mild (*n*=203)**	**Moderate (*n*=56)**	**Severe (*n*=283)**	**Patchy (*n*=296)**	**Lobar (*n*=162)**	**Perihilar (*n*=67)**	**Other infiltrates (*n*=17)**
Oral only, n (%)	135 (66.5)	14 (25.0)	118 (41.7)	164 (55.4)	54 (33.3)	39 (58.2)	10 (58.8)
Days given, median [IQR]	6 [5–7]	6 [5–7]	7[(5–9]	7 [5–7]	7 [5–8]	7 [6–8]	5 [5–7]
IV only, n (%)	4 (2.0)	4 (7.1)	18 (6.4)	12 (4.1)	8 (4.9)	6 (9.0)	0 (0)
Days given, median [IQR]	2 [1–2]	3 [2–3]	3 [2–6]	2 [2–4]	3 [2–6]	2 [1.5–3]	2 [1–2]
Total oral and IV, n (%)	61 (30.0)	36 (64.3)	141 (49.8)	116 (39.2)	97 (59.9)	19 (28.4)	6 (35.3)
Days given, median [IQR]	7 [6–7]	7 [7–10]	7 [6–10]	7 [6–9]	7 [7–10]	7 [7–9]	7 [6–8]

Table 4-5 Cox-proportional hazard model with admission duration

Variable	Coefficient	Exp coefficient	SE	Wald test	LHR[§]	*P*
Hospitals[*] (as coded in Figure 4-1)					12.1	0.206
A	0.23	1.26	0.22	1.049	–	0.294
B	0.16	1.18	0.23	0.709	–	0.478
C	0.41	1.51	0.24	1.751	–	0.080
D	0.17	1.19	0.21	0.821	–	0.412
E	-0.03	0.96	0.22	-0.158	–	0.875
G	0.52	1.69	0.23	2.260	–	0.024
H	0.54	1.71	0.28	1.944	–	0.052
I	0.21	1.24	0.26	0.828	–	0.408
J	0.17	1.19	0.24	0.702	–	0.483
Disease severity[†]					148	0.001
Moderate	-1.75	0.17	0.20	-8.725	–	<0.001
Severe	-1.58	0.21	0.14	-10.912	–	<0.001
Antibiotic treatment[‡]					74.1	1.0
Oral only	-0.02	0.97	0.42	-0.058	–	0.954
IV only	-1.07	0.34	0.47	-2.275	–	0.023
Both IV and oral	-0.86	0.42	0.42	-2.04	–	0.041

Reference categories: [*]hospitals, site F; [†]disease severity, mild; [‡]antibiotics, not given.

[§]LHR, likelihood ratio.

Figure 4-1 Proportions of children who had severe disease and of those who received intravenous (IV) antibiotics in each hospital

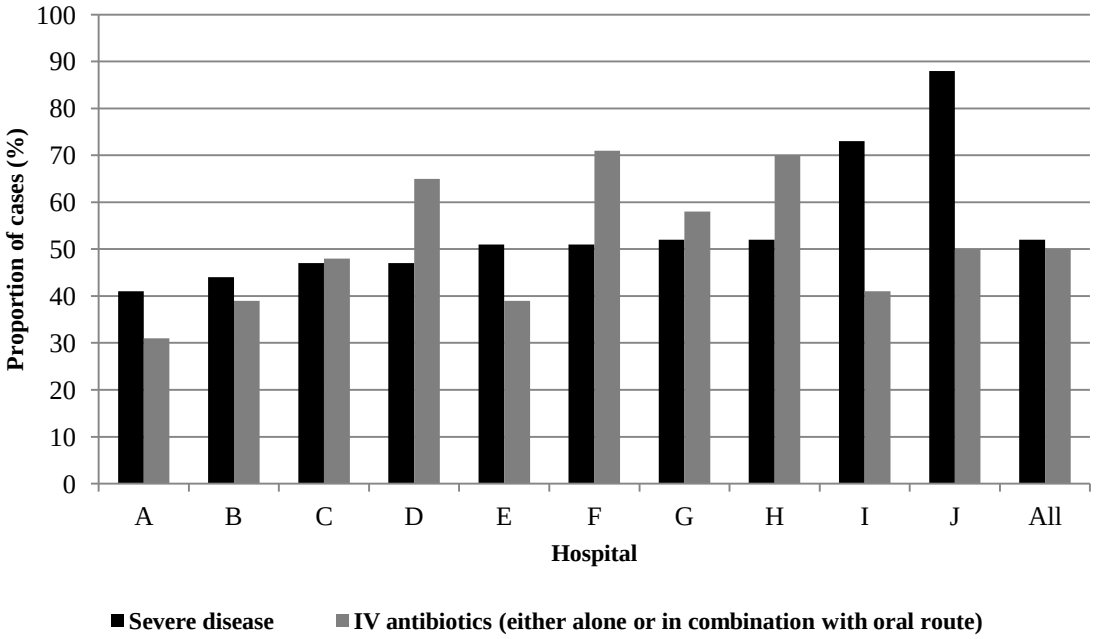
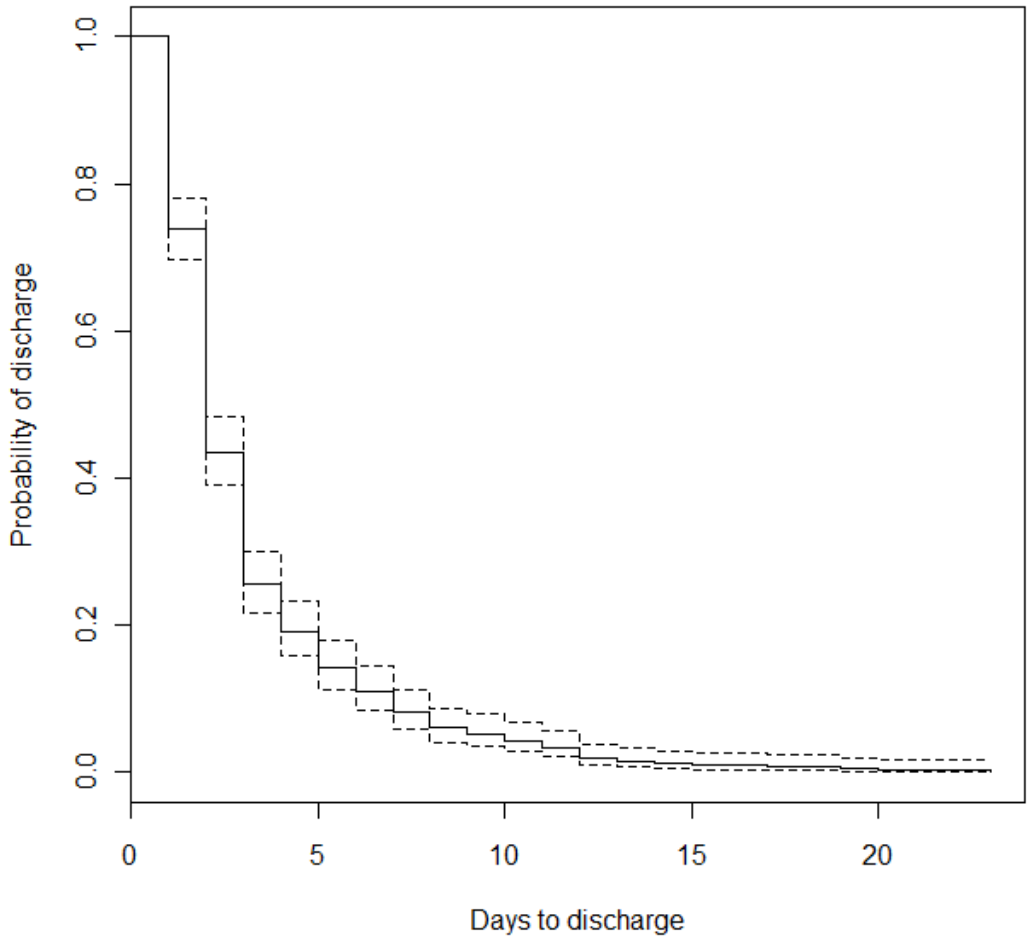


Figure 4-2 Probability of discharge from hospital in relation to duration of admission with associated 95% confidence intervals



Solid line, probability of discharge from hospital; broken lines, 95% CIs.

4.2 Discussion

This survey completes the audit cycle started with a pre-guidelines survey in 2001–2002 of presentation and management of children seen in hospital with pneumonia,[157] comparing selected standards.[160] Clinical management of children with pneumonia has changed significantly between 2002 and 2008. This included a reduction in the number of investigations performed, and a change in the type and administration of antibiotics to a decrease in IV and a concomitant increase in oral antibiotics.

Factors potentially influencing the change of clinical practice include the introduction of the 7-valent pneumococcal conjugate vaccination programme, the publication of the BTS management guidelines and an expanding literature on oral/IV antibiotic use.[161, 162, 270] Although this survey does not provide information on the management of paediatric CAP at the primary care/community level, it gives invaluable findings on the attitude of clinicians on managing this infection at hospitals where the local management policies may be driven by the published BTS guidelines.[160]

Drivers of change are complex. Some are likely to be literature driven; others probably reflect the complex relationships around perceived benefits and risks of IV cannulation, venepuncture and differing usefulness of investigations. It is interesting that fewer blood tests in terms of FBC and blood cultures were taken, but just as many CRP samples were ordered. This may reflect the fact that some children did not have IV access, as there was no association between blood sample collection and IV antibiotic use in the post-guidelines. This association however was significant pre-guidelines. The BTS guidelines including the recently updated version [1] made no specific recommendations around FBC, but blood cultures were (and are) specifically encouraged, whilst CRP is not.[160] In this survey, the correlation between high CRP of

>100 mg/L with pleural effusion demonstrates the usefulness of CRP in differentiating between uncomplicated and complicated pneumonia. Hence, it could be argued that CRP should be included in the further guidelines.

The reduction in collection of blood cultures perhaps reflects the feeling that bacterial pneumonia is less likely given the introduction of pneumococcal conjugate vaccine. However, the proportion of positive blood cultures has remained the same, suggesting that blood cultures, even with a low yield, are useful in terms of diagnosis. It is disappointing that the apparent perception of usefulness appears to favour obtaining CRP than blood culture, when blood culture is usually the only routine investigation that can potentially provide a rapid microbiological diagnosis. Although clinicians were not asked directly, the shift towards less testing of respiratory secretions for either viruses or bacteria could reflect the feeling that the results would not affect the decision on antibiotic use following detection of bacterial nasopharyngeal carriage.

More positive changes are seen with antibiotic usage. These included a significant reduction in the use of antibiotics prior to admission. This is in line with the observed substantial decline since 1990s in the prescription of antibiotics in primary care for lower respiratory tract infection in children.[271] This fall in antibiotic prescriptions predate the published BTS management guidelines of pneumonia in 2002.[160] They reflect a continued fall in the use of antibiotics despite a marginal increase in antibiotic prescription during the period between 2003 and 2006, primarily for non-specific upper respiratory tract infections, for which national guidance aimed at primary care was introduced in 2008.[271, 272] Intravenous antibiotics were used far less frequently than oral, with a substantial increase in the use of Amoxicillin overall and orally, at the expense of IV Cefuroxime and oral cephalosporins, which decreased from one fifth to

2%. In contrast, oral macrolides remain frequently prescribed particularly to those aged under five, similar to previous data,[157] although not recommended as first line treatment.[160] Evidence for the safety and efficacy of oral antibiotics even in severe pneumonia in children accumulated over the 6 years between surveys, including a Cochrane review in 2006 [161] and the PIVOT trial in 2007 [162]. Recent review of data sets from four studies of the management outcomes of severe pneumonia in children aged under three years in the community was associated with few complications, supporting the management of such cases with oral antibiotics in primary care settings.[163]

The selection of initial antibiotic route was influenced by disease severity and lobar changes, possibly reflecting that these criteria were considered markers of bacterial infection. The fact that lobar changes were associated with high mean value of inflammatory markers may support this. However, the use of IV antibiotics in relation to severe disease varied by site. This illustrates a variation in departmental practice, despite accumulating evidence of the benefits and safety of oral antibiotics in severe pneumonia.[162, 270, 273] Other factors that could have influenced the decision to give IV antibiotics, such as the level of training of admitting medical staff or the knowledge of the published guidelines, could not be ascertained with the data collected. In line with these findings, Gerber and colleagues conducted a major retrospective study on the variability of antibiotic use across 40 children's hospitals in the USA.[274] They found substantial variations in the prescription of antibiotics between sites, including both the proportion of children exposed to antibiotics (38%–72%) and the duration of treatment (368–601 antibiotic-days per 1000 patient-days). Also more recently considerable variability of antibiotic selections for management of CAP in children was observed among paediatric infectious disease consultants.[275] The variability in antibiotics use

highlights the need to implement and monitor effective antibiotic stewardship policies across and within hospitals to reduce the over or underuse of them, thus reducing the risks of development of antibiotic-resistant bacteria and treatment failures.[276]

Both severe disease and use of IV antibiotics alone or in combination with oral were found to be risk factors for longer hospital stay. However, adjusted analysis of Cox regression models showed that the severe disease was the main over-riding reason for extended hospital stay. Recent studies and pooled review data have shown that viruses play remain important causative pathogens of CAP in children.[1, 2, 37, 277, 278] These findings can give plausible explanation that a significant proportion of children with pneumonia in the present survey may have had viral rather than bacterial infections. Accordingly, the lack of association of the use of IV antibiotics as a risk factor for an extended hospital stay in the likelihood ratio analysis, whereas disease severity was, points to the fact that antibiotic treatment did not alter the course of viral pathogens which can cause severe pneumonia in children.[21, 279, 280]

4.2.1 Strengths and limitations

This survey provides invaluable evaluation of the presentation and management of childhood CAP seen in hospital over a year period with particular focus on the investigations performed and use of antibiotics. Lack of data collection and interviewing of admitting clinicians about their decisions for performing investigations and selection of the type and administration route of antibiotics limited the interpretations of potential factors surrounding the observed changes on these areas.

4.3 Conclusions

It is important for treating doctors to appreciate that intravenous antibiotics appear to be associated with increased hospital stay and to consider carefully the type and route of antibiotic to prescribe when admitting children with pneumonia to hospital. The large variation in intravenous antibiotic use and hospital stay between hospitals is highlighted and should be explored further. This is not explained fully by disease severity in our survey. In addition, a cost analysis focusing on the impact of reduced hospitalisation, intravenous antibiotic use and preadmission antibiotics would provide useful economic information.

Chapter 5 Aetiology of Childhood Pneumonia

5.1 Results

A total of 401 children were enrolled, 241 and 160 in the pre- and post-vaccine studies respectively. All had at least one microbiological investigation performed. There were similar demographic characteristics between the pre- and post-vaccine studies; including median age (2.5 versus 2.6 years), proportions of males (57% versus 56%) and aged under five years (75.5% versus 69%). The proportion of children who were referred directly in the pre-vaccine study from primary care was 86%, whereas 14% of children were referred in from secondary care district general hospital in the North East of England. This is compared to 78% and 22% respectively in the post-vaccine study (OR 1.8, 95% CI 1.04–2.98, p=0.041). Lobar consolidation was more often present post-vaccine in 61% compared to 23% of pre-vaccine (OR 0.2, 95% CI 0.13–0.30, p<0.001). More children developed empyema post-vaccine (25%, *n*=40) than pre-vaccine (7%, *n*=17).

A presumptive causative pathogen was established in 89% of all children post-vaccine, compared to 55% pre-vaccine (OR 0.2, 95% CI 0.09–0.27, p<0.001) when the results of all tests were combined. This significant difference in detection rates was similar for definite infective causes; being 61% in post-vaccine and 48.5% pre-vaccine (OR 0.6, 95% CI 0.41–0.92, p=0.019). Figure 5-1 summarises the aetiological and radiological classifications and Table 5-1 lists the results of the diagnostic tests performed. The differences in the numbers of tested samples such as serology were related to the availability of sufficient serum to perform as many as possible tests. Parents often declined returning for these convalescent samples (all in the post-vaccine study), contributing to the variability of investigations performed. Bronchoscopy fluids were available from 14 and 4 children in the pre- and post-vaccine studies respectively.

Forty-one children were not eligible for the pneumococcal conjugate vaccination due to age criteria whilst its uptake was 94% (112/119) among eligible children (89 had PCV7, 10 PCV13 and 13 received combined doses of each with age-appropriate schedule). Of those vaccinated with PCV7 either routinely or according to the catch up programmes, 83 of them received age-appropriate doses (57 had full schedule) whereas six children had partial schedule with one dose less for their age. Among those who had PCV13, one received full schedule, one child had one dose less for age, and eight had not completed but had appropriate doses for their age.

Figure 5-1 Summary of the aetiological and radiological classifications

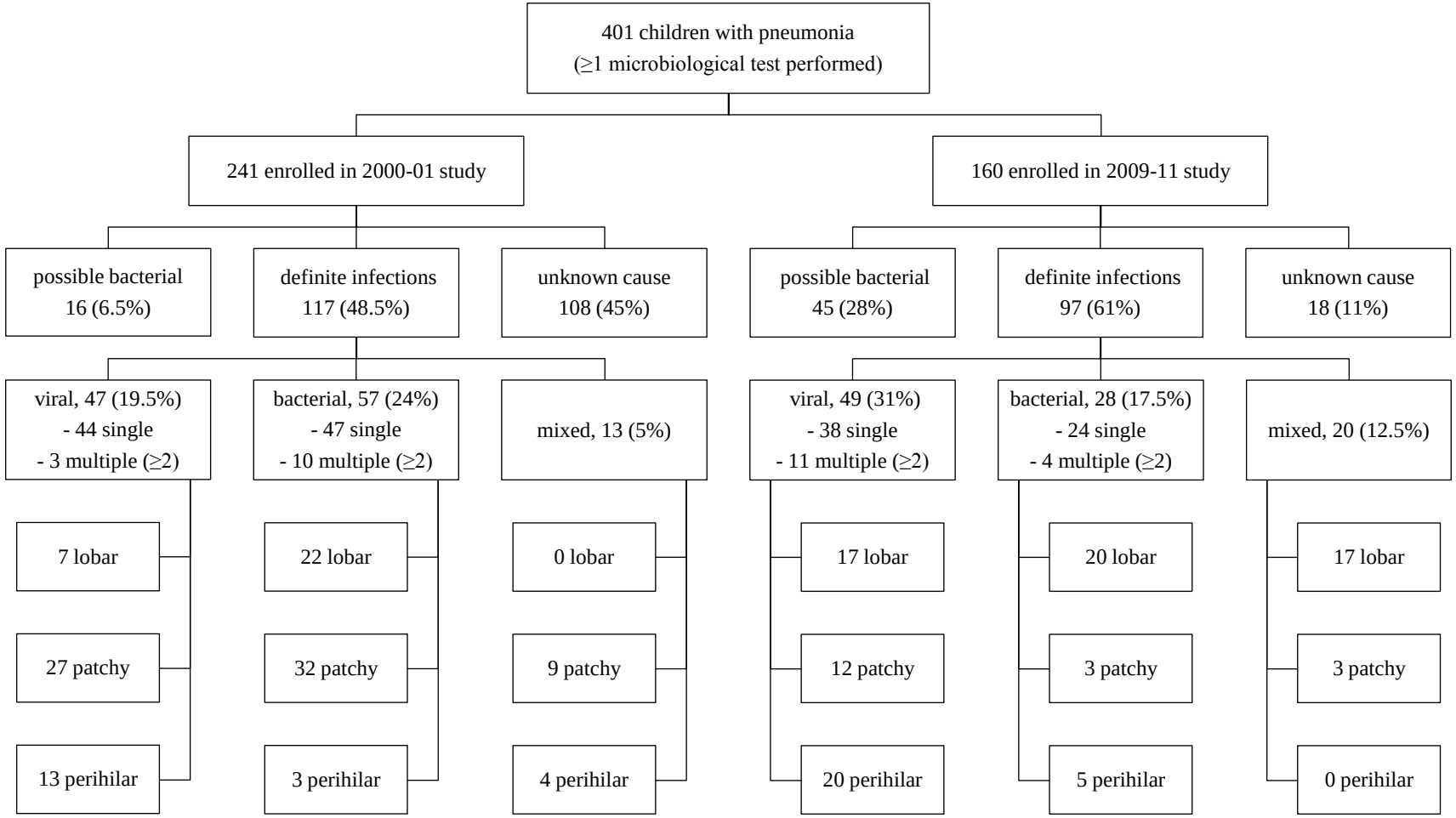


Table 5-1 Results of the diagnostic tests performed

	2001–2002 study (*n*=241)		**2009–2011 study (*n*=160)**	
Tests	**Tests, *n***	**Positive, *n* (%)**	**Tests, *n***	**Positive, *n* (%)**
Blood and serology	238	75 (31.5)	138	32 (23.2)
Blood, overall	***236***	***36 (15.3)***	***136***	***13 (9.6)***
Bacterial culture	185	6 (3.2)	126	7 (5.6)
S. pneumoniae PCR	228	30 (13.2)	86	7 (8.1)
16S rRNA PCR	0	0	89	1 (1.1)
Serology, overall	***181***	***49 (27.0)***	***105***	***22 (21.0)***
Acute serology				
Mycoplasma IgM antibody	34	11 (32.4)	77	8 (10.4)
ASOT	158	12 (7.6)	80	9 (11.3)
Mycoplasma/Chlamydia	128	8 (6.3) / 0	51 / 39	0
Legionella/Q-fever	0	0	50 / 42	0
Influenza A/B	158	1 (0.6) / 2 (1.2)	68 / 62	7 (10.3) / 0
RSV[*]/Adenovirus	158	2 (1.2) / 2 (1.2)	52 / 46	0
Epstein-Barr virus	1	0	0	0
Convalescent serology				
ASOT	52	2 (3.8)	0	0
Mycoplasma/Chlamydia	14	3 (21.4) / 0	0	0
Influenza-A/B	101	1 (1.0) / 0	0	0
RSV/Adenovirus	101	6 (6.0) / 6 (6.0)	0	0
Epstein-Barr virus	1	1 (100)	0	0
Respiratory secretions, overall	***175***	***59 (33.7)***	***151***	***121 (80.1)***
Viral screen	158	44 (27.9)	141	63 (44.7)
Bacterial culture	96	15 (15.6)	141	29 (20.6)
Pneumolysin RT (real-time)-PCR	0	0	121	76 (62.8)
H. influenzae RT-PCR	0	0	121	36 (29.8)
M. pneumoniae RT-PCR	0	0	121	5 (4.1)
C. pneumoniae/*B. pertussis* RT-PCR	0 / 1	0 / 1 (100)	121	0
Urinary pneumococcal antigen	***14***	***1 (7.1)***	***106***	***30 (28.3)***
Pleural fluids, overall	***17***	***4 (23.5)***	***40***	***27 (67.5)***
Bacterial culture	17	2 (11.8)	40	10 (25.0)
Pneumococcal antigen	17	2 (11.8)	30	7 (23.3)
Pneumococcal RT-PCR	0	0	30	18 (60.0)

[*]RSV, respiratory syncytial virus.

5.1.1 Definite viral infections

Table 5-2 shows the number of identified pathogens with age group distribution. Viral (31%) and mixed infections (12.5%) were significantly higher post-vaccine than pre-vaccine; being respectively 19.5% (OR 0.6, 95% CI 0.35–0.90, p=0.021) and 5% (OR 0.4, 95% CI 0.19–0.82, p=0.015). The detection of viruses using a combination of PCR and serological assays post-vaccine (57%, 85/149) was higher than that of testing with immunofluorescence and serology pre-vaccine (30.5%, 65/213). This improvement in viral detection was thought to be due to the application of PCR assays (44.7%) replacing immunofluorescence testing (27.9%) on respiratory secretions (OR 0.6, 95% CI 0.29–0.77, p=0.003). Post-vaccine, acute viral serological assays were only positive in seven with influenza A virus infection, whereas pre-vaccine, combined acute and convalescent serology identified infections with eight each of RSV and adenovirus, four influenza A/B viruses, and one Epstein-Barr virus.

Post-vaccine, RSV was detected in 21% (31/147) of samples, of which 19 were type A with rhinovirus (8.5%), influenza (7%) and adenoviruses (7%). These figures were comparable with those pre-vaccine for adenovirus and influenza A/B (6% each); but higher than that for RSV (15%). Of the 142 definite pathogens post-vaccine, 71 (50%) viruses were detected among those aged under five years, compared to finding in the pre-vaccine study (36%, 54/149). hMPV was not detected in any of the 48 tested pre-vaccine respiratory samples, but was identified in one child in the post-vaccine study.

Table 5-2 Detected definite pathogens by age group

	2001–2002 study			2009–2011 study		
Pathogens	***n*/N***		***n*/N (%)**	***n*/N**		***n*/N (%)**
	< 5 y	**5–16 y**		**< 5 y**	**5–16 y**	
Bacterial						
S. pneumoniae	28/180	7/58	35/238 (14.7)	14/93	10/45	24/138 (17.4)
M. pneumoniae	9/128	13/48	22/176 (12.5)	2/51	6/30	8/81 (9.9)
Group A *Streptococcus*	5/151	9/51	14/202 (7.0)	6/91	8/42	14/133 (10.5)
S. aureus	3/141	2/48	5/189 (2.6)	1/89	2/41	3/130 (2.3)
H. influenzae	0/141	2/48	2/189 (1.0)	3/89	0/41	3/130 (2.3)
Bordetella pertussis	1/1	0	1/1 (100)	0/85	0/36	0/121
M. catarrhalis	1/141	0/48	1/189 (0.5)	2/89	1/41	3/130 (2.3)
S. intermedius	1/141	0/48	1/189 (0.5)	0/89	1/41	1/130 (0.8)
Alpha haemolytic *Streptococcus*	1/141	0/48	1/189 (0.5)	0/89	0/41	0/130
K. pneumoniae	0/141	0/48	0/189	1/89	0/41	1/130 (0.8)
Viral						
RSV (not typed)	29/163	3/50	32/213 (15.0)	0	0	0
RSV type A	0	0	0	19/102	0/45	19/147 (13.0)
RSV type B	0	0	0	11/102	1/45	12/147 (8.2)
Influenza A and B viruses	9/163	4/50	13/213 (6.0)	7/103	4/44	11/149 (7.4)
Adenovirus	11/163	2/50	13/213 (6.0)	10/101	0/44	10/145 (6.9)
Parainfluenza 1-4	5/133	0/25	5/158 (3.2)	5/98	1/43	6/141 (4.3)
Human metapneumovirus	0/37	0/11	0/48	1/98	0/43	1/141 (0.7)
Epstein-Barr virus	0	1/1	1/1 (100)	NT†	NT	–
Varicella zoster virus	0	1/1	1/1 (100)	NT	NT	–
Rhinovirus	NT	NT	–	10/98	2/43	12/141 (8.5)
Pandemic influenza A H1N1	NT	NT	–	4/98	3/43	7/141 (5.0)
Bocavirus	NT	NT	–	2/85	2/36	4/121 (3.3)
Coronavirus (type OC43)	NT	NT	–	2/85	1/36	3/121 (2.5)
Total	103	46	149	100	42	142

*N, total number of performed tests that their positive results classified as definite infections.

†NT, not tested.

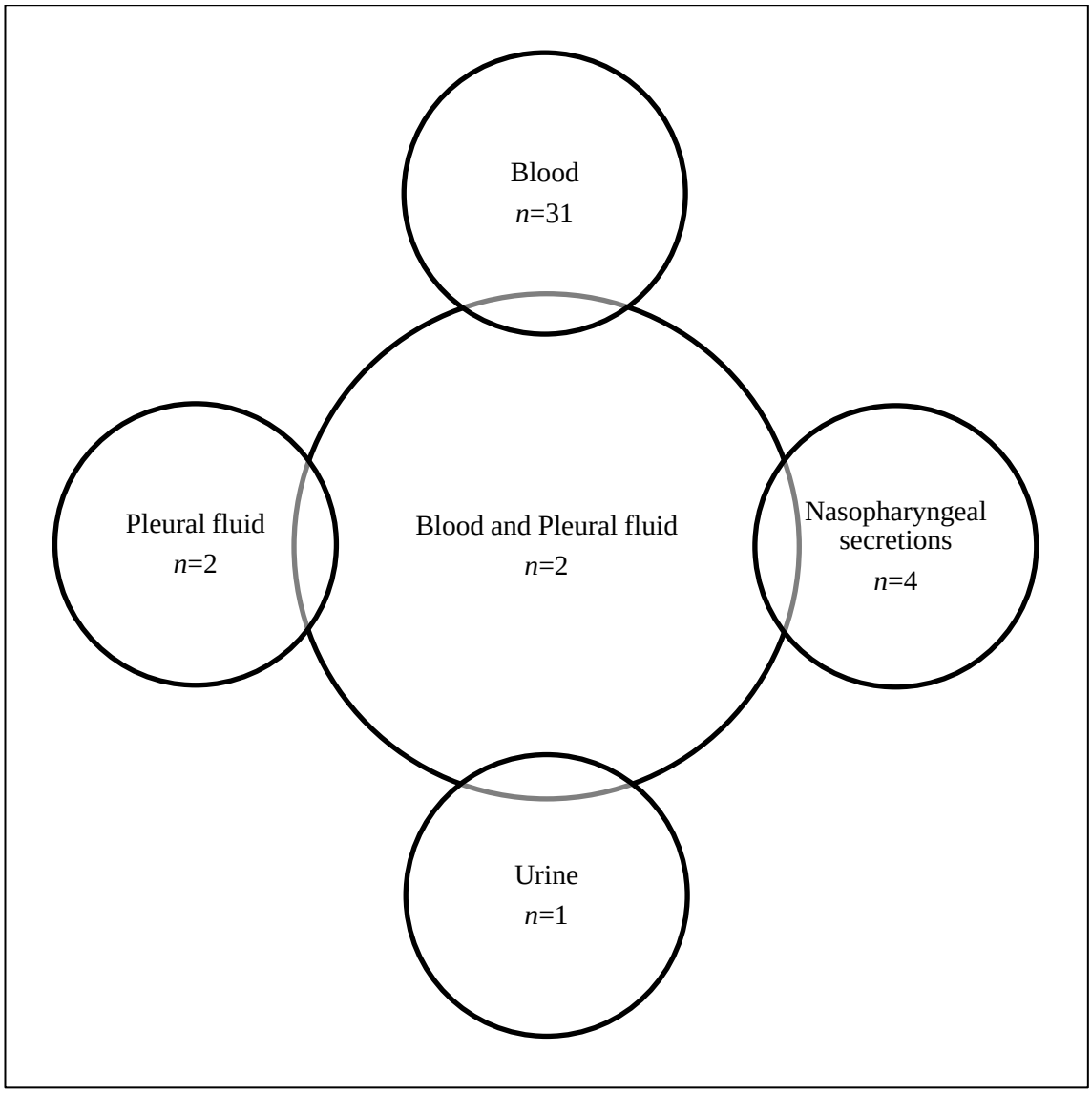
5.1.2 Definite bacterial infections

There were no difference in the rates of bacterial infections between post-vaccine at 17.5% of the total compared to 24% pre-vaccine (p=0.258). Identified overall pneumococcal infections as a definite cause were not different between both studies (p=0.557). They represent 17.4% among children tested post-vaccine (14/93 [15%] and 10/45 [22.2%] in those aged under and over five years respectively). This was compared to 14.7% pre-vaccine (28/180 [15.6%] and 7/58 [12%] among those aged under and over five years respectively). In the post-vaccine study, diagnosis of pneumococcal infection improved when PCR assays were used (21/97, 21.6%) compared to culture (8/132, 6%) (p=0.0004). A serotype was identified in 75% (18/24) post-vaccine. These were serotypes 1 (44.4%), 3 (27.8%), 19A (22.2%) and 7A/F (5.6%). The rate of positive blood culture post-vaccine was almost double (5.6%) that in pre-vaccine (3.2%). Figure 5-2 shows Venn diagram distribution of different single and concordant identification sites of the overall pneumococcal infections.

Group A streptococcal infections were confirmed in 10.5% of children tested post-vaccine and 7% pre-vaccine. These infections were associated with severe disease, and in two-thirds of them with empyema. *M. pneumoniae* was identified from acute serology in 9.9% of children tested post-vaccine, with 4% (2/51) in those aged under five and 20% (6/30) over five years. The rate of detected mycoplasma infection in the pre-vaccine study was 12.5% when paired acute and convalescent samples were available, with 7% (9/128) in those aged under five and 27% (13/48) over five years old. Figure 5-3 shows Venn diagram distribution of different single and concordant identification sites of definite group A streptococcal infections.

Figure 5-2 A Venn diagram showing different single and concordant identification sites of the overall pneumococcal infections (2001 and 2009)

- **2001–2002 study**



- **2009–2011 study**

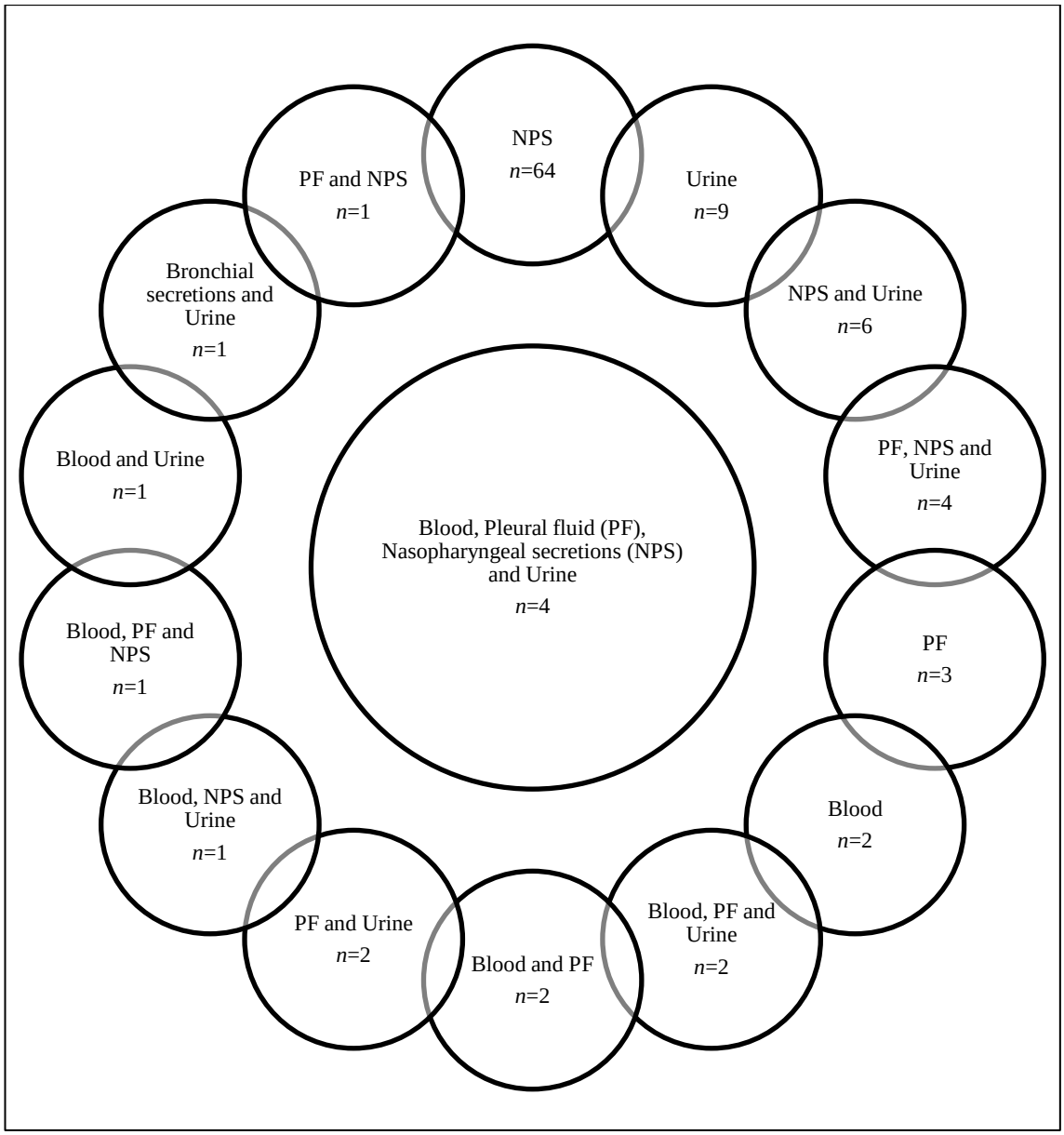
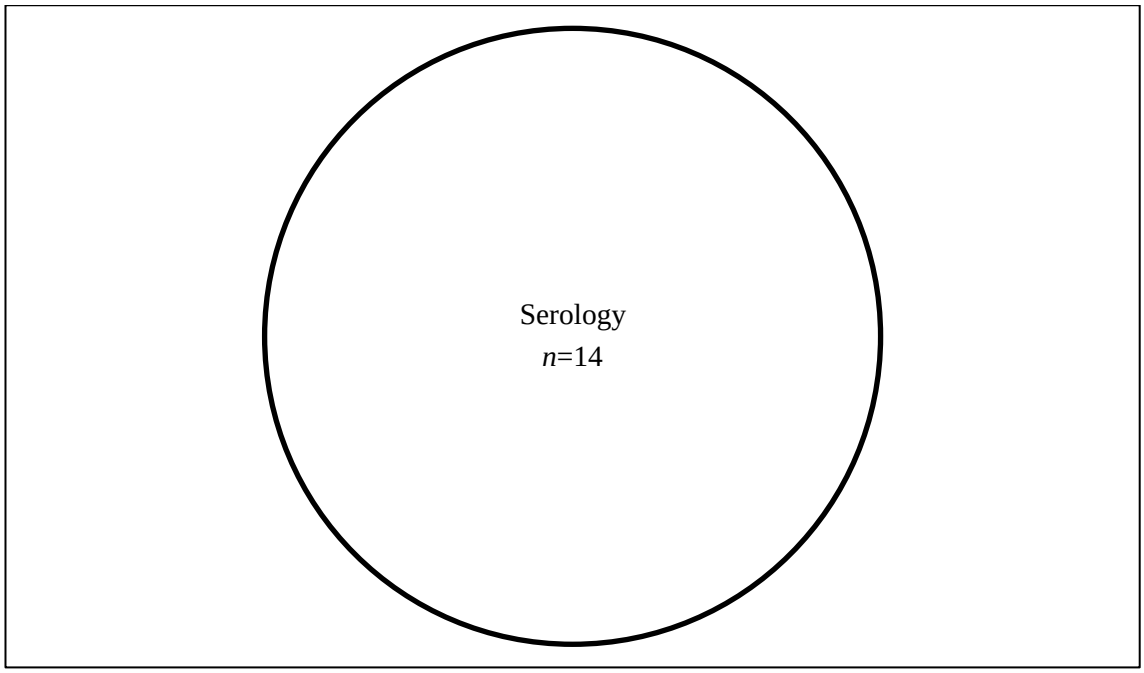
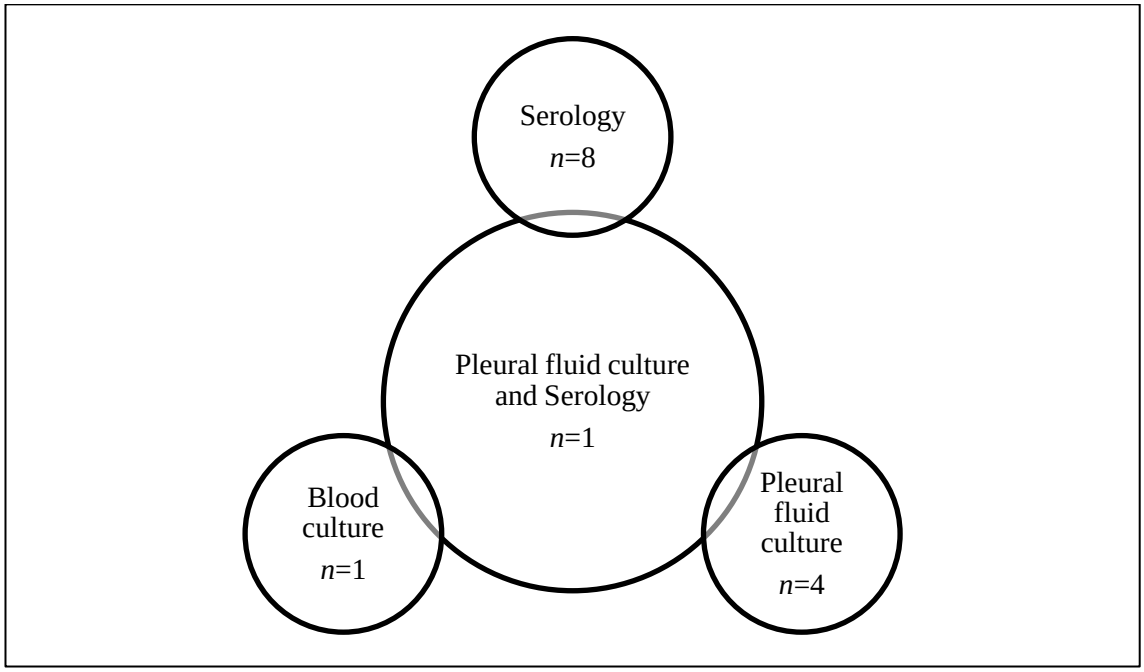


Figure 5-3 A Venn diagram showing different single and concordant identification sites of group A streptococcal infections (2001 and 2009)

- **2001–2002 study**



- **2009–2011 study**



5.1.3 Bacteria from nasopharyngeal secretions

Bacterial cultures of 96 samples of nasopharyngeal secretions yielded *S. pneumoniae* in 4.2% and non-typeable *H. influenzae* in 9.4% in the pre-vaccine study. This is similar to 7% and 7.8% respectively in the post-vaccine study. Detection rates increased considerably with the application of PCR assays on the samples to 62.8% and 29.8% for *S. pneumoniae* and *H. influenzae* respectively (Table 5-1). Mean (± SD) value of *S. pneumoniae* PCR was 38.2 ± 4.4 cycle threshold (Ct) with a median 38.6 (range, 28.5–46), whereas for *H. influenzae* PCR it was 31.9 ± 4.9 Ct (median, 32.4; range, 19.5–39.3). Where both bacterial culture and bacterial PCR for *S. pneumoniae* were performed on the same sample, there was no significant difference in the mean Ct levels between the positive and negative cultures [34.8 ± 6.5 versus 38.6 ± 4.2, p=0.072]. There was only one sample positive for *H. influenzae* by both culture and PCR.

5.2 Discussion

This is the first study to describe the aetiology of CAP in UK children prior to and following the introduction of the pneumococcal conjugate vaccination programme. The timing of this comprehensive study three years after the introduction of PCV7 and during the first year of PCV13 provides a baseline for future comparative studies of the pneumonia aetiology in the same setting. The causative pathogens identified were predominately viruses in both studies with the detection of pneumococcal infections increasing from pre-to post-vaccine studies presumably as a consequence of the application of molecular diagnostic methods.

The three previous UK studies investigating the aetiology of pneumonia in children prior to the introduction of the PCV7 were able to identify the aetiology of pneumonia in up to 54% of cases.[28, 29, 176] In addition to the blood culture, one tested blood for *S. pneumoniae*, *Mycoplasma* and *Chlamydophila* using PCR and identified 8% of children with pneumococcal pneumonia.[28] Another study identified 6% pneumococcal infection using pneumolysin ELISA on blood.[29] However, none of these studies investigated comprehensively the bacterial aetiology or evaluated the serotypes involved in pneumococcal pneumonia.

In the post-vaccine study a likely pathogen was identified in 89% of children. This is comparable to detection rates around 80% in studies which used serological and/or molecular approaches,[11, 13, 15, 177] but is higher than the rates previously found prior to the introduction of the conjugate vaccine.[12, 178] The improved detection rate between the pre- and post-vaccine studies appears to be related to the different laboratory approaches used and compared to other studies likely to be related to methods, study duration and seasonality.

Whilst molecular diagnostic methods have improved respiratory virus detection and bacterial detection from normally sterile sites, the interpretation of results can be more problematic when it is applied to nasopharyngeal secretions and other respiratory samples. In the post-vaccine study most of the respiratory samples that were positive for *S. pneumoniae* (62.8%) and *H. influenzae* (29.8%) had higher Ct levels (>30) and therefore may represent nasopharyngeal carriage rather than definite infection.[1] This detection rate by PCR on nasopharyngeal secretions is similar to that previously reported 69%.[281] It has recently been recognised that the pneumolysin gene can be detected in non-pneumococcal Viridans-group streptococci, particularly *S. pseudopneumoniae* and *S. mitis*.[57] Therefore, this increased level of positivity may have also been caused by cross-reactivity with other Viridans-group streptococci. Hence the results of PCR-based approaches on nasopharyngeal secretions to diagnose pneumonia must be interpreted with caution particularly if such tests are used to inform decisions regarding clinical management.

The rates of pneumococcal infection from the two data sets are lower than studies in other countries,[1] but are higher than previously described in the UK.[28, 29] Improvement of pneumococcal identification with the application of PCR assays when compared to culture alone is consistent with previous studies.[58, 188, 282, 283] This is similar to the reported increase in a recent Italian study (from 3.8% to 15.4%).[18] It is interesting that despite the overall decrease in the incidence and hospitalisation of pneumonia since the introduction of PCV7,[115, 144] the rates of pneumococcal infection were comparable between the two studies. Replacement with non-PCV7 serotypes causing invasive pneumococcal disease is well recognised,[284] and this may explain our findings in the face of reduction in disease incidence. Where pneumococcal

serotyping was possible with the majority being identified from pleural fluid samples, all serotypes recovered were non-PCV7 but covered in PCV13 (Chapter 6). This is similar to data from the USA on children with empyema where 98% were non-PCV7 serotypes which are primarily similar to the serotypes in this study.[108] Despite the lack of comprehensive serotype data, this suggests that PCV13 could substantially reduce IPD.[125, 140, 285]

Serological evidence of *Mycoplasma* infection was detected in 9.9% and 12.5% of children in both studies, rates that are similar to the published literature.[12, 29] *M. pneumoniae* is traditionally considered a pathogen of older children and in these studies was identified more frequently in those over five years of age. No other serological evidence was identified of other 'atypical' organisms although this may have been as a consequence of the lack of convalescent sera. *S. aureus* and GAS infections were often associated with severe pneumonia and empyema.[104, 286, 287] In keeping with previous findings, GAS can be found in up to 7% of children with pneumonia compared to 7% and 10.5% in the pre- and post-vaccine two data sets.[29, 288] With the introduction of PCV and decrease in pneumococcal pneumonia it is possible that the relative proportion of bacteria such as GAS and *S. aureus* as well as *M. pneumoniae* to cause severe pneumonia will increase.

Viruses either alone or as co-pathogens were detected in 25% and 43% of children in the pre- and post-vaccination studies respectively, with RSV being the most commonly detected pathogen as previously reported.[1, 277, 278] This was followed by rhinovirus, influenza and adenovirus at approximately 7% each, similar to data previously described for the same region.[29] Diagnosis of viral infection was achieved mainly through the testing of respiratory secretions rather than by serology which was only

positive in seven cases of influenza A virus. Most of the viruses detected were identified in those aged under five years, consistent with other studies.[13, 174] In the post-vaccine study, viral screening was expanded to include eight viruses with their subgroups including pandemic H1N1 and to delineate their contribution in causing CAP in UK children. Considering the timing of the second recruitment period, pandemic influenza A H1N1 was not implicated in many cases of pneumonia as a single pathogen. The low isolation rates of bocavirus, coronavirus and hMPV highlight the minimal contribution of these viruses in the aetiology of pneumonia in UK children. The rates of mixed viral-bacterial infection were variable between the two studies and likely to be dependent on the screening methods used to identify the causative pathogens.[1]

Although the source of referral of the enrolled children was statistically significant between the studies; being more from primary care than secondary care hospitals in the region in the pre- than post-vaccine study, it is unlikely that there would be sufficient bias in the aetiology data caused by the referral pathways. The change in epidemiology after the introduction of pneumococcal conjugate vaccination programme was not the focus of the aetiology study of the present research. The aetiology study was designed and focused to describe the proportion of pathogens in childhood CAP across a period of change in vaccination practice. In fact between the two study periods, the referral patterns have changed in the counties of Newcastle upon Tyne and Northumbria, such as primary care referrals from Ashington town were previously made to Royal Victoria Infirmary whereas after the pre-vaccine study this has changed the North Tyneside District General Hospital. When removed the children admitted at Royal Victoria Infirmary from Ashington town based on postcode in the pre-vaccine study and reanalysed the data, there was no statistical difference in the sources of referral between the pre- and post-vaccine study.

That this aspect of the aetiological studies was not designed to show incidence of pneumonia, in contrast to the observed substantial reduction in the annual incidence of lobar pneumonia following the conjugate vaccination programme in Canada,[79] the post-vaccine study reported increased lobar findings. This could be attributed to either the relative implication of non-PCV7 pneumococcal serotypes in the aetiology of pneumonia in children or due to the recognized variation in the interpretation of paediatric chest radiographs,[38, 210, 211] which in the pre- and post-vaccine study were reviewed and reported by two senior radiologists at the regional centres in Newcastle (designated as the “gold standard”) who were blinded to both the first reports by local radiologists at each site and specific clinical data.

5.2.1 Strengths and limitations

There are several limitations to these data, such as potential seasonal bias to the data of post-vaccine study where the recruitment was carried out over 18 months which included two winter seasons (48% of enrolled children). Although the post-vaccine study covered two winter seasons, enrolled children were fewer than the pre-vaccine study which could be a true reflection of decreased disease incidence and hospitalisation. The findings from the post-vaccine study may have been hampered by the lack of convalescent sera which may have led to the underestimation of the role of atypical bacteria in childhood pneumonia. But this effect is probably minimal as mycoplasma infection was only detected in three children by paired serum samples pre-vaccine. Another limitation is the variation between the two studies in the diagnostic methods used and the pathogens investigated. Lack of serotype data of the identified pneumococci from the pre-vaccine study limits the actual comparison with the serotype profile after the conjugate vaccine implementation. However, this research provides information on the changes of aetiology of pneumonia over two time periods. It also highlights the requirement of using multiple laboratory investigations in order to identify the likely causative pathogens. The improvement in the yield of several diagnostic approaches used in the post-vaccine study compared to the pre-vaccine study, particularly with more PCR-based assays used, is in line with recent studies over the last 15 years.[1, 2, 20, 289]

5.3 Conclusions

Although viruses are the most common cause of pneumonia, around one fifth of children had bacterial infections. The combined use of culture, serology and PCR-based diagnostic tests significantly improved the identification of definitely causative pathogens in childhood pneumonia. Despite the widespread use of PCV7 and PCV13, infection with non-vaccine pneumococcal serotypes continued to be a significant cause of pneumonia in UK children. This requires continued surveillance for the emergence of serotype replacement.

Chapter 6 Identification and Typing of Pneumococci

6.1 Results

A total of 225 children were enrolled, all of whom had at least one microbiological investigation performed. Of these, 60 with normal chest radiograph and 5 transferred from Cumbria region were excluded (three with empyema and two for intensive care management of complicated pneumonia with severe respiratory distress). Thus leaving 160 children for final analysis; 56% males, 69% under five, median age 2.6 years. All children received antibiotics and none died. Based on age, pneumococcal vaccination uptake among 119 eligible children was 94% (89 had PCV7, 10 PCV13 and 13 received combinations of each) (Table 6-1). Lobar consolidation was the commonest radiological finding in 61%. Forty (25%) children had empyema, while pleural effusion was reported in 42.5% of the chest radiographs.

Table 6-1 Number of received doses of PCV

Received PCV[*] doses	***n***
PCV7 (*n*=89)[‡]	
1 dose	13
2 doses	19
3 doses	57
PCV13 (*n*=10)[§]	
1 dose	4
2 doses	5
3 doses	1
Both PCV7 and PCV13 (*n*=13)	
1 dose of each	1
2 PCV7 + 1 PCV13 doses	11
1 PCV7 + 2 PCV13 doses	1

[*]PCV, pneumococcal conjugate vaccine

[‡]Six children had partial schedule with one dose less for their age.

[§]One child had one dose less for age.

6.1.1 Laboratory diagnostic testing

A summary of the performed laboratory diagnostic procedures is presented in Table 6-2. Blood samples were obtained from 136 children and blood culture testing performed on 126 samples. *S. pneumoniae* was isolated from five samples (4%) and single isolates of *Staphylococcus aureus* and group A *Streptococcus* (GAS) were also isolated from two additional samples. Of the 86 blood samples tested in the pneumococcal PCR assay, seven (8.1%) gave a positive signal in the test, whereas 16S rRNA PCR was only positive in one of 89 tested samples. Pleural fluid samples from 40 children were tested by culture with *S. pneumonia*e being isolated from three (7.5%) samples, as well as GAS from four samples, *S. aureus* from two samples and *S. intermedius* from one sample. Nucleic acid extracted from the thirty pleural fluid samples was tested in the pneumococcal PCR assay and 18 samples (60%) gave a positive signal in the test.

For the 151 respiratory secretions analysed, *S. pneumoniae* PCR was positive in 62.8% (76/121, of which only four had cycle threshold [Ct] <30), and culture in 7% (10/141). Out of 12 samples cultured from tracheobronchial secretions one was positive (8.3%) for *S. pneumoniae*, 5 samples grew 7 other bacteria either singly or in multiples. For nasopharyngeal secretions, mean (± SD) Ct of pneumolysin PCR was 38.2 ± 4.4 with a median 38.6 (range, 28.5–46). Where both bacterial culture and PCR for *S. pneumoniae* were performed on the same sample, there was no significant difference in the mean Ct levels between the positive and negative cultures (34.8 ± 6.5 versus 38.6 ± 4.2, p=0.072). Pneumococcal antigen testing was performed on urine samples from 106 patients and evidence of pneumococcal antigen in urine was detected in 28.3%. Overall presumptive pneumococcal infection was detected in 64% of children (103/160).

S. pneumoniae was the definite cause of pneumonia in 17.4% (24/138) of children; 15% (14/93) tested in those under five, 22.7% (10/44) tested among those over five. Twenty children of those with 24 identified definite pneumococcal infections had empyema. Definite pneumococcal infections among those who only had PCV7 was 19.8% (16/81), compared to 5.3% (1/19) for those who either had PCV13 only or both (OR 4.4, 95% CI 0.55–35.70, p=0.182). Cultures from sterile sites (including blood, pleural fluids and tracheobronchial secretions) isolated *S. pneumoniae* in 6% (8/132) of cases; compared with pneumococcal PCR detection rate from sterile sites of 21.6% (21/97), p=0.0004. A serotype was identified in 18 (75%) of these; with serotype 1 (8, 44.4%), serotype 3 (5, 27.8%), serotype 19A (4, 22.2%) and serotype 7A/F (1, 5.6%). Where a serotype was detected from a normally sterile sites (*n*=18), all but one case had empyema. Among these children with the identified 18 serotypes, 14 had pneumococcal antigen in urine tested with 11 (78.6%) being positive. Of the five pneumococcal isolates from blood cultures, only three were available and processed for serotyping which showed serotypes 1, 3 and 19A, of which two were concordantly identified in pleural fluids. Apart from one child who had 2 doses of PCV7 and a booster with PCV13, others with these non-PCV7 serotypes were either unvaccinated (*n*=7) or PCV7-vaccinated (*n*=10).

Ten additional serotypes were identified from nasopharyngeal secretions and urine by xMAP assay. Of five pneumococcal isolates from nasopharyngeal secretions, only one serotype (23B) was identified. Out of the 17 assayed urinary Binax-positive samples, 9 (53%) serotypes were identified. These included serotypes 1 and 7A/F (*n*=4 each) and one serotype 19A. Interestingly within this group of nine recovered urinary pneumococcal serotypes, four children had empyema and concordant identification of same serotypes from the pleural fluids (two were serotype 1, one each of serotypes 7A/F

and 19A). Figure 6-1 shows Venn diagram distribution of different single and concordant identification sites of definite pneumococcal infections by age group.

The pneumococcal multiplex serotype-specific PCR was applied on culture-negative but pneumolysin PCR-positive nasopharyngeal secretions and blood. However, only those with Ct levels of ≤30 produced a discernible amplification product and samples with Ct levels above 30 were associated with multiple non-specific PCR products. The serotype-specific PCR could not be fully applied to determine the serotype in the blood samples due to insufficient sample volume to perform all the multiple steps. However, the multiplex PCR assay was partially applied on four blood samples; two of these were positive but unable to proceed with identification due to insufficient volumes.

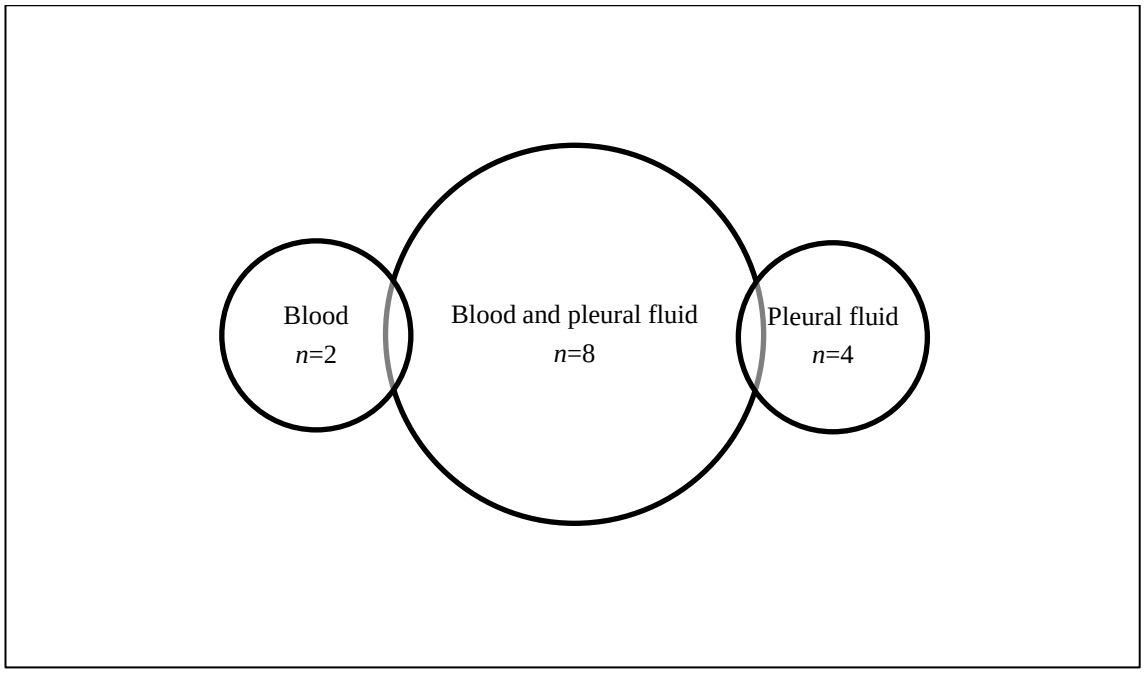
Table 6-2 Detection rates of pneumococcal infection from the performed tests

Tests	Patients, *n*	Positive (%)
Overall definite infections	***138***	***24 (17.4)***
Blood, overall	***136***	***11 (8.0)***
Culture (*S. pneumoniae*)	126	5 (4.0)
Pneumolysin real-time PCR	86	7 (8.1)
16S rRNA PCR	93	1 (1.1)
Pleural fluids, overall	***40***	***19 (47.5)***
Culture (*S. pneumoniae*)	40	3 (7.5)
Pneumococcal antigen	30	7 (23.3)
Pneumolysin real-time PCR	30	18 (60.0)
Respiratory secretions, overall	***151***	***82 (54.3)***
Nasopharyngeal secretions culture (*S. pneumoniae*)	141	10 (7.0)
Tracheobronchial secretions culture* (*S. pneumoniae*)	12	1 (8.3)
Pneumolysin real-time PCR	121	76 (62.8)
Urinary pneumococcal antigen	***106***	***30 (28.3)***

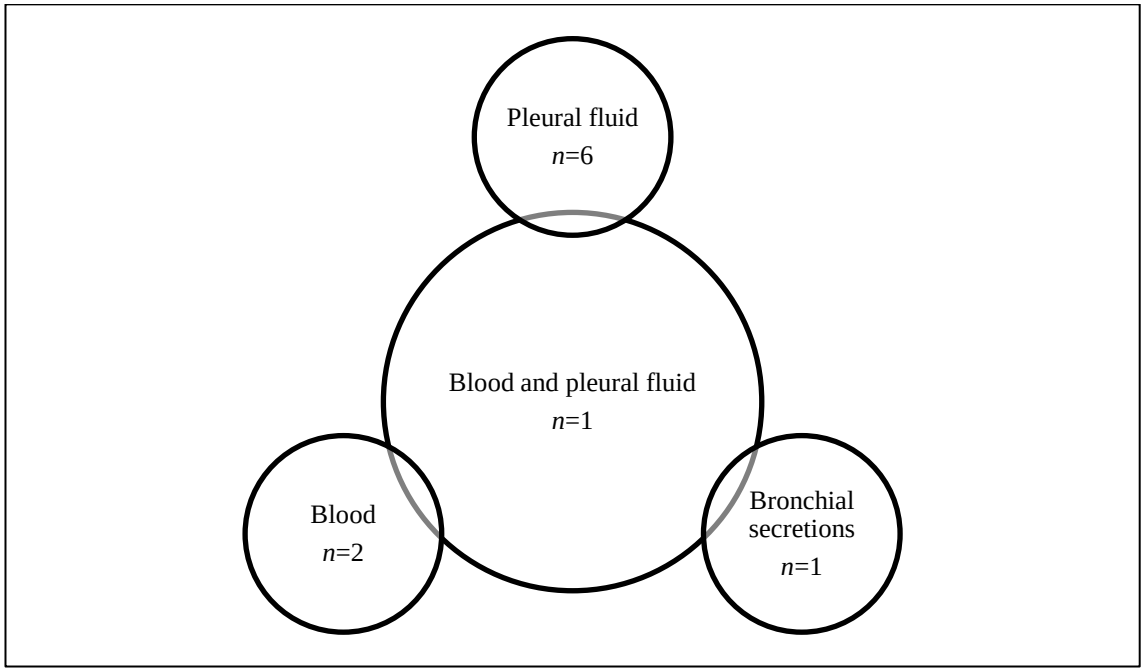
*Tracheobronchial secretions, collected via bronchoalveolar lavage and/or endotracheal tube.

Figure 6-1 A Venn diagram showing different single and concordant identification sites of definite pneumococcal infections by age group

- **Children aged <5 years old**



- **Children aged ≥5 years old**

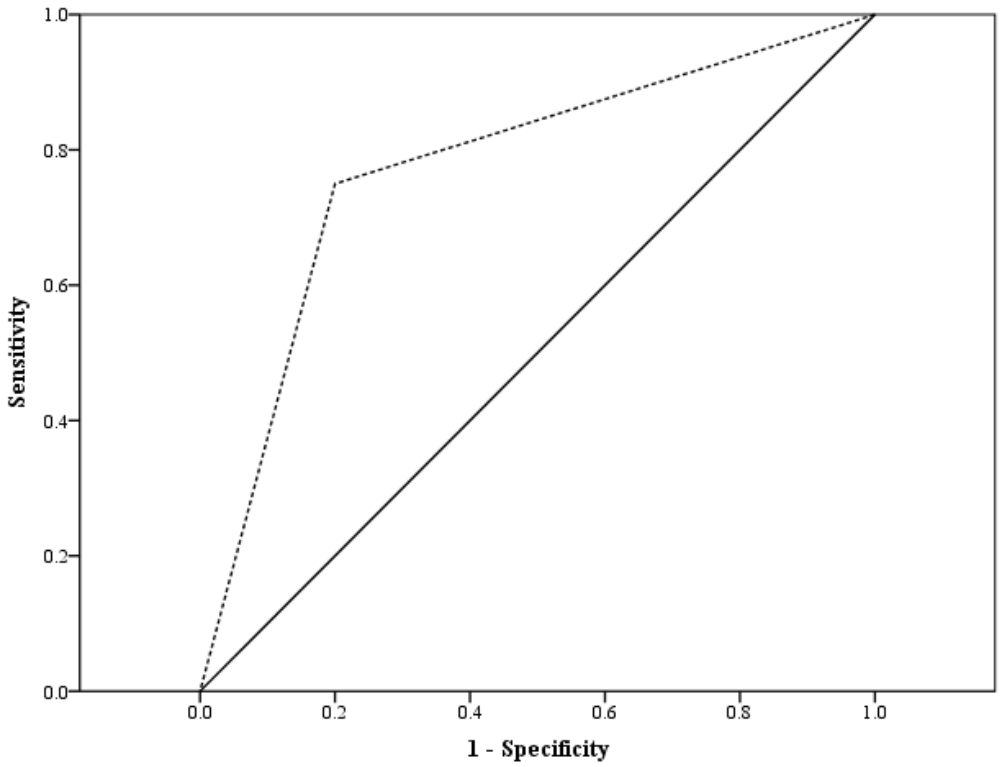


6.1.2 Correlation between pneumococcal diagnostic methods

Positive blood pneumococcal PCR alone was associated with positive urinary pneumococcal antigen (OR 10.3, 95% CI 1.11–95.15, p=0.025). When compared with the overall definitely identified pneumococcal infections from normally sterile sites, positive urinary pneumococcal antigen was significantly associated with the pneumococcal detection by culture (OR 6.5, 95% CI 1.18–36.02, p=0.028) and PCR assays (OR 11.8, 95% CI 3.21–43.55, p=0.00008). However, there was no significant association between positive blood PCR and respiratory PCR (OR 1.0, 95% CI 0.20–4.81, p=1.0), or urinary pneumococcal antigen and respiratory PCR (OR 1.2, 95% CI 0.46–3.34, p=0.804). Figure 6-2 shows the ROC curve of urinary pneumococcal antigen test for identifying definite pneumococcal infection. The performance of diagnostic accuracy of urinary Binax test for definite pneumococcal infection was acceptable, with an area under the curve of 0.78 (95% CI, 0.65–0.90, p<0.001). The test corresponds to high sensitivity (75%) and low specificity (20%) on the ROC curve.

None of the potential risk factors was significant in predicting positive status for PCR assays of blood and respiratory secretions or the collection of urine sample for the evidence of pneumococcal antigen during initial assessment at presentation. These tested risk factors included being male; aged under five; use of antibiotics or Ibuprofen prior to admission; referral to tertiary care; triage temperature >38^{o}C; oxygen saturation <92%; dullness on chest examination; presence of lobar consolidation or pleural effusions on chest radiograph; and CRP >100 mg/L (67 [50.8%] of 132 performed).

Figure 6-2 Receiver operating characteristic curve of urinary pneumococcal antigen test for definite pneumococcal infection



6.2 Discussion

This study describes diagnostic approaches to pneumococcal infections and provides the first information on serotype distribution of pneumococcal CAP in UK children after the introduction of the pneumococcal conjugate vaccination programme. The timing of the study towards the end of three years of PCV7 and during the first year of PCV13 gives a unique opportunity for future evaluation of the aetiology of pneumonia in the same setting. Non-PCV7 but PCV13 serotypes were the major contributor to the aetiology of pneumococcal pneumonia.

There are only three previous UK studies investigating the aetiology of pneumonia in children before pneumococcal vaccination was introduced, finding a cause for pneumonia between 24% and 54%.[28, 29, 176] One of these studies utilised blood PCR for *S. pneumoniae*, *Mycoplasma* and *Chlamydophila* and identified 8% of children with pneumococcal pneumonia.[28] Another study identified 6% pneumococcal infection using pneumolysin ELISA on blood.[29] None of these studies comprehensively explored bacterial aetiology or evaluated the serotypes involved in pneumococcal pneumonia.

Most of the nasopharyngeal secretion samples which were positive for *S. pneumoniae* by PCR had relatively high Ct levels (>30), which may represent nasopharyngeal carriage.[118, 290, 291] It has recently been recognised that the pneumolysin gene can be detected in non-pneumococcal Viridans-group streptococci, particularly *S. pseudopneumoniae* and *S. mitis*.[57] Therefore, this increased level of positivity may have also been caused by cross-reactivity with other Viridans-group streptococci. Definite pneumococcal infections were detected in approximately 18% of enrolled children. Although this is less than studies in other countries, it is more than previously

described in the UK.[28, 29] Diagnosis of pneumococcal infection was increased considerably when pneumolysin PCR was used,[58, 188, 282, 283] improving the detection rate of 6% by culture alone to 23%. This is similar to the increase (3.8% to 15.4%) reported in a recent Italian study.[18] However in this population of children, 16S rRNA PCR did not improve bacterial detection, with one only positive in a child who had proven pneumococcal empyema. The low positivity rate of this test may be related to prior use of antibiotics or pathogen viability, as tests were performed on batched samples. The cell wall of gram-positive bacteria is difficult to denature during DNA extraction which can make detection by 16S rRNA PCR lack sensitivity.[292-294]

Although the number of serotyped definite pneumococcal isolates was low with the majority being identified from pleural fluids, all were not covered within PCV7 (serotypes 1, 3, 7, and 19A), but are included in the new PCV13 vaccine. This is similar to data from the USA on children with empyema where 98% were non-PCV7 serotypes and primarily similar to the serotypes in this study.[108] Despite the lack of comprehensive serotype data, this suggests that PCV13 could substantially reduce IPD.[125, 140, 285]

It is acknowledged that the detection of antigen of *S. pneumoniae* in urine can represent nasopharyngeal carriage status.[191, 196] However, the significant association between this marker in the urine and the detection of pneumococci from normally sterile sites either by culture or PCR assays may suggest that despite its recognized low specificity, urinary pneumococcal antigen could still have a diagnostic role in the aetiological work up of CAP in children.[189] This is particularly helpful in resource-limited settings where PCR assays are not easily affordable or logistically possible. In this study, four of

the identified serotypes in urine had the same serotype detected in the blood and/or pleural fluids, which makes urine detection likely to represent a real infection. The lack of association between pneumococcal PCR assays in blood and respiratory secretions reflects the nasopharyngeal carriage which is common in children.[117, 118]

6.2.1 Strengths and limitations

This is the first study in the UK to explore serotype data in children with pneumococcal CAP. It illustrated the improvement in detection rate of *S. pneumoniae* by using a comprehensive range of diagnostic techniques including PCR-based assays. These provide important information for the public health policy makers for planning strategies to combat this infection in children and for the design of new generations of pneumococcal conjugate vaccines. An important limitation was the insufficient volume of the samples which limited progress with the steps of the pneumococcal multiplex serotype-specific PCR, despite efficient procedures taken towards its development and validation. I acknowledge that numbers of positive pneumococcal PCR assays were small and this could be underestimated by the application of only pneumolysin PCR on blood and respiratory secretions. However, the results provide useful information on the overall contribution of *S. pneumoniae* in pneumonia among children in this setting.

6.3 Conclusions

Non-PCV7 serotypes were the major contributor to the aetiology of pneumococcal pneumonia in UK children in the period after the PCV7 was introduced. This suggests that the replacement of PCV7 with PCV13 likely to be associated with a significant reduction in the incidence of pneumococcal-related pneumonia. Continued surveillance is required to monitor for the emergence of serotype replacement.

Chapter 7 Inter-observer Variability in the Interpretation of Chest Radiographs

7.1 Results

A total of 169 children were identified and treated for pneumonia and/or empyema (53% males, 73% aged under five years, mean (± SD) age of 3.8 ± 3.72 years, and age range from 0.05 to 16.7 years). Of those, 46 had chest radiograph reported as normal on the first reports, but on the second reading six (13%) had abnormal changes (i.e. false negative); four lobar and two patchy. All of the false negative cases received antibiotic treatment (median, 7 days), and none developed any complication. Fourteen (11.4%) were initially reported as having radiological changes, were reported as normal radiographs on the second review (i.e. false positive) (Table 7-1).

All radiologists agreed that all chest radiographs were suitable for interpretation. There was significant inter-observer variability in the interpretation of chest radiographs (k=0.70, p<0.001), with patchy (48.8%) and perihilar (28.1%) changes being the main components of this variability (Table 7-1). Although few (n=5) were first reported by radiology trainees, there was no difference in reporting when these were reported by the second radiologist. The two interpretations varied when the first reports were performed by senior radiologists, particularly consultant pediatric radiologists who had an overall 26.7% disagreement with the reviewing cardiothoracic radiologist and lowest (15.8%) with consultant thoracic radiologists (Table 7-2). Levels of disagreement were highest among children aged under five years compared to those aged over five years (26%, k=0.66 versus 11%, k=0.83, p<0.001). There was no disagreement on reporting lobar findings in the under five years age group, disagreement was mainly related to patchy and perihilar changes.

Pleural effusion was present at first reading of the films in 10% (17/169) compared to 22% (37/169) on review. Variation in reporting of pleural effusion was 11.8% (k=0.57,

p<0.001). However, if the presence of a pleural effusion was reported in the first report there was no disagreement about this in the second report. In contrast 13.2% of pleural effusions were reported only on the second report and not in the first report. Initial reporting of pleural effusion by radiology trainees was not different to reports at second reading (*k*=1, p=0.200). In addition there was good agreement between first and second reports of pleural effusion when initially read by consultant thoracic radiologists (*k*=0.17, p=0.368). Whilst there were significant differences in first reporting of effusion by consultant paediatric radiologists (*k*=0.78, p<0.001) or consultant general radiologists (*k*=0.41, p=0.002) compared to second reading, the proportions of disagreement were respectively low of 5.8% and 15.3%.

Table 7-1 Inter-observer variability and agreement in chest radiographs reporting

First reading		**Second reading (gold standard)**				**Disagreement***
Radiographic changes	***n* (%)**	**Lobar**	**Patchy**	**Perihilar**	**Normal**	***n* (%)**
Lobar	48 (28.4)	47	1	0	0	1 (2.1)
Patchy	43 (25.4)	7	22	5	9	21 (48.8)
Perihilar	32 (19.0)	4	0	23	5	9 (28.1)
Normal	46 (27.2)	4	2	0	40	6 (13.0)
Total	169	62	25	28	54	37 (22.0)

*Fisher's exact test, p<0.001; Kappa=0.70 (proportion of cases on which readers would be expected to agree).

Table 7-2 Comparison of chest radiographs reporting by the grade of readers

First reading		**Second reading (gold standard)**				**Disagreement**	
Radiographic changes	***n* (%)**	**Lobar**	**Patchy**	**Perihilar**	**Normal**	***n* (%)**	**Kappa (*P*)**
Radiology trainees	5 (3)	1	1	2	1	0	1.00 (0.105)
Lobar	1	1	0	0	0	0	
Patchy	1	0	1	0	0	0	
Perihilar	2	0	0	2	0	0	
Normal	1	0	0	0	1	0	
Consultant general radiologists	59 (35)	18	12	9	20	11 (18.6)	0.75 (<0.001)
Lobar	16	15	1	0	0	1 (6.3)	
Patchy	17	0	11	3	3	6 (35.3)	
Perihilar	8	1	0	6	1	2 (25.0)	
Normal	18	2	0	0	16	2 (11.1)	

First reading		Second reading (gold standard)				Disagreement	
Radiographic changes	***n* (%)**	**Lobar**	**Patchy**	**Perihilar**	**Normal**	***n* (%)**	**Kappa (*P*)**
Consultant paediatric radiologists	86 (51)	35	7	13	31	23 (26.7)	0.63 (<0.001)
Lobar	26	26	0	0	0	0	
Patchy	18	5	5	2	6	13 (72.2)	
Perihilar	17	2	0	11	4	6 (35.3)	
Normal	25	2	2	0	21	4 (16.0)	
Consultant thoracic radiologists	19 (11)	8	5	4	2	3 (15.8)	0.78 (<0.001)
Lobar	5	5	0	0	0	0	
Patchy	7	2	5	0	0	2 (28.6)	
Perihilar	5	1	0	4	0	1 (20.0)	
Normal	2	0	0	0	2	0	

7.2 Discussion

There was a substantial inter-observer variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children. This has been recognised since radiology reporting was initiated in the middle of last century,[210, 211] and continues despite the acceptance of the recommended WHO criteria for reporting chest radiographs of pneumonia in children.[150, 151]

The diagnosis of pneumonia in children based on a combination of clinical and radiological features is important for prompt management.[159] Yet, subtle radiographic changes can be difficult to recognise or interpret and failure to diagnose pneumonia may result in inappropriate management.[212, 213] The initial interpretation of chest radiographs is usually performed by clinicians with the radiologists' reports following later, often after the patient has been discharged from hospital.[212] Interpretation by clinicians could be biased by inadequate training in radiology and lack of clinical information may limit the accuracy of reporting by the radiologists.[214] For research purposes blinded interpretation of the chest radiograph may improve detection of subtle changes and differentiating normal biological variants.[215] Making clinical information available may reduce inter-observer variability but does not result in marked improvement in the overall accuracy.[216]

This study shows that most inter-observer variability is related to the interpretation of patchy and perihilar changes, which need careful viewing and the availability of clinical information during interpretation.[217] It is well recognised that abnormal chest radiographs may be interpreted as normal,[217] but surprisingly four of the normal reports had lobar changes on review. Similarly, 13% had a previously undetected pleural effusion. The variation in reporting of chest radiographs for those aged under

five years confirms the particular challenge of making a radiological diagnosis of pneumonia in this age group.[213, 219] The overall inter-observer variation is in line with other previously reported findings on interpretation variability including pleural effusion.[38, 219] It is widely accepted in the literature that chest radiographs cannot reliably differentiate viral from bacterial aetiology of pneumonia.[1, 2] Therefore these variations on the interpretation of chest radiographs do not significantly affect the clinical outcomes and management decisions of pneumonia in children.[1, 2, 209, 220, 221]

It is interesting that irrespective of the level of experience there continues to be significant variability in interpretation between reporters, particularly senior radiologists.[213, 222] A previous study showed that qualified radiologists had less inter-observer variability on reporting of chest radiographs compared to radiology trainees and physicians.[223] Despite the specialized training in paediatric radiology and advanced technology, human error remains a likely factor.[211] The level of variability between the senior radiologists could be a reflection of inconsistency in the application of WHO criteria, as this has been shown to decrease inter-observer variability.[224] However, the rate of false negative reports between the two interpretations of chest radiographs is a well recognised problem [217] which may jeopardize the results of epidemiological studies by underestimating the true burden of pneumococcal pneumonia.[225] In previous pneumococcal vaccine efficacy studies the radiographic evidence of pneumonia was observed in up to 34% of the enrolled children.[226] It has been suggested that using the WHO criteria would make any differences in the results reflect geographical variations in disease epidemiology or vaccine effects rather than methodological factors.[151] Despite the application of this classification, the concordance rate between two trained reviewers was only 48%

(250/521).[227] The degree of variability of reporting chest radiographs from the present study demonstrates that methodological differences are still a problem in the epidemiological studies of pneumonia in children.

7.2.1 Strengths and limitations

The findings were limited by heterogeneity amongst a range of radiologists (general and specialized radiologists) involved in the first reporting, with only one radiologist performing second reporting together with differences in clinical information provided at first and second readings. However, having them agree mostly on the interpretation of lobar changes, with the main variability related to non-end-point changes as recently shown among a group of 13 paediatricians and two radiologists,[295] make the impact of these limitations is minimal. On the other hand the agreement between readers was improved when the WHO criteria [151] was modified to consider the presence of any lung infiltrate irrespective of its features as end-point pneumonia.[296] All of these reported findings highlight the importance to have defined diagnostic radiological criteria of pneumonia that can be universally used in epidemiological studies and clinical practice.

7.3 Conclusions

There is substantial inter-observer variability in the reporting of chest radiographs particularly in young children with pneumonia which appears unrelated to the level of training and experience of those reporting. These findings add to the recognised variability in the literature demonstrating that there may be a need for evaluation of the WHO categorization of radiological pneumonia in children to improve the validity and encourage widespread adoption of the criteria.

Chapter 8 Final Summary

8.1 Community-acquired pneumonia in children

Community-acquired pneumonia is a common childhood infection and is a frequent cause of admission to hospital, particularly in the young age group.[1] It causes a substantial morbidity and mortality respectively in developed and low resource settings.[4, 9] This research provides invaluable epidemiological and aetiological data on this infection in children in the North East of England where the disease was investigated in a similar prospective study in 2001–2002.[59, 157] The annual incidence and management outcomes of CAP from the survey in 2001–2002 were previously published,[59, 157] while the aetiological data were analysed together with the new data from the repeated research. There were several public health interventions to combat this infection or standardise it management; such as the introduction of routine pneumococcal conjugate vaccination programme in the UK with PCV7 in 2006 [124] which subsequently replaced by PCV13 in 2010 [125] and the publication of the national management guidelines from the British Thoracic Society (BTS) in 2002 [160].

Data from the epidemiological survey were the first UK published prospective evaluation of the effect of PCV7 on the incidence of childhood CAP. It reports an 18% reduction in both the annual incidence of CAP presenting to hospital and hospitalisation rate between 2001 and 2009. The major reduction in pneumonia admissions (38%) was observed in those aged under two years. There was also a lower incidence of pneumonia among PCV7-vaccinated children under five years old than those annual unvaccinated. Analyses of the presentation and management outcomes showed that clinical management of children with pneumonia has changed significantly between 2002 and 2008. There has been a reduced number of investigations performed, a change in the type of antibiotics, a decrease in intravenous and a concomitant increase in oral antibiotics. Possible influencers on change of clinical practice include the introduction

of the 7-valent pneumococcal conjugate vaccine (PCV7) to the UK immunisation programme in 2006, the publication of the BTS management guidelines and an expanding literature on oral/IV antibiotic use.[161, 162, 270]

This is the first study to describe the aetiology of CAP in UK children prior to and following the introduction of pneumococcal conjugate vaccination programme. The timing of this comprehensive study three years after the introduction of PCV7 and during the first year of PCV13 provides a baseline for future comparative studies of the aetiology of pneumonia in the same setting. The causative pathogens identified were predominately viruses in both studies with the detection of pneumococcal infections increasing from the pre- to post-vaccine studies as a result of the application of molecular diagnostic methods. This study also describes different diagnostic approaches to pneumococcal infections and provides the first information on serotype distribution of pneumococcal CAP in UK children after the introduction of the pneumococcal conjugate vaccination programme. *S. pneumoniae* was the definite cause of pneumonia in 17.4% of children; 15% in those under five and 22.7% among those over five years. Non-PCV7 but PCV13 serotypes were the major contributor to the aetiology of pneumococcal pneumonia.

The observed substantial inter-observer variability in the interpretation of chest radiographs for the diagnosis of childhood pneumonia highlights the on going debate when defining the radiologically confirmed pneumonia as entry criteria to studies investigating the epidemiology and aetiology of pneumonia in children.

8.1.1 Overall strengths and limitations

8.1.1.1 Epidemiology survey

The strengths of this survey include the use of a multi-centre large scale approach, well-validated disease definition and previously studied population allowing accurate historical comparisons. Its significant limitation is that while the introduction of PCV7 is the major change between the two surveys, the ecological nature of the survey means that the decrease in disease incidence cannot be causally attributed to PCV7 alone.[50, 266] Further potentially relevant factors include natural variations in disease incidence, other public health interventions such as anti-smoking campaigns,[267, 268] variation in national and local health policies, changes in admission criteria, referral pathways and threshold for radiological investigation, and the implementation of national guidelines for the management of CAP in children by the BTS in 2002.[160] The later factor might have resulted in more children being managed in primary care including accident and emergency departments. While cannot rule out these factors using the methodology in this survey, I feel it is unlikely that any of these factors would have reduced the incidence of pneumonia to the degree observed. Furthermore, it would be speculated that these factors would alter the overall incidence rate regardless of age group. The fact that no significant difference was found in the annual incidence of pneumonia in the over five age group, by definition non-vaccine recipients, therefore increases the likelihood that the observed changes were attributable to PCV7.

It could be speculated that changes in the incidence of viral disease or vaccination may have contributed to the observed differences in the annual rates of pneumonia, though this is unlikely given that the neither age group (and specifically the under two age group) are routinely vaccinated against respiratory viral disease. No specific data were

collected on influenza vaccination status but it is most likely that the overwhelming majority of enrolled children were unvaccinated. It has also been hypothesised that a considerable proportion of viral pneumonia may in fact have co-infection with bacterial pathogens including *S. pneumoniae* as shown by Michelow and colleagues [11] which could potentially ameliorate the effect of variations in the incidence of seasonal influenza or other viral infections.

The inclusion of a further group of the other infiltrates/abnormalities chest radiographic feature to the WHO definition of radiological pneumonia could have overestimated the incidence of pneumonia within our population. However, the number of such individuals was low and represented only 3% of all cases of pneumonia within the studied cohort. It would be therefore suggested that this should not have significantly influenced these findings, given the magnitude of the changes reported in this survey. In contrast to the observed substantial reduction in the annual incidence of lobar pneumonia following the conjugate vaccination programme in Canada,[79] this survey reported increased lobar findings. This could be attributed to either the relative implication of non-PCV7 pneumococcal serotypes in the aetiology of pneumonia in children or due to the recognized variation in the interpretation of paediatric chest radiographs,[38] which in this survey were reported by local radiologists that differed between sites and from the original survey. Although the diagnosis of end-point pneumonia was dependent on reading non-standardised chest radiograph reports by local radiologists, the application of standardised criteria provided by the WHO on defining the radiological end-point pneumonia would allow more accurate comparative data in epidemiological studies for assessment of the impact of pneumococcal vaccination.[150, 151]

Furthermore, this survey provides invaluable evaluation of the presentation and management of childhood CAP seen in hospital over a year period with particular focus on the investigations performed and use of antibiotics. Lack of data collection and interviewing of admitting clinicians about their decisions for performing investigations and selection of the type and administration route of antibiotics limited the interpretations of potential factors surrounding the observed changes on these areas.

8.1.1.2 Aetiology study

There are several limitations to these data, such as potential seasonal bias to the data of post-vaccine study where the recruitment was carried out over 18 months which included two winter seasons (48% of enrolled children). Although the post-vaccine study covered two winter seasons, enrolled children were fewer than the pre-vaccine study which could be a true reflection of decreased disease incidence and hospitalisation. The findings from the post-vaccine study may have been hampered by the lack of convalescent sera which may have led to the underestimation of the role of atypical bacteria in childhood pneumonia. But this effect is probably minimal as mycoplasma infection was only detected in three children by paired serum samples pre-vaccine. Another limitation is the variation between the two studies in the diagnostic methods used and the pathogens investigated. Lack of serotype data of the identified pneumococci from the pre-vaccine study limits the actual comparison with the serotype profile after the conjugate vaccine implementation. However, this research provides information on the changes of aetiology of pneumonia over two time periods. It also highlights the requirement of using multiple laboratory investigations in order to identify the likely causative pathogens. The improvement in the yield of several diagnostic approaches used in the post-vaccine study compared to the pre-vaccine study, particularly with more PCR-based assays used, is in line with recent studies over the last 15 years.[1, 2, 20, 289]

This is the first study in the UK to explore serotype data in children with pneumococcal CAP. It illustrated the improvement in detection rate of *S. pneumoniae* by using a comprehensive range of diagnostic techniques including PCR-based assays. These provide important information for the public health policy makers for planning strategies to combat this infection in children and for the design of new generations of pneumococcal conjugate vaccines. An important limitation was the insufficient volume of the samples which limited progress with the steps of the pneumococcal multiplex serotype-specific PCR, despite efficient procedures taken towards its development and validation. I acknowledge that numbers of positive pneumococcal PCR assays were small and this could be underestimated by the application of only pneumolysin PCR on blood and respiratory secretions. However, the results provide useful information on the overall contribution of *S. pneumoniae* in pneumonia among children in this setting.

8.1.1.3 Radiology study

The findings were limited by heterogeneity amongst a range of radiologists (general and specialized radiologists) involved in the first reporting, with only one radiologist performing second reporting together with differences in clinical information provided at first and second readings. However, having them agree mostly on the interpretation of lobar changes, with the main variability related to non-end-point changes as recently shown among a group of 13 paediatricians and two radiologists,[295] make the impact of these limitations is minimal. On the other hand the agreement between readers was improved when the WHO criteria [151] was modified to consider the presence of any lung infiltrate irrespective of its features as end-point pneumonia.[296] All of these reported findings highlight the importance to have defined diagnostic radiological criteria of pneumonia that can be universally used in epidemiological studies and clinical practice.

8.1.2 Overall conclusions

The introduction of the pneumococcal conjugate vaccination programme was associated with a reduction in both the annual rates of pneumonia and hospitalisation. The identification rates of confirmed causes of CAP overall and pneumococcal infections have increased with application of more PCR assays when compared with data from 2001–2002 study in the same setting. All identified pneumococcal serotypes being non-PCV7 but PCV13 serotypes which make the introduction of PCV13 likely to be associated with a substantial reduction in the annual incidence of pneumococcal-related pneumonia.

There is substantial inter-observer variability in the reporting of chest radiographs particularly in young children with pneumonia which appears unrelated to the level of training and experience of those reporting. These findings add to the recognised variability in the literature demonstrating that there may be a need for evaluation of the WHO categorization of radiological pneumonia in children to improve the validity and encourage widespread adoption of the criteria.

8.2 Research impacts and future studies

The outcomes of this research have several potential impacts on informing healthcare workers and policymakers:

1. Information on the changes of rates of CAP and hospitalisation, aetiology and pneumococcal serotypes provide important opportunity for epidemiologists and public health policymakers to design and plan future strategies and new generations of pneumococcal conjugate vaccine to combat this infection in children.
2. Outcomes on the presentation and management of CAP in children following the publication of BTS guidelines provide figures that are useful for practice improvement. These outcomes were reflected to the hospitals involved.

I would suggest the following areas to be investigated in future studies:

1. Repeat the survey into the incidence of CAP and hospitalisation in the same hospitals every five years to evaluate the impact the introduction of new generation of pneumococcal conjugate vaccine.
2. Continued surveillance is required to monitor for the emergence of serotype replacement in childhood pneumococcal-related pneumonia.
3. Further studies are required to explore the clinical decision making to determine the reasons behind the wide variation in IV antibiotic use seen, as this is not explained fully by disease severity in this research.
4. A cost-effectiveness analysis focusing on the impact of reduced hospitalisation, IV antibiotic use and preadmission antibiotics would provide useful economic information.

5. Future studies should not only aim to replicate the methodology used here, but also, in addition, be designed to take into account the impact of the following factors on the incidence of pneumonia as determined in these surveys [59, 297]:
    a. Variable reporting of chest radiographs between sites and across different study periods.
    b. Referral patterns for pneumonia (how many children seen only in primary care or by accident and emergency staff).

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Appendix 1 Ethics Approval

Newcastle & North Tyneside 2 Research Ethics Committee
Room G14
Dental School
Framlington Place
Newcastle upon Tyne
NE2 4BW

Telephone: 0191 222 3581
Facsimile: 0191 222 3582
Email: gillian.mayer@ncl.ac.uk

2 April 2008

Dr Julia Clark
Consultant Paediatric Infectious Disease COPD
Newcastle General Hospital

Dear Dr Clark

Query re collecting data for research - full postcode for epidemiological data

In response to your recent query the Chairman notes the following points:

1. The vaccine has been introduced on a population basis into the immunisation schedule across the UK, so there is no research intervention here.

2. If data will be gathered on children admitted with pneumonia and the researchers will gather clinical and demographic data prospectively, as previously done, on an anonymised basis, then providing this is the case, and they are just simply repeating what they did before the vaccine was introduced, I don't feel they need formal review by a REC.

This is assuming that the full postcode is the method of collecting the socioeconomic data. If this is the case, I don't think they need informed consent to collect this.

Yours sincerely

G Mayer

Gillian Mayer
Committee Co-ordinator

Newcastle & North Tyneside 1 Research Ethics Committee
Newcastle Dental School
Room G14
Dental School
Framlington Place
Newcastle
NE2 4BW

Telephone: 0191 222 3581
Facsimile: 0191 222 3582

22 December 2008

Dr Julia Clark
Consultant in Paediatric Immunology and Infectious Diseases
Newcastle upon Tyne Hospitals NHS Trust
Newcastle General Hospital
Westgate Road
Newcastle upon Tyne
NE4 6BE

Dear Dr Clark

Full title of study:	**The Impact of Pneumococcal Vaccine (Prevenar) on the epidemiology of Childhood Pneumonia in the North East of England**
REC reference number:	**08/H0906/105**

The REC gave a favourable ethical opinion to this study on 09 December 2008.

Further notification has been received from a local site assessor following site-specific assessment. On behalf of the Committee, I am pleased to confirm the extension of the favourable opinion to the new site. I attach an updated version of the site approval form, listing all sites with a favourable ethical opinion to conduct the research.

R&D approval

The Chief Investigator or sponsor should inform the local Principal Investigator at each site of the favourable opinion by sending a copy of this letter and the attached form. The research should not commence at any NHS site until approval from the R&D office for the relevant NHS care organisation has been confirmed.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

08/H0906/105	**Please quote this number on all correspondence**

Yours sincerely

Ms Anne Taylor
Committee Co-ordinator

Email: anne.taylor7@nhs.net

Enclosure:	*Site approval form*

Copy to:	Ms A Tortice Joint Research Office (Research & Development) Newcastle upon Tyne Hospitals NHS Foundation Trust Research & Development 4th Floor, Leazes Wing Royal Victoria Infirmary Newcastle upon Tyne

13th November 2008

Dr F J Hampton
Consultant Paediatrician
Paediatric Unit
Women & Children Division
The James Cook University Hospital

Dear Dr Hampton

ID: 2008075 - The Impact of Pneumococcal Vaccine (Prevenar) on the epidemiology of Childhood Pneumonia in the North East of England

Your project was reviewed at the Research Approval Board on 12th November 2008. I am happy to say that on this occasion your project was approved.

Documents reviewed and approved were:

- Protocol - Version 1 dated 29/07/2008
- Parent Information Sheet - Version 1 dated 15/07/2008
- Children's Information Sheet (11-16 years) - Version 1 dated 15/07/2008
- Children's Information Sheet (10 years and younger) - Version 1 dated 15/07/2008
- Assent Form for Children - Version 1 dated 15/07/2008
- Consent Form (Child) - Version 1 dated 15/07/2008
- Consent Form (Parent / Carer 1) - Version 1 dated 15/07/08
- Consent Form (Parent / Carer 2) - Version 1 dated 15/07/08
- GP Letter - no version number or date

I would like to take this opportunity to remind you that STHNHS Trust manages all research in accordance with the requirements of the Research Governance Framework. As a researcher working in the Trust you must comply with all reporting requirements, systems and duties of action put in place by the Trust to deliver Research Governance. You will be expected to read and familiarise yourself with conditions of approval as well as incident reporting procedures in relation to your project.

Please note it is the responsibility of all researchers to adequately cover the ongoing costs of their project. If external funding is not available or becomes unavailable then these costs must be covered by their departmental budget. There is at present no possibility of the R&D department covering any shortfall in these costings.

Enclosed are labels which need to be affixed to the front of Patient notes to indicate they are taking place in a clinical trial/study/registry. These are the only labels that should be used and any issued from another source should be discarded. This will indicate to Health Records that these notes should be kept for a minimum of 15 years. If you require additional labels, please contact Research & Development.
If the R&D Department can be of any further assistance, please do not hesitate to contact myself or Trish Watson, R&D Administrator on (01642) 282585.

Yours sincerely

Dr S Graham
Chairman of Research Approval Board

Cc Dr Katherine Eastham
Teaching & Education Fellow
Education Centre
North Tyneside General Hospital
Rake Lane
Tyne & Wear
NE29 8NH

The Newcastle upon Tyne Hospitals NHS
NHS Trust

LRF/HA/196

10 December 2008

The Freeman Hospital
High Heaton
Newcastle upon Tyne
NE7 7DN

Tel: 0191 233 6161
Fax: 0191 213 1968

Dr J Clark
Consultant in Paediatric
Immunology and Infectious Diseases
Newcastle General Hospital

Dear Dr Clark,

Trust Approval for R&D Project: 4687

Title of Project:	**The impact of conugate pnenmococcal vaccine (prevenar) on the epidemiology of childhood pneumonia in the North East**
Principal Investigator	**Dr Julia Clark**
Funder (proposed):	**Wyeth Pharmaceuticals**
Sponsor (proposed):	**The Newcastle upon Tyne Hospitals NHS Foundation Trust**

The Trust grants approval for the above project, dependent upon:

(i) you, as Principal Investigator, agreeing to comply with the Department of Health's Research Governance Framework for Health and Social Care, and understanding their responsibilities and duties (a copy of responsibilities prepared by the Trust R&D Office is enclosed)

(ii) you, as Principal Investigator, ensuring compliance of the project with all other legislation and guidelines including Caldicott Guardian approvals and compliance with the Data Protection Act 1998, Health and Safety at Work Act 1974, any requirements of the MHRA (*eg* CTA, EudraCT registration), and any other relevant UK/European guidelines or legislation (*eg* reporting of suspected adverse incidents).

Sponsorship

The Newcastle upon Tyne Hospitals NHS Foundation Trust will act as Sponsor for this project, under the Department of Health's guidelines for research in health and social care.

In addition, the Trust has a Research Governance Implementation Plan, agreed with the Department of Health, in order to fully comply with Research Governance and fulfil the responsibility of a Sponsor.

As the Trust is acting as Sponsor for the research and where some of the research is taking place outside of Newcastle upon Tyne, then all costs must be met for research governance audit visits to those sites. It is the responsibility of the PI to provide confirmation to the Trust of who will pay these costs. Audit is required under the Research Governance Framework for Health and Social Care. (Please note that the Trust randomly audits 10% of all its active research annually.)

You must notify the R&D Office if any changes to the protocol, etc. are agreed with the Ethics Committee or if there are any associated changes in cost relating from such alterations. It is imperative that the R&D Office retains a *complete* and up-to-date set of all such material.

It is also the Principle Investigator's responsibility to ensure that all staff involved have Honorary Contracts, where necessary, issued prior to commencing the research. Please be aware that Honorary Contracts will not be issued without a favourable ethical opinion and funding.

In addition, unless otherwise agreed with the Trust, the research will be covered for negligence under the CNST (Clinical Negligence Scheme for Trusts), however cover for no-fault harm is the responsibility of the Principal Investigator to arrange if required.

Please also note that for any NHS employee who generates Intellectual Property *in the normal course of their duties*, it is recognised that the Intellectual Property Rights remain with the employer and not the employee.

Yours sincerely,

Sir Leonard R Fenwick CBE
Chief Executive

Enc:

CC: Mrs C Hughes, Finance Department, Room 203, Cheviot Court, Freeman Hospital
Dawn Reed, Chief Executive's Office, Freeman Hospital
Professor A Cant, Clinical Director, Children's Services, Newcastle General Hospital

Appendix 2 Questionnaire

Completed by				**Hospital**				**Study No.**			
Date & time of admission		/ / __ __:__ __ am / pm					**Date of discharge**			/ /	
Pre-admission duration of illness (days)							**Postcode**				
Seen by GP / A&E		Y / N	**if yes, days since initial assessment**								
Date of birth		/ /	**Father's occupation & age**								
Sex	M / F	**Weight (kg)**		**Mother's occupation & age**							
No. of bedrooms in the house			**Household size (total no. living at the same house)**								
Pre-admission antibiotics		Y / N	**Name**				**Start Date**			**End Date**	
							/ /			/ /	
							/ /			/ /	
Received Prevenar		Y / N	**Doses**		**Pre-admission Ibuprofen**			Y / N	**Tamiflu**		Y / N

RISK FACTORS (✓)												
Prematurity		**Gestation**		**Immunodeficiency**		**Chronic lung disease**						
Cystic fibrosis		**Sickle**		**Bronchiectasis**		**Steroids**		**Other**				
Parental smoking		**Child smoking**		**Nursery school**		**Childminding**						

CLINICAL PRESENTATION		
Respiratory rate		**Clinical findings (free text)**
Temperature		
SaO_2 (in air) %		

PERFORMED INVESTIGATIONS (circle)						
FBC	**BC**	**CRP**	**ESR**	**Serum Na**	**Secretions: virology IF**	**Secretions: m,c&s**
Other (specify):						
Radiologist's chest x-ray report (report text or attach copy)						

CLINICAL PROGRESS (✓)							
Home on antibiotics		**Home without antibiotics**		**Transferred**	where:	**Died**	

IF ADMITTED									
Oxygen	Y / N	**No. of days**		**Ibuprofen**	Y / N	**Confirmed H1N1 virus**			Y / N
NG feeds	Y / N	**No. of days**		**IV fluids**		Y / N	**No. of days**		
Antibiotics	Y / N	**IV / Oral**	**Name**				**No. of days**		
		IV / Oral	**Name**				**No. of days**		
		IV / Oral	**Name**				**No. of days**		
		IV / Oral	**Name**				**No. of days**		
Organism Isolated (any)	Y / N	**Name:**		**Site: blood culture / NPS / Sputum / other**					
		1. **2.** **3.**		**1.** **2.** **3.**					

COMPLICATIONS							
Pleural effusion	Y / N	**PICU admission**		Y / N	**if yes, duration (days)**		
Empyema	Y / N	**Fluid resuscitation**	Y / N	**IPPV**	Y / N	**if yes, duration (days)**	
Other (specify)							

Appendix 3 Information and Consent Forms

CHILDREN'S INFORMATION SHEET (10 YEARS AND YOUNGER)

Causes of Pneumonia in Children

The child should be helped to read the following information please:

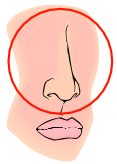
What is the research study all about?
We are asking you if you would like to take part in a research study to find out what bugs cause chest infections in children.

Why am I being asked to take part?
You are being seen in hospital because you have an infection in your chest which is making you feel unwell. We want to do a special study to try to find out more about the kinds of bugs that cause infections like yours in children. This will help us know the best medicines to give.

Do I have to take part?
You do not have to take part in the study if you don't want to. If you decide to take part you can stop at any time. Just tell you parents, doctor or a nurse. They will not be cross with you.

What will happen to me if I take part?
We would like to take some special tests from you to look for the bugs causing your chest infection **even if your doctor would not normally have done them.**

- The first test is to look for bugs from your chest. To do this a nurse will suck some secretions (the runny stuff!) from the back of your nose with a small tube.
  If you are able, you could cough some spit into a pot instead.



The Nose

- The second is to look for bugs in your blood.
  If your doctor has asked for a blood test anyway then a little extra blood will be taken at the same time.
  If your doctor did not ask for a blood test then a little blood test will be taken. This test is just for this study and would not be done otherwise. It is not very sore, is very quick, and we can use a special cold spray or cream so you don't feel the prick as much.

- We also need to test some urine – we will ask you to collect some wee in a potty or special pot. We will show you what to do.

What are the possible benefits of taking part?
We cannot promise that the study will help you but the information we get may help treat children with chest infections better in the future

What if there is a problem?
If you have any questions or there is a problem during the study please talk to your parents or the doctors and nurses who will be able to help you.

Will anyone else know that I'm doing this?
We will only tell people who have a right or need to know that you are taking part.

Did anyone else check the study is OK to do?
Before any research is allowed to happen it has to be checked by a group of people called a Research Ethics Committee. They make sure that the research is fair. The study has been checked by the Research Ethics Committee.

We hope you will let us do these extra tests. The doctors and nurses will look after you just the same whether these extra tests are done or not. Please have a think if you'd like to take part and talk to your family, doctors and nurses about the study if you'd like to. Thank you for reading this and we hope you soon feel better

CHILDREN'S INFORMATION SHEET (11–16 YEARS)

Causes of Pneumonia in Children

What is the research study all about?
We are asking you if you would like to take part in a research study to find out what bugs cause chest infections in children.

Why am I being asked to take part?
You are being seen in hospital because you have an infection in your chest which is making you feel unwell. Lots of different kinds of bugs can cause this infection. Some medicines are best for one bug, others for a different one and some do not need any medicine at all! It is hard to know which bug you have. To try to find out some special tests are sometimes done. Sometimes we never know exactly which bug caused your infection. All children admitted to hospitals in the Newcastle and Middlesbrough between October 2008 and March 2010 will also be asked if they would like to take part in the study.

Why are we doing the research study?
We want to do a special study to try to find out more about the kinds of bugs that cause infections like yours in children. This will help us know the best medicines to give. It will also let us find out if a new vaccine for babies has helped stop chest infections in children.

Do I have to take part?
You do not have to take part in the study if you don't want to. You can also stop taking part in the study at any time during the study without giving a reason and without it affecting how the doctors and nurses look after you.

What will happen to me if I take part?
We would like to take a number of tests from you to look for the bugs causing your chest infection **even if your doctor would not normally have done them.**

- The first test is to look for bugs from your chest. To do this a nurse will suck some secretions (the runny stuff!) from the back of your nose with a small tube. If you are able, you could cough some spit into a pot instead.

- The second is to look for bugs in your blood.
  If your doctor has asked for a blood test anyway then a little extra blood will be taken at the same time.
  If your doctor did not ask for a blood test then a little blood test will be taken. This test is just for this study and would not be done otherwise. It is not very sore, is easy and quick to do, and we can use a special cold spray or cream so you don't feel the prick as much.

- We also need to test some urine – we will ask you to collect some wee in a special pot. We will show you what to do.

What are the possible benefits of taking part?
We cannot promise that the study will help you but the information we get may help treat children with chest infections better in the future.

What if there is a problem?
If you have any questions or there is a problem during the study please talk to your parents and either you or they can ask to speak to one of the Researchers who will do their best to help you.

Contact
Dr Julia Clark, Consultant in Paediatric Immunology and Infectious Diseases, NGH
0191 2336161, extension 23116

Will anyone else know that I'm doing this?
We will keep your information in confidence. This means that we will only tell people who have a right or need to know. Any information sent out of the hospital will have your name and address removed so you cannot be recognised from it.

Who is funding the study?
This study is being funded by a company called Wyeth who make a vaccine (prevenar) which helps to protect children from pneumonia. The money is being used to pay for the extra tests and to pay a nurse's salary to undertake the extra work involved. None of the doctors or nurses looking after you will make any extra money if you agree to take part.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people called a Research Ethics Committee. They make sure that the research is fair. The study has been checked by the Research Ethics Committee.

We hope you will let us do these extra tests. The doctors and nurses will look after you just the same whether these extra tests are done or not. Please have a think if you'd like to take part and talk to your family, doctors and nurses about the study if you'd like to. Thank you for reading this and we hope you soon feel better.

PARENT INFORMATION SHEET

Title of Project: The impact of pneumococcal vaccination on the epidemiology of childhood pneumonia

Investigators: Dr Julia Clark, Newcastle General Hospital
Dr Fiona Hampton, James Cook University Hospital

Summary
Together with doctors in Middlesbrough we are doing a study of the causes of pneumonia in children and how these might have changed since the introduction of pneumococcal vaccination into the national childhood immunisation schedule. We are inviting you, on behalf of your child, to take part in this research study. Before you decide whether to take part it is important that you understand why the research is being done and what it involves for your child. This information sheet is to help you with this. If anything is not clear or you have any further questions after reading this sheet please talk to the doctors or nurses looking after your child.

What is pneumonia?
There are several types of pneumonia and pneumonia is one type of chest infection. Most children with pneumonia are not seriously ill and may get better by themselves or with antibiotic medicine. Some children may need injections of antibiotics or other types of treatment that mean a stay in hospital.

What causes pneumonia?
Pneumonia may be caused by many different germs. Some germs are viruses and some are bacteria. Viruses do not need any treatment but bacteria need antibiotics. In most children with pneumonia the germ causing the infection is never found. Because we do not know which germs are causing the infections we have to judge whether the child needs antibiotics or not and which antibiotics would be best. We judge this by examining the children and by using information about which germs caused similar infections in other children in earlier studies.

Why do we need to do a study?
We need to see if the causes of pneumonia in children have changed since the introduction of a pneumococcal vaccine into the national childhood immunisation schedule. We have information about causes of pneumonia in the North East of England before the vaccine was introduced and want see if these have changed. New tests that have been developed in the last few years mean we can now find more germs quite easily. A study done now will therefore give us a lot of very useful information without too many tests for the children.

Why have I been given this information?
You have been given this information sheet because the doctors looking after your child think that your child has pneumonia. They may think this because of what they see when examining your child or from the chest x-ray or both. We are asking if you and your child will help us in our study of the causes of pneumonia.

What will happen if I agree to help in the study?

If you agree to take part then these things will happen:

1. **Your child will have some secretions sucked from the nose with a small tube and sent to the laboratory.**

This is a test we do regularly on nearly all small children with poorly chests and some of the older ones, even when they are not helping with a study. If you do not wish to take part in this study your child may still need to have this test done as part of routine care.
The secretions will be tested for signs of viruses and bacteria.

If your child is able to cough up some spit into a pot we will send this specimen to the laboratory instead of secretions from the nose.

2. **Your child will have some blood tests taken and sent to the laboratory.**

Many children with pneumonia need blood tests as part of their routine care.
If your child needs blood tests then these will be taken to help with giving your child the best possible care. This will not depend on whether or not you decide to take part in this study. If you decide to take part in this study then that will mean that a little more blood is taken at the same time.
If your child does not need blood tests as part of routine care then taking part in this study will mean blood tests especially for this study. We would use a special cold spray or local anaesthetic cream to numb the skin prior to the blood test.
This blood will be tested for signs of viruses and bacteria living in the blood and also used as a baseline for the tests in "3".

3. **Anonymous records will be kept of some parts of the examination of your child when he or she comes into hospital and throughout the hospital stay. Records will also be kept of the treatment your child receives in hospital and any medicines your child takes home.**

These records of your child's age, weight, temperature, breathing rate and so on will later be matched with any germs we find when doing our tests. In this way we can help build a picture of how each germ may affect children. The treatment records will help us to see if we are using the best treatment for the germs which affect most children.

4. **You and your partner will have a small swab taken from the back of the nose.**

It is not known how a particular type of the pneumococcus bug, responsible for many of the complicated pneumonias we see, is picked up by children. It is thought that it may be transmitted to the child from the nose of a parent / carer. We will be looking for this bug in the child's specimens (1-3 above), and are keen to see if either parent / carer carries this bug up their nose too. This will involve taking a small swab (a cotton bud on the end of a stick) from the back of the nose. The swab will be introduced into each nostril. It only takes a few seconds to do and should not cause any discomfort, but some adults find that it tickles, causes them to sneeze, or makes their eyes water.

Additional points:

1. **What possible effects are there on my child by taking part in this study?**

This study is looking at the causes of pneumonia not the treatment. Your own child will be monitored, his or her treatment will not be affected by you agreeing to take part in the study. Your own child will therefore not benefit or be put at any risk by taking part in the study. Our hope is that we may benefit future children with pneumonia, but we cannot benefit those who help in this study.

2. Will the information about my child be kept confidential?
All information collected about your child during this study will be kept strictly confidential. Any information which leaves the hospital will have your child's name and address removed so that he or she cannot be recognised from it.

3. Financial concerns.
This study is being funded by a company called Wyeth who make a vaccine (prevenar) which helps protect children from pneumonia. The money is being used to pay for the extra tests on the children's blood and nasal secretions which the hospital would not do as part of routine care. The money is also being used to pay a nurse's salary to undertake extra work in collecting information and second blood tests. None of the doctors or nurses asking you to help with the study will make any extra money if you agree to take part.

4. Will I learn the results?
We will inform your GP that your child is taking part in the study and will send you and your GP a letter once we know the results for your child. This may not be until several months after your child has been ill. Remember that even after all our tests it is possible we will not know the name of the germ in your child's illness.

5. What will happen to the results of the study?
It is intended to publish the results of the study in a general paediatric journal. Your child's details will not be identified in any publication. You can find out which journal the study will be published in by contacting the Study Team after March 2010.

6. What will happen to the study samples?
Specimens collected for research purposes, that are not part of routine clinical care, will be stored by the Research Team until the end of the project. The specimens may be used for future research, however this would require further approval by the Research Ethics Committee (see point 9).

7. What if there is a problem?
If you have any concern about any aspect of the study you should ask to speak to one of the Researchers who will do their best to answer your questions. If you remain unhappy you wish to complain formally you can do this through the NHS complaints procedure. Details can be obtained from the hospital.
Contact: Dr Julia Clark, Consultant in Paediatric Immunology and Infectious Diseases, NGH
0191 2336161 ext 23116

8. What happens if I no longer want my child to participate in the study?
You can withdraw from the study at any point without having to give a reason and without your child's clinical care being affected in any way. Specimens provided for research purposes can also be withdrawn from the study at your request.

9. Who has reviewed the study?
All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. The study has been reviewed and given a favourable opinion by the Research Ethics Committee.

10. Other questions.
If you have any further questions about the study then please talk to one of the doctors or nurses on the ward. If they cannot answer your question directly they will ask one of the doctors or nurses organising the study to come and speak to you.

11. What now?
If you agree to take part in the study then please let the doctors and nurses know and sign the attached consent form. If you agree to take any part at all in the study you will be providing us with some very useful information for treating children with pneumonia in the future. We will be very grateful for this, as will the children. We would like to thank you very much for helping us with our study. If you do not agree to take part we would still like to thank you for reading this information sheet and thinking about helping us.

ASSENT FORM FOR CHILDREN

Title of Project: The impact of pneumococcal vaccination on the epidemiology of childhood pneumonia

Name of Researcher: Dr Julia Clark

Child (or if unable parent to circle on their behalf) / young person to circle all they agree with:

Have you read (or had read to you) about this project?	Yes / No
Has somebody else explained this project to you?	Yes / No
Do you understand what this project is about?	Yes / No
Have you asked all the questions you want to?	Yes / No
Have you had your questions answered in a way you understand?	Yes / No
Do you understand it's OK to stop taking part at any time?	Yes / No
Are you happy to take part?	Yes / No

If any answers are 'no' or you don't want to take part then don't sign your name
If you do want to take part can you sign your name below?

Your name_____

Date_____

The doctor who explained the project to you needs to sign too:

Print Name_____

Sign_____

Date_____

Thank you for your help.

CONSENT FORM (CHILD)

Title of Project: The impact of pneumococcal vaccination on the epidemiology of childhood pneumonia

Name of Researcher: Dr Julia Clark

Please initial box

1. I confirm that I have read and understand the information sheet dated 12/11/2008 (version 2) for the above study and have had the opportunity to ask questions. ☐

2. I understand participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my child's medical care or legal rights being affected. ☐

3. I understand that sections of any of my child's medical notes may be looked at by responsible individuals where it is relevant to my taking part in research. I give permission for these individuals to have access to my child's records. ☐

4. I agree to my child taking part in the main part of the above study. ☐

5. I also agree to my child's GP being informed of participation in the study ☐

-
Name of Patient

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Name of Parent	Date	Signature
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Name of Person taking consent (if different from researcher)	Date	Signature
---	---	---
Researcher	Date	Signature

Appendix 4 Publications and Presentations

Publications

1. Elemraid MA, Sails AD, Eltringham GJA, Perry JD, Rushton SP, Spencer DA, Thomas MF, Hampton F, Eastham KM, Gennery AR, Clark JE. Aetiology of paediatric pneumonia after the introduction of pneumococcal conjugate vaccine. *Eur Respir J* 2013. DOI:10.1183/09031936.00199112.[298]
2. Elemraid MA, Sails AD, Thomas MF, Rushton SP, Perry JD, Eltringham GJA, Spencer DA, Eastham KM, Hampton F, Gennery AR, Clark JE. Pneumococcal diagnosis and serotypes in childhood community-acquired pneumonia. *Diagn Microbiol Infect Dis* 2013;76:129-32.[299]
3. Elemraid MA, Pollard K, Thomas MF, Simmister C, Spencer DA, Rushton SP, Gennery AR, Clark JE. Children's participation in clinical research: observations from prospective studies. *Nurs Child Young People* – accepted on 25/01/2013.
4. Elemraid MA, Rushton SP, Shirley MDF, Thomas MF, Spencer DA, Eastham KM, Hampton F, Gorton R, Pollard K, Gennery AR, Clark JE. Impact of the 7-valent pneumococcal conjugate vaccine on the incidence of childhood pneumonia. *Epidemiol Infect* 2012. DOI:10.1017/S0950268812002257.[297]
5. Elemraid MA, Pollard K, Thomas MF, Gennery AR, Eastham KM, Rushton SP, Hampton F, Singleton P, Gorton R, Spencer DA, Clark JE. Validity of using Hospital Episode Statistics data on monitoring disease trends. *Thorax* 2011;66:827.[300]

Submitted Manuscripts

1. Elemraid MA, Muller M, Spencer DA, Rushton SP, Gorton R, Thomas MF, Eastham KM, Gennery AR, Clark JE. Variability in the interpretation of chest radiographs for the diagnosis of paediatric pneumonia in epidemiological studies.
2. Elemraid MA, Rushton SP, Thomas MF, Spencer DA, Eastham KM, Gennery AR, Clark JE. Changing clinical practice: management of paediatric community-acquired pneumonia.
3. Elemraid MA, Rushton SP, Clark JE, Perry JD, Thomas MF, Sails AD, Gennery AR, Spencer DA. Parental pneumococcal nasopharyngeal carriage of children with pneumonia.
4. Elemraid MA, Rushton SP, Clark JE, Perry JD, Thomas MF, *S*ails AD, Gennery AR, Spencer DA. A case-control study to assess the urinary pneumococcal antigen test in childhood pneumonia.

Posters

1. Elemraid MA, Pollard K, Thomas MF, Simmister C, Spencer DA, Rushton SP, Gennery AR, Clark JE. Children's participation in clinical research: factors influencing parental consent.
   Arch Dis Child 2012;97:Suppl 1 A107. DOI:10.1136/archdischild-2012-301885.253.
   - RCPCH Annual Meeting, Glasgow – May 2012
2. Elemraid MA, Eastham KM, Rushton SP, Shirley MDF, Thomas MF, Spencer DA, Hampton F, Gorton R, Pollard K, Gennery A, Clark JE. Impact of heptavalent pneumococcal conjugate vaccine on the incidence of childhood pneumonia seen in hospital in the North East of England.
   Thorax 2011;66:Suppl 4 A137. DOI:10.1136/thoraxjnl-2011-201054c.171.
   - British Thoracic Society Winter Meeting, London – December 2011
3. Elemraid MA, Eltringham GJA, Sails AD, Perry JD, Thomas MF, Spencer DA, Eastham KM, Shirley MDF, Rushton SP, Hampton F, Gorton R, Gennery AR, Clark JE. Clinical significance of inflammatory markers and radiological features in predicting the aetiology of community-acquired pneumonia in children.
   Pediatric Research 2011;70:436. DOI:10.1038/pr.2011.661.
   - Annual Meeting of the European Society for Paediatric Research, Newcastle – October 2011

Presentations

1. Impact of heptavalent pneumococcal conjugate vaccine on the epidemiology of childhood pneumonia in Northern England.
    - Paediatric Immunology and Infectious Diseases Research Symposium, Newcastle – January 2012 (Regional)
    - British Thoracic Society Winter Meeting, London – December 2011 (National)
    - Paediatric Teaching Programme, Great North Children's Hospital, Newcastle – April 2012 (Local)
    - Paediatric Research Presentations, Great North Children's Hospital, Newcastle – November 2011 (Regional)
    - British Paediatric Allergy, Immunology and Infectious Diseases Group, Edinburgh – November 2010 (National)
2. Impact of pneumococcal conjugate vaccination programme on the epidemiology and aetiology of childhood pneumonia in the North East of England.
    - Annual Meeting of the European Society for Paediatric Infectious Diseases, The Hague, Holland – June 2011 (International)
3. Limited impact of the 7-valent pneumococcal vaccine on paediatric empyema in the North of England. Elite poster presentation.
    - Annual Meeting of the European Society for Paediatric Infectious Diseases, The Hague, Holland – June 2011 (International)
4. Impact of the 13-valent pneumococcal vaccine on the incidence of paediatric empyema in the North of England. Elite poster presentation.
    - Annual Meeting of the European Society for Paediatric Infectious Diseases, The Hague, Holland – June 2011 (International)
5. The childhood pneumonia study.
    - Institute of Cellular Medicine Research Seminars, Newcastle University – June 2011 (Local)

Collaborative Work – Publications

1. Blain AP, Thomas MF, Shirley MDF, Simmister C, Elemraid MA, Gorton R, Pearce MS, Clark JE, Rushton SP, Spencer DA. Spatial variation in the risk of hospitalization with childhood pneumonia and empyema in the North of England. *Epidemiol Infect* 2013. DOI:10.1017/S0950268813001015.[301]
2. Thomas MF, Sheppard CL, Guiver M, Slack MP, George RC, Gorton R, Paton JY, Simmister C, Cliff D, Elemraid MA, Clark JE, Rushton SP, Spencer DA. Emergence of pneumococcal 19A empyema in UK children. *Arch Dis Child* 2012;97:1070-2.[138]

Collaborative Work – Posters

1. Thomas MF, Simmister C, Cliff D, Elemraid MA, Clark JE, Rushton SP, Gorton R, Paton JY, Spencer DA. Comparison of primary pleural drainage strategies in paediatric empyema.
   - British Thoracic Society Winter Meeting, London – December 2011
2. Thomas MF, Sheppard CL, Guiver M, George RC, Simmister C, Cliff D, Gorton R, Elemraid MA, Clark JE, Rushton SP, Paton JY, Spencer DA. Changes in pneumococcal serotype distribution of paediatric empyema in the age of pneumococcal conjugate vaccines.
   - British Thoracic Society Winter Meeting, London – December 2011
3. Spencer DA, Thomas MF, Sheppard CL, Guiver M, George RC, Gorton R, Paton JY, Simmister C, Cliff D, Elemraid MA, Clark JE, Rushton SP. Emergence of pneumococcal serotype 19A as a cause of severe complicated pneumonia with empyema in children in England.
   - British Thoracic Society Winter Meeting, London – December 2011
4. Thomas MF, Blain AP, Shirley MDF, Simmister C, Elemraid MA, Gorton R, Pearce MS, Clark JE, Rushton SP, Spencer DA. Geographical variation in the risk of childhood pneumonia and relationships to socio-economic and health deprivation.
   - European Respiratory Society Annual Congress, Holland – September 2011
5. Thomas MF, Rushton SP, Shirley MDF, Elemraid MA, Clark JE, Gorton R, Spencer DA. Understanding the changing epidemiology of paediatric empyema: the relationship with pneumonia.
   - Health Protection Agency Conference, UK – May 2011

Appendix 5 Copies of the Study Manuscripts

Aetiology of paediatric pneumonia after the introduction of pneumococcal conjugate vaccine

Mohamed A Elemraid[*,#], Andrew D Sails[†], Gary JA Eltringham[†], John D Perry[‡], Stephen P Rushton[§], David A Spencer[¶], Matthew F Thomas[§,¶], Katherine M Eastham[**], Fiona Hampton[##], Andrew R Gennery[*,#], Julia E Clark[*,#]
On behalf of the North East of England Paediatric Respiratory Infection Study Group

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Contents: Words (abstract: 200, text: 3437), References: 40, Tables: 3, Figures: 1
Keywords: Aetiology; children; pneumococcal conjugate vaccine; pneumonia; *Streptococcus pneumoniae*
Running head: Aetiology of pneumonia pre and post PCV

Message of the study

Aetiology of community-acquired pneumonia in children following the implementation of pneumococcal conjugate vaccination programme.

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Authorship

JEC and DAS developed the original study concepts and with ADS, KME, FH, ARG, MFT and MAE contributed in the study design and logistic support. MAE and JEC collected the data and managed the study databases. ADS, JDP, GJAE and MAE performed the laboratory analyses. MAE, SPR and JEC participated in data analysis and interpretation. All authors were involved in the writing of all drafts and approved the final version for submission.

Acknowledgements

We are indebted to the paediatric staff for their facilitation of the recruitment. We thank the research nurses; J Kelly and C Barwick, K Pollard, P Singleton and C Simmister for their assistance with data collection. Thanks are also due to Drs R Lee and M Muller at the Department of Radiology for reviewing chest radiographs of 2001 and 2009 studies respectively; and A Nicholson for the laboratory support at the Department of Microbiology, Freeman Hospital Newcastle. Our thanks also go to Dr R George and C Sheppard for running the xMAP immunoassay for serotyping the pneumococcal isolates at the Health Protection Agency in London.

Support statement and statement of interest

The work was supported by Pfizer Vaccines UK (No. 0887X1-4479). JEC and DAS received unconditional research support from Pfizer. The sponsor had no role in the study design, data collection, analysis or interpretation, and writing of the manuscript.

ABSTRACT

We describe the aetiology of community-acquired pneumonia in children before and after the introduction of the pneumococcal conjugate vaccination programme in 2006.

Prospective studies were conducted in 2001–2002 (pre-vaccine) and 2009–2011 (post-vaccine) of children aged 0–16 years with radiologically-confirmed pneumonia seen in hospital. Investigations included culture, serology, immunofluorescence antibody and urine antigen testing; with an increased use of PCR assays and expanded panels of pathogens in the post-vaccine study.

241 and 160 children were respectively enrolled in the pre- and post-vaccine studies (73% aged <5 years). Identification of a causative pathogen was higher post-vaccine (61%) than pre-vaccine (48.5%) [p=0.019]. Rates of bacterial infections were not different between post- and pre-vaccine studies (17.5% versus 24%, p=0.258). Viral (31%) and mixed infections (12.5%) found more often post-vaccine than pre-vaccine (19.5% [p=0.021] and 5% [p=0.015] respectively). Rates of identified pneumococcal infections were comparable between pre- and post-vaccine studies (14.7% versus 17.4%, p=0.557). Diagnosis of pneumococcal infection post-vaccine improved when PCR was used compared to culture (21.6% versus 6%, p=0.0004). Serotypes were identified in 75% (18/24) post-vaccine which are included in PCV13 but not PCV7.

Infection with non-vaccine pneumococcal serotypes continued to be a significant cause of pneumonia in UK children.

INTRODUCTION

The range of implicated pathogens in paediatric community-acquired pneumonia (PCAP) is wide and includes viruses, bacteria or co-infection with both [1, 2]. Studies of pneumonia frequently report low levels of pathogen identification although improved knowledge of pneumonia aetiology is essential for development of targeted management and effective public health strategies such as vaccination [3, 4]. In the UK, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced routinely in September 2006 and replaced by PCV13 from April 2010. The vaccine schedule is three doses administered at 2, 4 and 13 months of age. When introduced, those over and under one year of age received one and two doses respectively as part of a catch up programme for children aged under two years.

Identifying the aetiology of PCAP is challenging with a large number of potential pathogens, some of which may also be carried as commensal organisms, which can complicate the interpretation of the results of testing nasopharyngeal samples [5]. Conventional methods such as blood culture and serology often have limited sensitivity due to inadequate sample volume or lack of convalescent sera [3]. Molecular diagnostics are now routinely used in the assessment of viral respiratory infections and similar techniques have been developed for the detection of bacterial respiratory infections [6, 7]. Resti and colleagues demonstrated a significant improvement in the identification of pneumococcal pneumonia in children by PCR on blood samples (15.4%) when applied simultaneously with blood culture (3.8%) [8]. In a recent study of Italian children aged under five years, overall bacteremic pneumococcal pneumonia was identified in 14.3%, which was established by PCR in 92%, blood culture 1% and both in 7% [9].

The introduction of PCV was expected to decrease the incidence of pneumonia in children, and this is supported by a region-wide epidemiological prospective survey [10]. We present data from studies conducted over two periods before (2001–2002) and after (2009–2011) the addition of conjugate pneumococcal vaccination. These were designed to describe the proportion of causative pathogens in PCAP and describe how the identification of causative pathogens could be improved with the application of more PCR-based assays. As the disease incidence declined, we therefore planned a

longer recruitment period in the post-vaccine study in order to have a larger cohort with representative aetiological data.

METHODS

Participants

Two prospective studies were undertaken from August 2001 to July 2002 and October 2009 to March 2011. Enrolled children were from the North East England (excluding Cumbria) who were aged 0–16 years and presented with clinical and radiological features suggestive of pneumonia. They were admitted to the paediatric services at the Great North Children's Hospital (formerly Newcastle General and Royal Victoria Infirmary), the regional cardiothoracic centre, Freeman Hospital Newcastle, or the James Cook Hospital in Middlesbrough. The cohort of 2001–2002 study was a proportion of children with pneumonia seen at these recruitment sites as part of a previously published regional epidemiological survey [11]. They were consented and enrolled in the aetiological study with an extended panel of investigations. Written informed consent was obtained from parents and assent from older children. Ethical approvals were granted by the Newcastle and North Tyneside Research Ethics Committee and Research Approval Board at South Tees Hospitals NHS Trust, Middlesbrough. Caldicott approvals were also obtained.

Research teams of doctors and nurses led and ascertained the standardised diagnosis of pneumonia and the recruitment procedures. Recruitment methodology and enrolment criteria were consistent across the two studies and included children with any history, signs or symptoms suggestive of lower respiratory tract infection including any of fever, tachypnoea (defined age-specific respiratory rates), dyspnoea, cough, respiratory distress, chest wall retractions and auscultatory findings such as crackles, bronchial breathing or reduced breath sounds together with chest radiographic findings consistent with pneumonia as determined initially by the local paediatrician. Data on C-reactive protein and full blood count indices were used when clinically indicated by the admitting teams to inform the diagnosis of pneumonia. Exclusion criteria included resident outside of North East England; clinical diagnosis of bronchiolitis; hospitalisation in the preceding three weeks or normal chest radiograph after formal reporting by a radiologist.

All chest radiographs were reviewed by second consultant radiologists (one for each study) at the regional centre in Newcastle who were blinded to both clinical data and the first reports. Radiological findings were categorised into lobar, patchy or perihilar according to the WHO criteria [12]. Pneumococcal conjugate immunisation history including the valency of PCV was obtained from parents and cross-checked with the child's parent held health records. General practice surgeries were contacted to clarify doses given if there was uncertainty. Immunisation history was not collected in the pre-vaccine study.

Laboratory procedures

Blood samples were collected for serum, blood culture (BacT/ALERT®, bioMérieux, France) and pneumococcal PCR testing. Approximately four weeks later blood was collected for convalescent serology. Parents often declined returning for these convalescent samples (all in the post-vaccine study), contributing to the variability in the number of investigations performed. Nasopharyngeal secretions included aspirates (NPA) from infants, and/or swab (NPS) from older children. NPA sample was placed in 0.9% sodium chloride transport solution or swabs (Medical Wire & Equipment Co Ltd, UK). Tracheobronchial secretions (collected via endotracheal tube or bronchoalveolar lavage) and pleural fluids were tested when obtained. The nature of collected samples were standardised across all ages and in both studies. Recovery of bacterial pathogens from nasopharyngeal secretions, sputum, or by urinary pneumococcal antigen was not considered evidence of definite infection due to the risk of physiological colonisation [3, 13]. We therefore only present and discuss positive results that were classified as likely causative pathogens of pneumonia according to defined diagnostic criteria (Table 1). Any positive results from the potentially colonised above mentioned sites were added together separately and rates were grouped among "unknown causes" in Figure 1 for reader's information.

Where tests were not part of routine clinical care, samples were stored at –20°C for subsequent analysis. Investigations were performed in the Microbiology Laboratory, Newcastle upon Tyne Hospitals NHS Trust and the Health Protection Agency (HPA) Public Health Laboratory, Newcastle. Apart from locally performed routine diagnostic tests, samples from Middlesbrough were transported to Newcastle via daily transport services. Pneumococcal isolates from blood and respiratory secretions including pleural

fluids were serotyped by multiplexed immunoassay using xMAP beads for detection of serotype-specific *Streptococcus pneumoniae* antigens at the national HPA Respiratory and Systemic Infection Laboratory in London [14].

Viral laboratory diagnostic tests

Pre-vaccine, immunofluorescence antibody testing (IFAT) was applied to respiratory secretions using Chemicon SimuFluor FITC for respiratory syncytial virus (RSV), influenza A and B, parainfluenza 1-3, and adenovirus and human metapneumovirus (hMPV) was tested for using IFAT utilising an in-house pool of anti-hMPV monoclonal antibodies [15]. Viral screening was performed in the post-vaccine study using an in-house multiplex real-time PCR assay. The target panel was expanded to include pandemic influenza A subtype H1N1, parainfluenza virus 4, rhinovirus, coronavirus (229E, OC43 and NL63), and bocavirus plus the viruses previously tested for by IFAT. Viral serological tests included respiratory syncytial virus (RSV), influenza A and B virus, and adenovirus.

Bacterial laboratory diagnostic tests

Total nucleic acid was extracted from blood samples from both studies and tested using a *S. pneumoniae* specific PCR assay targeting the pneumolysin (ply) gene [16]. An acute complement fixation test (CF) antibody screen for 'atypical' bacteria and respiratory viruses was also performed which included *Mycoplasma* IgM antibody, *M. pneumoniae, C. pneumoniae and C. psittaci*, as well as, *Coxiella burnetii*, and *Legionella pneumophila* serogroup 1-6 in the post-vaccine study. Antistreptolysin O titre (ASOT) was assayed in both studies by Rheumajet ASO kit (Launch Diagnostics, UK).

Statistical analysis

Data were analysed using Epi Info^{TM} 7. Summary of the isolated pathogens was presented as frequencies and categorised as viral, bacterial or mixed viral-bacterial infections. Detection rates of pathogens are expressed as proportions of those tested, and results were compared in relation to age group. The age group classification was under/above five years as by the start of post-vaccine study; children in the under five age group should have been vaccinated with PCV. This would allow the investigation of the relative contribution of pneumococcal infection in causing pneumonia, as well as the

role of other pathogens. Where there was a common methodology for diagnosis between studies, identification rates of infections were compared using Fisher's exact test with odds ratios (ORs) and 95% confidence intervals (CIs).

A subgroup analysis within the post-vaccine study was performed on enrolled children before the PCV13 was introduced in April 2010. This is to compare the rates of infection groups in relation to the data from pre-vaccine study and all post-vaccine study (October 2009 to March 2011).

RESULTS

A total of 401 children were enrolled, 241 and 160 in the pre- and post-vaccine studies respectively. All had at least one microbiological investigation performed. There were similar demographic characteristics between the pre- and post-vaccine studies; including median age (2.5 versus 2.6 years), proportions of males (57% versus 56%) and aged under five years (75.5% versus 69%). Figure 1 summarises the aetiological and radiological classifications and Table 2 lists the results of the diagnostic tests performed. Lobar consolidation was more often present post-vaccine in 61% compared to 23% pre-vaccine ($p<0.001$). A likely causative pathogen was established in 61% of children post-vaccine, compared to 48.5% pre-vaccine (OR 0.6, 95% CI 0.41–0.92, $p=0.019$) when the results of all tests were combined.

Forty-one children were not eligible for the pneumococcal conjugate vaccination due to age criteria whilst its uptake was 94% (112/119) among eligible children (89 had PCV7, 10 PCV13 and 13 received combined doses of each with age-appropriate schedule). Of those vaccinated with PCV7 either routinely or according to the catch up programmes, 83 of them received age-appropriate doses (57 had full schedule) whereas six children had partial schedule with one dose less for their age. Among those who had PCV13, one received full schedule, one child had one dose less for age, and eight had not completed but had appropriate doses for their age.

Viral infections

Table 3 shows the number of identified pathogens with age group distribution. Viral (31%) and mixed infections (12.5%) were significantly higher post-vaccine than pre-vaccine; being respectively 19.5% ($p=0.021$) and 5% ($p=0.015$). The detection of

viruses using a combination of PCR and serological assays post-vaccine (57%, 85/149) was higher than that of testing with immunofluorescence and serology pre-vaccine (30.5%, 65/213). This improvement in viral detection was thought to be due to the application of PCR assays (44.7%) replacing immunofluorescence testing (27.9%) on respiratory secretions (p=0.003). Post-vaccine, acute viral serological assays were only positive in seven with influenza A virus infection, whereas pre-vaccine, combined acute and convalescent serology identified infections with eight each of RSV and adenovirus, four influenza A/B viruses, and one Epstein-Barr virus.

Post-vaccine, RSV was detected in 21% (31/147) of samples, of which 19 were type A, with rhinovirus (8.5%), influenza (7%) and adenoviruses (7%). These figures were comparable with those pre-vaccine for adenovirus and influenza A/B (6% each); but higher than that for RSV (15%). Of the 142 identified causative pathogens post-vaccine, 71 (50%) viruses were detected among those aged under five years, compared to the finding in pre-vaccine study (36%, 54/149). hMPV was not detected in any of the 48 tested pre-vaccine respiratory samples, but was identified in one child in the post-vaccine study.

Bacterial infections

There were no difference in the rates of bacterial infections between post-vaccine at 17.5% of the total compared to 24% pre-vaccine (p=0.258). Identified overall pneumococcal infections were not different between both studies (p=0.557). They represent 17.4% among children tested post-vaccine (14/93 [15%] and 10/44 [22.7%] in those aged under and over fives respectively). This was compared to 14.7% pre-vaccine (28/180 [15.6%] and 7/58 [12%] among those under and over fives respectively). In the post-vaccine study, diagnosis of pneumococcal infection improved when PCR was used (21/97, 21.6%) compared to culture (8/132, 6%) (p=0.0004). A serotype was identified in 75% (18/24) in the post-vaccine study. These were serotypes 1 (44.4%), 3 (27.8%), 19A (22.2%) and 7A/F (5.6%). The rate of positive blood culture post-vaccine was almost double (5.6%) that in pre-vaccine (3.2%).

Group A streptococcal infections were confirmed in higher proportion of children (10.5%) post-vaccine than the pre-vaccine (7%). These infections were associated with severe disease, and in two-thirds of them with empyema. *M. pneumoniae* was identified

from acute serology in 9.9% of children post-vaccine, with 4% (2/51) in those under five and 20% (6/30) over five years. The rate of detected mycoplasma infection was higher in pre-vaccine (12.5%) when paired acute and convalescent samples were available, with 7% (9/128) in those under five and 27% (13/48) over five years old.

Subgroup analysis (October 2009 to March 2010)

Among of 67 children enrolled during this period, the causative pathogen was identified in 37 (55%). *S. pneumoniae* was identified in 18.3% (11/60) compared to 16.7% (13/78) during the first year of PCV13 (p=0.824). Rates of infections were 22.4% bacterial, 22.4% viral and 10.5% mixed with both. The rate of bacterial infection is similar to the figures from pre- and entire post-vaccine studies. There was no difference between the rates of viral infections before and after the introduction of PCV13 during the post-vaccine study (p=0.079).

DISCUSSION

This is the first published study to describe the aetiology of CAP in UK children prior to and following the introduction of the PCV7. The timing of this comprehensive study three years after the introduction of PCV7 and during the first year of PCV13 provides a baseline for future comparative studies of the pneumonia aetiology in the same setting. The causative pathogens identified were predominately viruses in both studies with the detection of pneumococcal infections increasing from pre-to post-vaccine studies presumably as a consequence of the application of molecular diagnostic methods.

Previous UK studies investigating the aetiology of pneumonia prior to the introduction of the PCV7 were able to identify the causative pathogens in up to 54% of children [17-19]. In addition to the blood culture, one tested blood for *S. pneumoniae*, *Mycoplasma* and *Chlamydophila* using PCR and identified 8% of children with pneumococcal pneumonia [18]. Another study identified 6% pneumococcal infection using pneumolysin ELISA on blood [19]. The identification rate of 61% for the likely causative pathogens in the post-vaccine study is similar to that reported by Don and colleagues who used serological assays [20]. However, this is lower than detection rates around 80% from studies which used serological and/or molecular approaches [21-23]. But this is higher than the rates previously found prior to the introduction of the

conjugate vaccine [24, 25]. The improved detection rate between our two studies appears to be related to the different laboratory approaches used.

Whilst molecular diagnostic methods have improved respiratory virus detection and bacterial detection from normally sterile sites, the interpretation of results can be more problematic when it is applied to nasopharyngeal secretions and other respiratory samples [3, 13]. Additionally, pneumococcal pneumolysin (*ply*) DNA can be detected in the blood of healthy children colonized with pneumococcus [26]. The pneumolysin gene can also be detected in non-pneumococcal Viridans-group streptococci, particularly *S. pseudopneumoniae* and *S. mitis* [5]. Potential confounders with using a pneumolysin PCR in this study therefore include false positives associated with pneumococcal carriage or cross-reactivity with other Viridans-group streptococci. Given the very low pneumococcal carriage rate in this population (7%) the former is unlikely. Although not well explored, viral carriage is also a clinical possibility, leading to positive PCR results which do not necessarily correlate to the observed pneumonia [27]. Hence the results of PCR-based approaches can be limited in making a definite diagnosis of causative pathogens in pneumonia.

The rates of pneumococcal infection from the two data sets are lower than studies in other countries [3], but are higher than previously described in the UK [18, 19]. Improvement of pneumococcal identification with the application of PCR when compared to culture alone is consistent with previous studies [9, 28-30]. This is similar to the reported increase in a recent Italian study (from 3.8% to 15.4%) [8]. It is interesting that despite the overall decrease in the incidence and hospitalisation of pneumonia since the introduction of PCV7 [31, 32], the rates of pneumococcal infection were comparable between the two studies. Replacement with non-PCV7 serotypes causing invasive pneumococcal disease is well recognised [33], and this may explain our findings in the face of reduction in disease incidence. Where pneumococcal serotyping was possible with the majority being identified from pleural fluids, all serotypes recovered were non-PCV7 but covered in PCV13. This is similar to data from the USA on children with empyema where 98% were non-PCV7 serotypes and primarily similar to the serotypes in our study [34]. Despite the lack of comprehensive serotype data, this suggests that PCV13 could substantially reduce invasive pneumococcal disease [35, 36].

Serological evidence of *Mycoplasma* infection was detected in 9.9% and 12.5% of children in both studies, rates that are similar to the published literature [19, 25]. *M. pneumoniae* is traditionally considered a pathogen of older children and in these studies was identified more frequently in those over five years of age. We identified no other serological evidence of other 'atypical' organisms although this may have been as a consequence of the lack of convalescent sera. *S. aureus* and GAS infections were often associated with severe pneumonia and empyema [37, 38]. In keeping with previous findings, GAS can be found in up to 7% of children with pneumonia compared to 7% and 10.5% in our two data sets [19, 39]. With the introduction of PCV and decrease in pneumococcal pneumonia it is possible that the relative proportion of bacteria such as GAS and *S. aureus* as well as *M. pneumoniae* to cause severe pneumonia will increase.

Viruses either alone or as co-pathogens were detected in 25% and 43% of children in the pre- and post-vaccination studies respectively, with RSV being the most commonly detected pathogen as previously reported [3, 40]. This was followed by rhinovirus, influenza and adenovirus at approximately 7% each, similar to data previously described for the same region [19]. Diagnosis of viral infection was achieved mainly through the testing of respiratory secretions rather than by serology which was only positive in seven children with influenza A virus. The improvement in detection of viruses in the post-vaccine study was mainly achieved by the application PCR assays for respiratory viral screening. Most of the viruses detected were identified in those aged under five years, consistent with other studies [2, 23]. In the post-vaccine study, viral screening was expanded to include eight viruses with their subgroups including pandemic H1N1 and to delineate their contribution in causing CAP in UK children. Considering the timing of the second recruitment period, pandemic influenza A H1N1 was not implicated in many cases of pneumonia as a single pathogen. The low isolation rates of bocavirus, coronavirus and hMPV highlight the minimal contribution of these viruses in the aetiology of pneumonia in UK children. The rates of mixed viral-bacterial infection were variable between the two studies and likely to be dependent on the screening methods used to identify the causative pathogens [3].

There are several limitations to our data, such as potential seasonal bias to the data of post-vaccine study where the recruitment was carried out over 18 months which

included two winter seasons (48% of enrolled children). Although the post-vaccine study covered two winter seasons, enrolled children were fewer than the pre-vaccine study which could be a true reflection of decreased disease incidence and hospitalisation. The findings from the post-vaccine study may have been hampered by the lack of convalescent sera which may have led to the underestimation of the role of atypical bacteria in paediatric pneumonia, but this effect is probably minimal as mycoplasma infection was only detected in three children by paired serum samples pre-vaccine. Lack of serotype data of the identified pneumococci from the pre-vaccine study limits the true comparison with the serotype profile after the conjugate vaccine implementation. Another limitation is the variation between the two studies in the diagnostic methods used and the pathogens investigated which makes the interpretation of comparative findings guarded. The significant improvement in the identification by the application of more PCR assays adds further evidence on the importance of using these techniques to monitor changes in the epidemiology of PCAP and pneumococcal serotype replacement.

In conclusion, although viruses are the most common cause of pneumonia, around one fifth of children had bacterial infections. The combined use of culture, serology and PCR-based diagnostic tests significantly improved the identification of causative pathogens in PCAP. Replacement of PCV7 with PCV13 was likely to be associated a significant reduction in pneumococcal disease as non-PCV7 but PCV13 serotypes predominated. This requires continued surveillance to monitor for the emergence of serotype replacement.

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TABLE 1 Laboratory investigations and diagnostic criteria

		Tests*		Diagnosis of causative pathogens
Sample	**Pathogen/antigen**	**2001-02 study**	**2009-11 study**	**Definite/probable**
Serum	Respiratory viruses	Complement fixation	Complement fixation	Acute titre ≥1/128 or 4-fold rise between paired sera
	Atypical bacteria			
	Mycoplasma	IgM antibody	IgM antibody	Positive
	Group A *Streptococcus*	ASOT (IU/mL)	ASOT (IU/mL)	Acute 2-fold rise or 4-fold rise between paired sera
Blood	Bacteria	Culture	Culture	Growth
	S. pneumoniae	Real-time PCR	Real-time PCR	Positive
Nasopharyngeal secretions/sputum	Respiratory viruses	IFAT	Real-time PCR	Positive
	Bacteria	Culture	Culture/real-time PCR	Not applicable
Tracheobronchial secretions (bronchoalveolar lavage/endotracheal)	Respiratory viruses	IFAT	Real-time PCR	Positive
	Bacteria	Culture	Culture/real-time PCR	Growth/Positive
Pleural fluids	Bacteria	Culture	Culture	Growth
	Pneumococcal antigen	ELISA	ELISA	Positive
	S. pneumoniae	Not tested	Real-time PCR	Positive

*ASOT, antistreptolysin O titre; IFAT, immunofluorescence antibody testing; ELISA, enzyme-linked immunosorbent assay.

TABLE 2 Results of the diagnostic tests performed

	2001-02 study (n=241)		2009-11 study (n=160)	
Tests	**Tests, n**	**Positive, n (%)**	**Tests, n**	**Positive, n (%)**
Blood and serology	238	75 (31.5)	138	32 (23.2)
Blood, overall	***236***	***36 (15.3)***	***136***	***13 (9.6)***
Bacterial culture	185	6 (3.2)	126	7 (5.6)
S. pneumonia PCR	228	30 (13.2)	86	7 (8.1)
Serology, overall	***181***	***49 (27.0)***	***105***	***22 (21.0)***
Acute serology				
Mycoplasma IgM antibody	34	11 (32.4)	77	8 (10.4)
ASOT	158	12 (7.6)	80	9 (11.3)
Mycoplasma/Chlamydia	128	8 (6.3) / 0	51 / 39	0
Legionella/Q-fever	0	0	50 / 42	0
Influenza-A/B	158	1 (0.6) / 2 (1.2)	68 / 62	7 (10.3) / 0
RSV*/Adenovirus	158	2 (1.2) / 2 (1.2)	52 / 46	0
Convalescent serology				
ASOT	52	2 (3.8)	0	0
Mycoplasma/Chlamydia	14	3 (21.4) / 0	0	0
Influenza A/B	101	1 (1.0) / 0	0	0
RSV/Adenovirus	101	6 (6.0) / 6 (6.0)	0	0
Respiratory secretions, overall	***175***	***59 (33.7)***	***151***	***121 (80.1)***
Viral screen	158	44 (27.9)	141	63 (44.7)
Bacterial culture (TBS)†	14	5 (35.7)	12	7 (58.3)
Pleural fluids, overall	***17***	***4 (23.5)***	***40***	***27 (67.5)***
Bacterial culture	17	2 (11.8)	40	10 (25.0)
Pneumococcal antigen	17	2 (11.8)	30	7 (23.3)
Pneumococcal Real-time PCR	0	0	30	18 (60.0)

*RSV, respiratory syncytial virus; †TBS, tracheobronchial secretions.

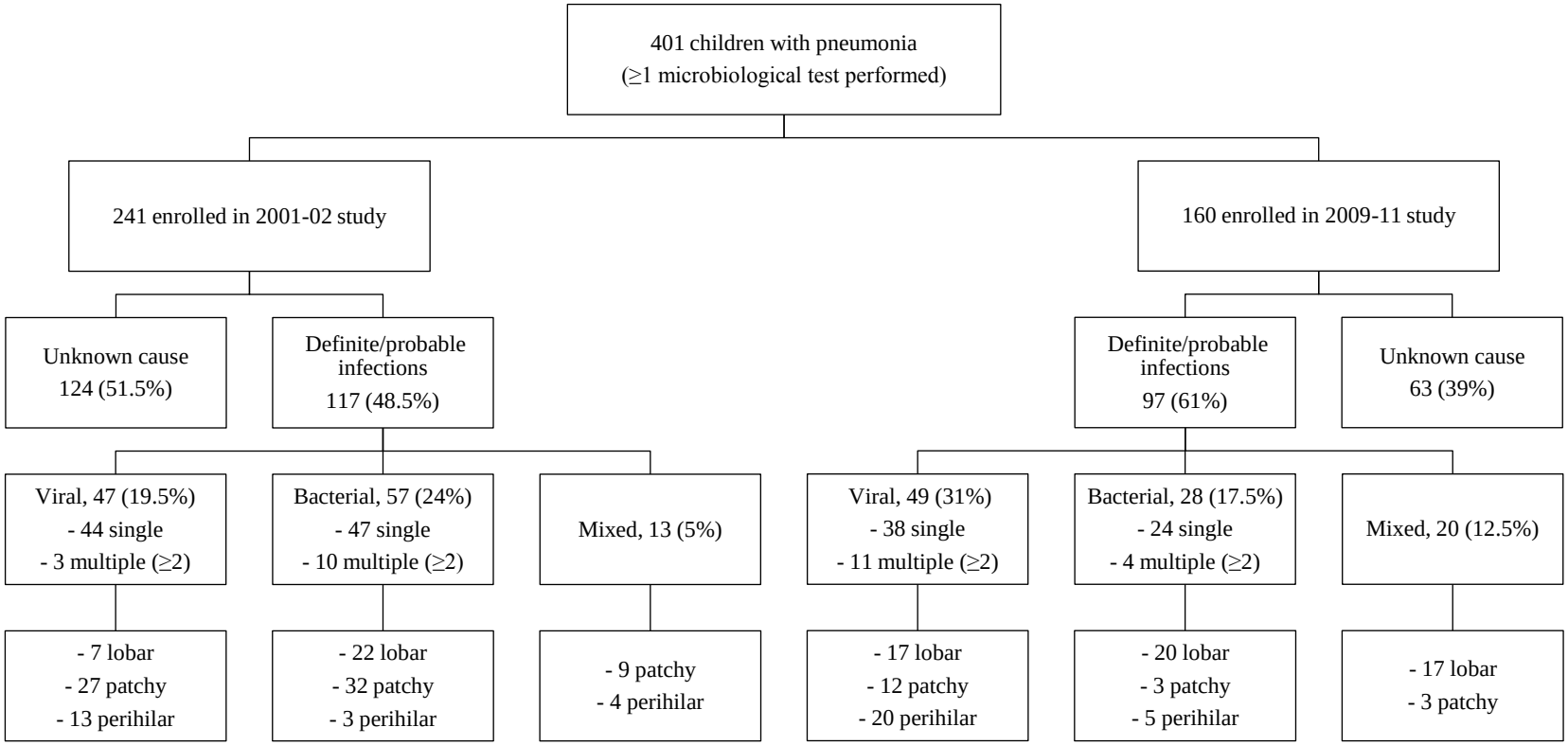
TABLE 3 Detected likely causative pathogens by age group

	2001-02 study			2009-11 study		
Pathogens	**< 5 y**	**5–16 y**	**n/N* (%)**	**< 5 y**	**5–16 y**	**n/N (%)**
Bacterial						
S. pneumoniae	28	7	35/238 (14.7)	14	10	24/138 (17.4)
M. pneumoniae	9	13	22/176 (12.5)	2	6	8/81 (9.9)
Group A *Streptococcus*	5	9	14/202 (7.0)	6	8	14/133 (10.5)
S. aureus	3	2	5/189 (2.6)	1	2	3/130 (2.3)
H. influenzae	0	2	2/189 (1.0)	3	0	3/130 (2.3)
Bordetella pertussis	1	0	1/189 (0.5)	0	0	0
M. catarrhalis	1	0	1/189 (0.5)	2	1	3/130 (2.3)
S. intermedius	1	0	1/189 (0.5)	0	1	1/130 (0.8)
Alpha-haemolytic *Streptococcus*	1	0	1/189 (0.5)	0	0	0
K. pneumoniae	0	0	0	1	0	1/130 (0.8)
Viral						
RSV (not typed)	29	3	32/213 (15.0)	0	0	0
RSV type A	0	0	0	19	0	19/147 (13.0)
RSV type B	0	0	0	11	1	12/147 (8.2)
Influenza A and B viruses	9	4	13/213 (6.0)	7	4	11/149 (7.4)
Adenovirus	11	2	13/213 (6.0)	10	0	10/145 (6.9)
Parainfluenza 1-4	5	0	5/158 (3.2)	5	1	6/141 (4.3)
Human metapneumovirus (hMPV)	0	0	0/48	1	0	1/141 (0.7)
Epstein-Barr virus	0	1	1/1 (100)	Not tested	Not tested	–
Varicella zoster virus	0	1	1/1 (100)	Not tested	Not tested	–
Rhinovirus	Not tested	Not tested	–	10	2	12/141 (8.5)
Pandemic influenza A H1N1	Not tested	Not tested	–	4	3	7/141 (5.0)
Bocavirus	Not tested	Not tested	–	2	2	4/121 (3.3)
Coronavirus (type OC43)	Not tested	Not tested	–	2	1	3/121 (2.5)
Total	103	46	149	100	42	142

*N, total number of performed tests that their positive results classified as definite/probable infections.

Figure Legend

FIGURE 1 Summary of the aetiological and radiological classifications


401 children with pneumonia
(≥1 microbiological test performed)
241 enrolled in 2001-02 study
160 enrolled in 2009-11 study
Unknown cause
124 (51.5%)
Definite/probable infections
117 (48.5%)
Definite/probable infections
97 (61%)
Unknown cause
63 (39%)
Viral, 47 (19.5%)
- 44 single
- 3 multiple (≥2)
Bacterial, 57 (24%)
- 47 single
- 10 multiple (≥2)
Mixed, 13 (5%)
Viral, 49 (31%)
- 38 single
- 11 multiple (≥2)
Bacterial, 28 (17.5%)
- 24 single
- 4 multiple (≥2)
Mixed, 20 (12.5%)
- 7 lobar
- 27 patchy
- 13 perihilar
- 22 lobar
- 32 patchy
- 3 perihilar
- 9 patchy
- 4 perihilar
- 17 lobar
- 12 patchy
- 20 perihilar
- 20 lobar
- 3 patchy
- 5 perihilar
- 17 lobar
- 3 patchy

Barriers and facilitators to parental decisions on consenting children to participate in clinical research

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Position titles:
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Statement of contribution

JEC and DAS developed the ideas of pneumonia and empyema studies respectively. JEC suggested examining issues surrounding consent within these studies. MAE, KP, MFT and CS collected and managed the data. All authors were involved in the interpretation of the results and the writing of this manuscript.

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Conflicts of interest

The sponsors had no role in the studies' design, and data collection, analysis or interpretation. JEC and DAS received unconditional research grants from Pfizer and GSK for DAS. JEC and DAS were the chief investigators respectively for the pneumonia and empyema studies.

Abstract

An important challenge when undertaking clinical research in children is the consent procedure. Understanding the factors influencing this process is vital in improving children's involvement in research. We report observed rates and reasons for recruitment refusal in two studies of childhood pneumonia and empyema, and describe the potential underlying factors contributing to refusal or recruitment facilitation. Each study team included a research nurse and medical registrar. Severity of child's illness appeared to determine parent's decision regarding participation in clinical research. We found that willingness of research teams to provide parents with adequate study information as well as the liaison of research team members with nursing and admitting medical staffs about suitable time to approach families were effective for successful recruitment.

Introduction

It is recognised that children are under-represented in clinical research [1]. This has impacted on the evidence base available for the management of sick children (Medical Research Council [2]. The barriers for research in children are well documented [1, 3]. One such barrier is the problem of obtaining informed consent (National Research Ethics Service [4]. Assessing a child's ability to understand and make decisions about participation in research is a challenge and varies between individuals [2]. It is a legal requirement that in young children, parents or guardians are asked to provide consent on their behalf before they can be enrolled in studies [2, 5, 6]. International good clinical practice guidelines provide unified standards in research governance that governments can adopt to protect human rights of participants and also to define the roles of funders, investigators and monitors (International Conference on Harmonisation [7].

In a previous study of British parents consenting to an interventional trial, the major reasons for participation given were benefit to other children in the future, contribution to science and benefit to their own child [8, 9]. Conversely, parents may feel an obligation to protect their children from potential harm or painful procedures [3, 8]. These factors are likely to influence the way in which parents rank the risks and potential benefits for their child, before making decisions regarding their child's enrolment [3, 8, 10, 11]. Given the urgent need to increase the involvement of children in clinical research, greater understanding of the process of consent is vital to address potential knowledge, attitudinal and psychosocial barriers to this procedure [11-14].

There is a considerable body of literature about children's consent to participation in research, particularly clinical trials [9, 11, 14-16]. However, most studies address the recruitment outcomes with minimal emphasis on strategies to improve the recruitment process [17]. Evidence from pooled review data showed that barriers to participation in research such as time constraints and demand of additional study procedures are related to both participants and researchers [15]. Approaches such as taking time to establish rapport with children and their parents, short consent forms, the presence of the research team to discuss the study with families and giving them the opportunity to request further information were found to improve recruitment rates [17]. Several other factors described in the literature appear important; trained research staff can significantly increase success in obtaining consent [18, 19] and complex information sheets and difficulties in understanding the process can be significant barriers to consent [8].

We examined data from two studies outside vaccine trials investigating childhood pneumonia and empyema both conducted in the same setting, in order to describe the potential reasons for consent refusal. The aim was also to report our experience on approaches that facilitated the recruitment procedures.

Methods

Study procedures

Data were collected prospectively from two studies investigating the impact of the routine introduction of pneumococcal conjugate vaccination programme in 2006 on the aetiology and epidemiology of childhood community-acquired pneumonia and empyema [20]. These studies were undertaken at the Newcastle upon Tyne Hospitals NHS Foundation Trust between April 2010 and March 2011. Informed consent was voluntarily obtained from a parent or guardian (person or authority who holds parental responsibility) [1, 2, 6, 7, 21]. If the consent was refused within that period then the following information was documented: reason for refusal if an explanation was offered (although parents were not asked to express their reasoning); who approached parents for consent; and the severity of pneumonia based on the national guidelines for the management of childhood pneumonia [22]. In line with this guidance all children with empyema were regarded as severe.

The aims of these studies were to investigate the effect of a pneumococcal conjugate vaccination programme on the aetiology of childhood pneumonia, and the changing epidemiology of childhood empyema. Children were eligible for the pneumonia study if aged between 0–16 years and seen in hospital with clinical and radiological features of pneumonia. Similarly, children were eligible for the empyema study if had clinical and radiological diagnosis of empyema and underwent pleural drainage.

A favourable ethical opinion by the Newcastle and North Tyneside Research Ethics Committee was obtained for the pneumonia study and by the Sunderland Research Ethics Committee for the empyema study. Approval was not granted to ask parents about the refusal, hence they were not asked directly and systematically about declining the enrolment.

Recruitment process

During the time of hospital admission, usually after the diagnosis had been confirmed, a member of the research team would visit the ward. This was either the research nurse (KP) or research registrar (MAE) for pneumonia study, or the research nurse (CS) or research registrar (MFT) for empyema study. Written information on the pneumonia study consisted of a four-page sheet for parents and a separate two-page information document for children under and those over ten years of age. While for empyema study consisted of a three-page parent information sheet and a three-page age appropriate information document for the child.

Before approaching the parents and child, liaison with medical and nursing staff helped to establish if it was a suitable time to speak to the family about the study. Consenting for the empyema study was generally delayed until the invasive procedures had been carried out. As all children with empyema were eligible for both studies, efforts were made to co-ordinate visits between the two separate research teams, to avoid multiple requests to families. Study information was then presented both verbally and in written format to the parents and unless the parents specifically requested to give consent immediately, a suitable time was established to return and complete the consent process if they wished to join the study. The verbal explanation took up to ten minutes, after which the parents were offered a chance to ask questions regarding the given information. Consents were obtained at the child's bedside. Where it was age appropriate, children were involved in the consent process and they could themselves accept or decline the study.

If written consent was obtained then children with pneumonia had a standard proforma completed for epidemiological, clinical and management characteristics, together with samples of nasopharyngeal secretions, urine and blood. Three questions regarding medical history were asked on empyema patients and a saliva sample obtained either by expectoration or by use of soft-tipped oropharyngeal saliva swabs for those unable to expectorate.

Results

A total of 116 children were eligible for enrolment to the pneumonia study and 28 were also eligible for the empyema study giving a total of 144 consent procedures. Ten (7%) consents were refused, 8 of them had severe pneumonia or empyema. Of 10 refusals,

five were seen by a research nurses and the remaining by a research registrars (Table 1). Of those with severe disease, consent refusals were 5% and 11% for pneumonia and empyema studies respectively. Refusal was linked to not wanting the child to undergo further tests, research delaying discharge, and anxiety regarding written consent and length of information sheets. It was also observed that if the child and family were approached prior to surgery then they would be reluctant to discuss their involvement in research at that time. Approximately two days postoperatively when the child became clinically stable was the optimum time to discuss the study and consent.

Often parents declined the option to read the information and felt able to give consent based on the verbal information offered together with discussion to answer questions that they might have. None asked for more information on either of the studies. Parents commented on the length of the information sheets as being long and complicated. Other reasons for parental refusals were related to lack of interest in participating in research or preventing their children from extra study procedures. Children were difficult to engage during the recruitment phase. Few were interested in the verbal explanation of the study and although age appropriate information was always offered not many looked at or read them.

Discussion

This paper reports consent refusal rates from two clinical studies, and describes the potential reasons contributing to the refusal. Although the acceptance rates for both studies were high, severity of a child's illness appears to influence parental decision on enrolment in clinical research. The high number of enrolled participants suggests effective recruitment strategies. We found that willingness of research teams to provide parents with adequate study information as well as the liaison of research team members with nursing and admitting medical staffs about suitable time to approach families were effective for this successful recruitment.

Informed consent and assent

The main elements of informed consent include adequate information, freedom of decision and capacity to understand [3, 6, 7, 23]. Informed consent must be sought from parents or legal guardians before enrolling children in research in the UK according to the research guidelines from the Medical Research Council [2, 7, 21] and the European Ethics Working Group in Paediatrics [24]. This happens after both written information

sheets and verbal explanation are provided. Our findings suggest that there is a balance between providing adequate information and over-lengthy information sheets which can often appear time-consuming and intrusive. In fact one parent after reading the information sheet felt that it was too long and declined the study despite earlier acceptance upon verbal explanation of the study. We initially created a one-page information sheet but the Ethics Committee advised provision of comprehensive information covering essential facts about the studies. Essential information required within consent for research is governed by a legal and statuary framework, thus any changes to this can only be made within this framework [7].

Assent is a concept that allows the participation of older children in the recruitment decision, which in turn encourages a sense of ownership and is defined as "positive agreement" [25, 26]. In the UK, assent is advocated if the researcher feels that the child is able to make a decision about participation in study [2]. It refers to "acquiescence and affirmative agreement to participate" as defined by the Royal College of Paediatric and Child Health and Medical Research Council respectively [1, 2]. Involving children in the initial discussion of consent process is important as children feel respected [27-30] and researchers should recognize that they are developing autonomous decision making capacity [31]. In practice, experience has highlighted that it is difficult to engage with children despite researchers' explanations [32] and there are concerns that the distinction of consent and assent is confusing [33]. As in our study, children were often observed not to be interested in verbal explanation or written information [34, 35]. They frequently referred to their parents to sign the consent on their behalf. Hence in older competent children beside the assent form if provided, consent was also obtained from their parents. This is unsurprising in the context of tired and ill children who may also be socially wary of new people [17].

Taking into account how many families were approached by each member of the team, the results showed the individual refusal rate of consent did not vary between different team members, suggesting that the role of the team member who obtained consent did not determine the likelihood of agreement to participation. Consent refusal was also not related to the role of the person seeking consent when research nurses were compared with medical registrars despite a variable knowledge and skills, a finding supported in a recent study [36]. Hence, our data supports the increasing role of nurses in the field of research. The research nurses (KP and CS) started their research careers with these

pneumonia and empyema studies respectively. Joining medical teams during the ward round following admission and having the research team introduced by them to the families was found to be helpful as we did not observe any refusal in such situations. This may show that both admitting and research staffs appear as a single team. Furthermore coordination with the ward-based non-research nursing staff was useful by building bridges between families and research teams as well as supporting the research teams to collect study samples. These overall factors facilitated the achievement of high enrolment rates to both studies.

Timing and environment

When a child is admitted to hospital with an illness and consent is sought soon after diagnosis, parents will be making decisions when they are stressed and vulnerable, whilst simultaneously trying to comfort their child [37]. The pneumonia and empyema research teams used approaches in accordance with the guidance on how to seek consent provided by the Royal College of Paediatrics and Child Health [1]. We provided parents with the study information sheets either before or at the same time of interview for consent. If at the initial meeting with parents it was felt to be an inappropriate time due to previous events, the child's condition or parental anxiety then information sheets were left with the parents and an appointment made to come back at a later time. It did not appear that more of these parents read the information than those given the information at time of interview. Parents reported not reading the information leaflets and the information provided did not appear to influence the questions that were asked by them. This would reflect the trust in person obtaining consent as previously reported [38]. Due to the nature of acute illness, when the admission period is often minimal, obtaining informed consent usually occurs during one meeting.

Ideally informed consent should be presented in a relaxed and non-coercive environment [2]. The environment in which we delivered study information and obtained consent was often at the child's bedside. This allowed the parent to stay with their child and the child to continue with their activity should they not want to listen to the researcher. Confidentiality may be a consideration when the child is on a bay with other children and families. From our experience on children with empyema or severe pneumonia timing is equally important as the environment chosen when gaining consent, highlighting the challenges when seeking consent for severely unwell children [39]. We found that if the child and family were approached prior to decortication

surgery then they would be reluctant to discuss their involvement in research at that time. At this time parents would often be distressed and vulnerable whilst simultaneously trying to comfort their sick child. Postoperatively when the child was less critical appeared to be the optimum time to gain consent and related specimens. It was interesting from the documented seven children of refusal that five of them had severe pneumonia. It could be argued therefore that parental anxiety and the parental role to protect their child from further harm was heightened, resulting in refusal.

There are limitations of the reported findings such as parents in this study were not directly asked why they did not want to participate. This could affect the conclusions on factors influencing consent as these may not have been uncovered. The research team reported the parental response and their personal experience which are subject to self report bias. These limitations highlight the tensions and barriers involved in trying to improve the consent process [11].

In conclusion, reasons for refusal of enrolment of children into research are complex, influenced by illness severity and study procedures. Consent forms should be simple for easy understanding. Our findings and observations suggest that increasing the research awareness within the department and involvement of non-research nursing and medical staffs are important elements for improvement of the recruitment outcomes in clinical research. Therefore this warrants a systematic evaluation of the role of non-research departmental staff in recruitment procedures.

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Table 1 Summary of consent refusals

Staff obtaining consent	**Refusals (%)**
Research Nurses	5/68 (7.3%)
Pneumonia (KP)	4/52 (7.7%)
Empyema (CS)	1/16 (6.2%)
Research Registrars	5/76 (6.6%)
Pneumonia (MAE)	3/64 (4.7%)
Empyema (MFT)	2/12 (16.7%)

Variability in the interpretation of chest radiographs for the diagnosis of paediatric pneumonia in epidemiological studies

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AUTHORSHIP

JEC and DAS developed the original study concept. MAE collected and managed the data. MM reviewed all chest radiographs. All authors were involved in the interpretation of the results and writing of this paper.

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DECLARATION OF INTEREST

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SUMMARY

In epidemiological studies, chest radiograph remains a major criterion for the classification of pneumonia in children and yet considerable variation in its interpretation is still happening. We aimed to report inter-observer variability in the interpretation of 169 chest radiographs in children suspected of having pneumonia. An 18-month prospective study was undertaken at two centres in Northern England. Chest radiographs were performed on eligible children aged ≤16 years with clinical features of pneumonia. The initial radiology report was compared with a subsequent assessment by a consultant cardiothoracic radiologist. Chest radiographic changes were categorised according to the World Health Organization (WHO) criteria. There was significant disagreement (22%) between the first and second reports (kappa=0.70, $P<0.001$), notably in those aged <5 years (26%, kappa=0.66, $P<0.001$). The most frequent sources of disagreement were the reporting of patchy and perihilar changes. The levels of disagreement between the two interpretations varied when the first reports were performed by consultant general, paediatric or cardiothoracic radiologists. Pleural effusion was present in first reading in 10% compared to 22% on review of these films. Variation in reporting of effusion was 11.8% (kappa=0.57, $P<0.001$). In conclusion, there is substantial inter-observer variability without apparent link to the level of training and experience. This highlights the need for experts from different countries to create a consensus to review and improve the wider applicability of the WHO radiological classification criteria which form the basis for recruitment in epidemiological studies of pneumonia in children.

INTRODUCTION

Chest radiograph is frequently performed when managing pneumonia in children [1], but usually does not affect the clinical outcome [2]. In epidemiological studies, the chest radiograph remains a major criterion in classification of pneumonia [3, 4]. Variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children is a recognised problem [5]. It has been suggested that if all radiologists followed the standardised World Health Organization (WHO) radiological criteria for classifying pneumonia [3], this would allow more accurate comparative data in epidemiological studies for assessment of the impact of pneumococcal vaccination [4]. Broadly four categories are defined: "End-point consolidation", "Other (non end-point) infiltrate", "Pleural effusion" and "No pneumonia".

We conducted a study to explore the effect of the implementation of pneumococcal conjugate vaccine on the aetiology of childhood community-acquired pneumonia. Radiological findings were part of the study entry criteria. The aim of this analysis was to characterise inter-observer variability in the interpretation of chest radiographs for the diagnosis of pneumonia in children according to the WHO radiological classification [3].

METHODS

Study design and participants

A prospective study to investigate the aetiology of pneumonia in children was undertaken from October 2009 to March 2011 in two teaching hospitals in North of England. Caldicott approval was granted and the study was ethically approved by the Newcastle and North Tyneside Research Ethics Committee (No: 08/H0906/105), and the Research Approval Board at South Tees Hospitals NHS Trust (No: 2008075).

Children aged ≤16 years who presented to paediatric services with signs and symptoms suggestive of lower respiratory tract infection including any of fever, tachypnoea, dyspnoea, cough, respiratory distress and auscultatory chest crackles, with chest radiographic findings consistent with pneumonia as determined initially by the admitting paediatrician were enrolled. Paediatricians were not asked to give specific radiological interpretations which were provided by radiologists. As this study was on the community-acquired pneumonia (CAP) aetiology, exclusions included being resident outside of North East England, clinical bronchiolitis, or hospitalization in the

preceding three weeks. Children with recent hospitalization were excluded in order to eliminate the potential risk of having hospital rather than community-acquired pneumonia. Children with underlying chronic chest diseases (such as cystic fibrosis) were also excluded to avoid any ambiguity in the interpretation of acute and chronic changes on chest radiographs. Research teams of doctors and nurses led and ascertained the standardised diagnosis of pneumonia and the recruitment process across the two sites. All enrolled children irrespective of the chest radiographic findings received treatment for pneumonia according to the management guidelines from the British Thoracic Society [6].

Radiology

All chest radiographs were anteroposterior views and first reported by radiologists locally as per routine clinical care and viewed electronically via the Picture Archiving and Communications System (PACS). There were uniform and regular quality assessments performed on the system performance including display characteristics. All reporters used similar workstations of radiological standards when reporting the chest radiographs. Using the full text written first reports, each radiograph was categorised into lobar (end-point consolidation), patchy, perihilar (non end-point consolidation/infiltrate) or normal (no pneumonia) according to the WHO criteria [3, 4]. Effusion with fluid in the pleural space between the lung and chest wall was considered as primary end-point and classified simply as either present or absent [4]. This does not include fluid in the horizontal or oblique fissures. First reports were generated with the benefit of clinical information, a standard institutional requirement for routine reporting. All radiographs were reviewed by a second consultant cardiothoracic radiologist (MM) at the regional centre (designated as the "gold standard") who was blinded to both the first report and specific clinical data. However, this radiologist knew the radiographs were from a child enrolled in the CAP study, thus clinically pneumonia had been suspected. Radiologists involved in performing the first and second reporting received the same training in radiology including the classification of radiological pneumonia. Those involved in first reporting included five radiology trainees, three consultants general, two paediatric and two cardiothoracic radiologists.

A workshop including MAE, MM, DAS and JEC was carried out before the application of WHO criteria [3] on the first reports and performing the second reading in order to discuss and refine the potential definitions which could be a source of disagreement

such as interstitial infiltrates of patchy or perihilar changes. The study team agreed that if more than one radiographic change were reported, then in line with WHO recommendations the most significant one is reported [3]. The WHO criteria were prioritised according to the clinical significance, as follows: lobar (end-point consolidation) in favour of other changes (non-end-point infiltrates) if both were present [3]. When more than one radiographic change was reported then the radiograph was classified overall according to the most significant category. Grouping of the first reports was carried out by MAE and there was no ambiguity on the wording of first reports that might cause confusion on categorization. Inter-observer variability in the interpretation of chest radiographs was measured by the comparison of first reports with their second reading. The intra-observer variation was not calculated because all radiologists read the radiographs only once.

Statistical analysis

Data analysis was performed using the PASW Statistics 19 program. The significance of inter-observer variability was assessed using fisher's exact test because there were small values <5 in the tables. Cohen's kappa index (*k*) was calculated to measure the agreement between the first and second readers above that which would be expected by chance.

RESULTS

A total of 169 children were identified and treated for pneumonia and/or empyema (53% males, 73% aged <5 years, mean age 3.8±3.72 years, and age range from 0.05 to 16.7 years). Of those, 46 had chest radiograph reported as normal on the first reports, but on the second reading six (13%) had abnormal changes (i.e. false negative); four lobar and two patchy. All of the false negative cases received antibiotic treatment (median, 7 days), and none developed any complication. Fourteen (11.4%) were initially reported as having radiological changes, were reported as normal radiographs on the second review (i.e. false positive) (Table 1).

All radiologists agreed that all chest radiographs were suitable for interpretation. There was significant inter-observer variability in the interpretation of chest radiographs (k=0.70, P<0.001), with patchy (48.8%) and perihilar (28.1%) changes being the main components of this variability (Table 1). Although few (n=5) were first reported by radiology trainees, there was no difference in reporting when these were reported by the

second radiologist. The two interpretations varied when the first reports were performed by senior radiologists, particularly consultant pediatric radiologists who had an overall 26.7% disagreement with the reviewing cardiothoracic radiologist and lowest (15.8%) with consultant thoracic radiologists (Table 2). Levels of disagreement were highest among children aged <5 years compared to those aged ≥5 years (26%, k=0.66 versus 11%, k=0.83, P<0.001). There was no disagreement on reporting lobar findings in the <5 years age group, disagreement was mainly related to patchy and perihilar changes.

Pleural effusion was present at first reading of the films in 10% (17/169) compared to 22% (37/169) on review. Variation in reporting of pleural effusion was 11.8% (k=0.57, P<0.001). However, if the presence of a pleural effusion was reported in the first report there was no disagreement about this in the second report. In contrast 13.2% of pleural effusions were reported only on the second report and not in the first report. Initial reporting of pleural effusion by radiology trainees was not different to reports at second reading (k=1, P=0.200). In addition there was good agreement between first and second reports of pleural effusion when initially read by consultant thoracic radiologists (k=0.17, P=0.368). Whilst there were significant differences in first reporting of effusion by consultant paediatric radiologists (k=0.78, P<0.001) or consultant general radiologists (k=0.41, P=0.002) compared to second reading, the proportions of disagreement were respectively low of 5.8% and 15.3%.

DISCUSSION

We found substantial inter-observer variability in the interpretation of chest radiographs for the diagnosis of paediatric pneumonia. This has been recognized since radiology reporting was initiated in the middle of last century [7, 8], and continues despite the acceptance of the recommended WHO criteria for reporting chest radiographs of pneumonia in children [3, 4].

The diagnosis of pneumonia in children based on a combination of clinical and radiological features is important for prompt management [9]. Yet, subtle radiographic changes can be difficult to recognise or interpret [10] and failure to diagnose pneumonia may result in inappropriate management [11]. The initial interpretation of chest radiographs is usually performed by clinicians with the radiologists' reports following later, often after the patient has been discharged from hospital [11]. Interpretation by clinicians could be biased by inadequate training in radiology and lack of clinical

information may limit the accuracy of reporting by the radiologists [12]. For research purposes blinded interpretation of the chest radiograph may improve detection of subtle changes and differentiating normal biological variants [13]. Making clinical information available may reduce inter-observer variability but does not result in marked improvement in the overall accuracy [14].

This study shows that most inter-observer variability is related to the interpretation of patchy and perihilar changes, which need careful viewing and the availability of clinical information during interpretation [15]. It is well recognised that abnormal chest radiographs may be interpreted as normal [15], but surprisingly four of the normal reports had lobar changes on review. Similarly, 13% had a previously undetected pleural effusion. The variation in reporting of chest radiographs for those aged <5 years confirms the particular challenge of making a radiological diagnosis of pneumonia in this age group [10, 16]. The overall inter-observer variation is in line with other previously reported findings on interpretation variability including pleural effusion [5, 16]. It is widely accepted in the literature that chest radiographs cannot reliably differentiate viral from bacterial aetiology of pneumonia [6, 17]. Therefore these variations on the interpretation of chest radiographs do not significantly affect the clinical outcomes and management decisions of pneumonia in children [2, 6, 17-19].

It is interesting that irrespective of the level of experience there continues to be significant variability in interpretation between reporters, particularly senior radiologists [10, 20]. A previous study showed that qualified radiologists had less inter-observer variability on reporting of chest radiographs compared to radiology trainees and physicians [21]. Despite the specialized training in pediatric radiology and advanced technology, human error remains a likely factor [8]. The level of variability between the senior radiologists could be a reflection of inconsistency in the application of WHO criteria, as this has been shown to decrease inter-observer variability [22]. However, the rate of false negative reports between the two interpretations of chest radiographs is a well recognized problem [15] which may jeopardize the results of epidemiological studies by underestimating the true burden of pneumococcal pneumonia [23]. In previous pneumococcal vaccine efficacy studies the radiographic evidence of pneumonia was observed in up to 34% of the enrolled children [24]. It has been suggested that using the WHO criteria would make any differences in the results reflect geographical variations in disease epidemiology or vaccine effects rather than

methodological factors [4]. Despite the application of this classification, the concordance rate between two trained reviewers was only 48% (250/521) [25]. The degree of variability of reporting chest radiographs from the present study demonstrates that methodological differences are still a problem in the epidemiological studies of pneumonia in children.

Our findings were limited by heterogeneity amongst a range of radiologists (general and specialized radiologists) involved in the first reporting, with only one radiologist performing second reporting together with differences in clinical information provided at first and second readings. However, having them agree mostly on the interpretation of lobar changes, with the main variability related to non-end-point changes as recently shown among a group of 13 paediatricians and two radiologists [26], make the impact of these limitations is minimal. On the other hand the agreement between readers was improved when the WHO criteria [4] was modified to consider the presence of any lung infiltrate irrespective of its features as end-point pneumonia [27]. All of these reported findings highlight the importance to have defined diagnostic radiological criteria of pneumonia that can be universally used in epidemiological studies and clinical practice.

In conclusion, there is substantial inter-observer variability in the reporting of chest radiographs particularly in young children with pneumonia which appears unrelated to the level of training and experience of those reporting. These findings add to the recognized variability in the literature demonstrating that there may be a need for evaluation of the WHO categorization of radiological pneumonia in children to improve the validity and encourage widespread adoption of the criteria.

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Table 1 Inter-observer variability and agreement in the chest radiographs reporting

First reading		**Second reading (gold standard)**				**Disagreement***
Radiographic changes	***n* (%)**	**Lobar**	**Patchy**	**Perihilar**	**Normal**	***n* (%)**
Lobar	48 (28.4)	47	1	0	0	1 (2.1)
Patchy	43 (25.4)	7	22	5	9	21 (48.8)
Perihilar	32 (19.0)	4	0	23	5	9 (28.1)
Normal	46 (27.2)	4	2	0	40	6 (13.0)
Total	169	62	25	28	54	37 (22.0)

*Fisher's exact test, $P<0.001$; Kappa=0.70 (proportion of subjects on which readers would be expected to agree).

Table 2 Comparison of chest radiographs interpretation by the grade of reporters

First reading		Second reading (gold standard)				Disagreement	
Radiographic changes	***n* (%)**	**Lobar**	**Patchy**	**Perihilar**	**Normal**	***n* (%)**	**Kappa (*P*)**
Radiology trainees	5 (3)	1	1	2	1	0	1.00 (0.105)
Lobar	1	1	0	0	0	0	
Patchy	1	0	1	0	0	0	
Perihilar	2	0	0	2	0	0	
Normal	1	0	0	0	1	0	
Consultant general radiologists	59 (35)	18	12	9	20	11 (18.6)	0.75 (<0.001)
Lobar	16	15	1	0	0	1 (6.3)	
Patchy	17	0	11	3	3	6 (35.3)	
Perihilar	8	1	0	6	1	2 (25.0)	
Normal	18	2	0	0	16	2 (11.1)	
Consultant paediatric radiologists	86 (51)	35	7	13	31	23 (26.7)	0.63 (<0.001)
Lobar	26	26	0	0	0	0	
Patchy	18	5	5	2	6	13 (72.2)	
Perihilar	17	2	0	11	4	6 (35.3)	
Normal	25	2	2	0	21	4 (16.0)	
Consultant thoracic radiologists	19 (11)	8	5	4	2	3 (15.8)	0.78 (<0.001)
Lobar	5	5	0	0	0	0	
Patchy	7	2	5	0	0	2 (28.6)	
Perihilar	5	1	0	4	0	1 (20.0)	
Normal	2	0	0	0	2	0	

Changing clinical practice: management of paediatric community-acquired pneumonia

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Running head: Management of PCAP

Abstract

Rationale and aim: To compare clinical features and management of paediatric community-acquired pneumonia following the publication of UK pneumonia guidelines in 2002 with data from a similar survey at the same hospitals in 2001–2002 (pre-guidelines).

Methods: A prospective survey of 11 hospitals in Northern England was undertaken during 2008–2009. Clinical and laboratory data were recorded on children aged ≤16 years who presented with clinical and radiological features of pneumonia.

Results: 542 children were included. There was a reduction in investigations performed (P<0.001) except C-reactive protein (P=0.448) between surveys. These included full blood count (76% to 61%); blood culture (70% to 53%) and testing of respiratory secretions for viruses (24% to 12%) and bacteria (18% to 8%). Compared to pre-guidelines, there was a reduction in the use of intravenous antibiotics as a proportion of the total prescribed from 47% to 36% (P<0.001) and a change in the route of antibiotic administration with increasing preference for oral alone (16% pre- compared to 50% post-guidelines, P<0.001).

Conclusion: Apart from the collection of blood culture which is encouraged and acute phase reactants that should not be measured routinely, these changes are in line with the guideline recommendations. Improvements in antibiotic use are possible and have implications for future antimicrobial stewardship programmes.

Introduction

Paediatric community-acquired pneumonia (PCAP) is a frequent cause of admission to hospital [1, 2]. Clinical features of pneumonia are often non-specific in young children [3, 4]. Management decisions are generally based on a combination of clinical signs, symptoms and radiological changes [3, 5]. National UK clinical guidelines for management of PCAP were published in 2002[6] and updated in 2011[1] by the British Thoracic Society (BTS). They synthesized evidence and expert opinion to produce best practice national standards, which included statements on investigations and antibiotics use (box 1) [6].

In the UK, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced routinely from September 2006. It was associated with a reduction in the incidence of PCAP and rate of hospitalisation [7]. We therefore aimed to explore changes in the management of children with pneumonia seen in hospital in the context of the national guidelines. Presentation and management outcomes of pneumonia in children in the present survey were compared to those previously described in a similar survey conducted in the same region in 2001–2002 [3], prior to the publication of the BTS management recommendations for PCAP in 2002 [6]. Such findings are important for doctors involved in the management of this infection and for experts updating these guidelines.

Box 1 Selected standards from 2002 BTS management guidelines of PCAP [6]

- Blood cultures should be performed in all children suspected of having bacterial pneumonia.
- Nasopharyngeal aspirates from all children under the age of 18 months should be sent for viral antigen detection with or without viral culture.
- Acute phase reactants should not be measured routinely.
- Amoxicillin is first choice for oral antibiotic therapy in children under the age of 5 years and macrolide antibiotics may be used as first line empirical treatment in children aged 5 and above.
- Antibiotics administered orally are safe and effective for children presenting with CAP.
- Intravenous antibiotics should be used in the treatment of pneumonia in children when the child is unable to absorb oral antibiotics (for example, because of vomiting) or presents with severe signs and symptoms.
- Appropriate intravenous antibiotics for severe pneumonia include Co-amoxiclav, Cefuroxime, and Cefotaxime.
- If clinical or microbiological data suggest that *S. pneumoniae* is the causative organism, Amoxicillin, Ampicillin, or Penicillin alone may be used.

Methods

Participants

A prospective survey of children aged ≤16 years who presented with clinical and radiological features of pneumonia at 11 hospitals (sites) was conducted in Northern England (excluding Cumbria) from August 2008 to July 2009 "post-guidelines". Exclusions included being resident outside the geographical study area; clinical bronchiolitis; hospital admission within three weeks of pneumonia admission; or normal chest radiograph. Disease severity was classified according to the BTS criteria [6]. Chest radiographic changes from the local radiologists' reports were classified into patchy, lobar or perihilar consolidations according to the WHO criteria [8]. 'Non-end-point changes' such as increased bronchovascular markings, peribronchial thickening, bronchial wall thickening, or peribronchial cuffing were grouped together in an additional category "other infiltrates/abnormalities". This cohort was included in a region-wide survey investigating the impact of PCV7 on the incidence of pneumonia confirmed on chest radiograph [9].

Results were compared with those from an identically performed survey, using the same recruitment methods and diagnostic criteria in 2001–2002 "pre-guidelines" [3]. Hospital reconfigurations reduced the number of units admitting children from 13 to 11 during the pre- and post-guidelines surveys respectively. The catchment population and referral pathways from primary care or accident and emergency departments to paediatric services remained the same. Ethical approval was obtained from the Newcastle and North Tyneside Research Ethics Committee with Caldicott approval granted from all sites.

Data collection and case ascertainment

A family doctor/general practitioner or medical staff at the accident and emergency departments saw children before referral for further assessment by the paediatric team. Children were managed entirely by their local paediatric team. Data were recorded on standard form and validated by reviewing ward admission diaries for children admitted with respiratory symptoms (eight sites), or by obtaining hospital coding data on pneumonia where admissions are carried out electronically (three sites). Hard copy and electronic records were reviewed to ascertain the data and resolve any missing or inconsistent data. Duplicates or those who did not fulfil the enrolment criteria were removed.

Statistical analysis

Descriptive analysis was performed using Epi Info™ 7. Fisher's exact test was used to compare categorical variables between groups and with those from the pre-guidelines survey [3]. A comparison of treatment approaches, clinical and radiological features for severe versus mild/moderate CAP was performed using logistic regression.

Results

A total of 542 were eligible for inclusion (58% males; 74% <5 years old). Similar to pre-guidelines (89%), 84% children were admitted. Ten children required admission to the intensive care for assisted ventilation; eight were under five. An underlying co-morbidity was present in 15% and asthma in 7%. No children died during either survey periods. The epidemiological outcomes for this cohort were described in a separate publication [9].

Table 1 summarises the clinical features at presentation across both surveys. Comparing post- with pre-guidelines surveys; fewer children presented with severe disease (*P*=0.023). Among those with pleural effusion, reported lobar changes were present in 77% post-guidelines compared to 42% pre-guidelines (OR=0.2; 95% CI 0.09 to 0.48; *P*=0.0002). Logistic regression analysis of the post-guidelines data suggested that children over two not given preadmission antibiotics were more likely to develop severe disease (*P*=0.010). Hospitalisation was associated with disease severity (*P*<0.001), but not with pyrexia (triage temperature >38^{o}C) or chest radiographic changes.

Investigations

There was an association between the collection of blood samples for investigation(s) and use of intravenous (IV) antibiotics pre-guidelines (*P*<0.001), but not post-guideline. There was a reduction in the number of investigations performed (*P*<0.001) except C-reactive protein (CRP) (*P*=0.448) between pre- and post-guidelines. Full blood count (FBC) decreased from 76% to 61%; blood culture from 70% to 53%; testing respiratory secretions for viruses from 24% to 12% and bacteria from 18% to 8%. The yield of blood culture was the same in both surveys (4% and 4.9%). Post-guidelines, viral PCR assays (immunofluorescence test in pre-guidelines) were performed on respiratory secretions from 66 children with 26 (39%) positive. Obtaining a viral respiratory screen was age-dependent and more frequently performed in those aged <2 (22%) than ≥2 years, but less often when compared with pre-guidelines (34%) [OR=0.5; 95% CI 0.33 to 0.75; *P*=0.001].

CRP was obtained in 322 (59%). Of which, 27% were >100 mg/L; 9% of infants, 58% of under five years old and 42% in the above five. Pleural effusion was associated with higher CRP greater than 100 mg/L (*P*<0.001). Lobar and patchy changes were associated with a CRP more than 150 mg/L (*P*<0.05). Mean values of CRP, total white cell count (WCC) and neutrophils were higher with lobar changes (*P*<0.001).

Management

Between the pre- and post-guidelines, IV antibiotics as a proportion of the total prescribed antibiotics decreased from 47% (501/1065) to 36% (318/891) [OR=1.6; 95% CI 1.33 to 1.93; *P*<0.001], and oral antibiotics alone increased from 16% to 50% [OR=4.4; 95% CI 3.37 to 5.71; *P*<0.001]. There was also a reduction in the use of IV route only from 8% to 5% [OR=1.8; 95% CI 1.08 to 2.86; *P*=0.025] and the use of both

oral and IV routes (P<0.001) between the pre- and post-guidelines respectively. Post-guidelines, Amoxicillin prescription both orally and intravenously increased (P<0.001) with a decrease in IV cephalosporins (Cefuroxime and Cefotaxime) (P<0.001) and total oral macrolides (Erythromycin, Azithromycin and Clarithromycin) (P<0.001). However, the individual use of Azithromycin or Clarithromycin remained the same, whilst decreased for Erythromycin (P<0.001).

Pre-guidelines, initial IV antibiotics were associated with severe disease, lobar changes, pleural effusion, or pyrexia (P<0.05), but not with oxygen saturation <93%. These associations were replicated in post-guidelines with the initial use of IV antibiotics being associated with severe disease (P=0.0003), lobar changes (P=0.018), or pleural effusion (P=0.041), but not with oxygen saturation <93% or pyrexia. Comparing post- with pre-guidelines; IV antibiotics were more likely to be given to those with lobar changes (35% versus 25%) [OR=0.6; 95% CI 0.45 to 0.85; P=0.004], but less likely to be given to children presenting with low oxygen saturations (25% versus 34%) [OR=0.6; 95% CI 0.45 to 0.89; P=0.009].

Mean duration of hospitalisation decreased from pre- to post-guidelines (4.7±7.16 versus 3.2±3.02 days, P<0.001). Those with severe disease, lobar changes or pleural effusion had a longer stay (P<0.001). All children irrespective of their age group who received any IV antibiotics (alone or in combination with oral) had a longer average duration of hospitalisation than those who had only oral (4.1±3.4 versus 2.0±1.9 days, P<0.001). Figure 1 shows the probability of discharge from hospital in relation to the duration of admission. Approximately 75% of children were likely to be discharged within two days of hospital admission, whilst hospital stay for up to five days was required for nearly 20% of children. Approximately 5% of children stayed for nearly three weeks because of complications and presence of pre-morbid medical illnesses.

Discussion

This survey provides invaluable evaluation of the presentation and management of PCAP seen in hospital over a year period. Clinical management of children with pneumonia has changed significantly between 2002 and 2008. There have been a reduced number of investigations performed, a change in the type of antibiotics, a decrease in IV and a concomitant increase in oral antibiotics. Reasons for these changes

are likely to be multifactorial such as the publication of the BTS management guidelines [6], an expanding literature on oral/IV antibiotic use [10-12], and the routine introduction of PCV7 in the UK in 2006.

Drivers of change are complex. Some are likely to be literature driven. Others probably reflect the complex relationships around perceived benefits and risks of IV cannulation, venepuncture and differing usefulness of investigations. It is interesting that fewer blood tests in terms of FBC and blood cultures were taken, but just as many CRP samples were ordered. The BTS guidelines including the recently updated version [1] made no specific recommendations around FBC, but blood cultures were (and are) specifically encouraged, whilst CRP is not [6]. In this survey, the correlation between high CRP of >100 mg/L with pleural effusion demonstrates the usefulness of CRP in differentiating between uncomplicated and complicated pneumonia of bacterial aetiology [13]. The reduction in collecting blood cultures perhaps reflects the feeling that bacterial pneumonia is less likely given the introduction of PCV7, which in the same population was associated with decreased disease incidence and rate of hospitalisation [9]. Although clinicians were not asked directly, the shift towards less testing of respiratory secretions for either viruses or bacteria could reflect the feeling that the results would not affect the decision on antibiotic use.

More positive changes are seen with antibiotic usage, encouraging for developing antimicrobial stewardship programmes. These included a significant reduction in the use of antibiotics prior to admission. This is in line with the observed substantial decline since 1990s in the prescription of antibiotics in primary care for lower respiratory tract infection in children [14]. This fall in antibiotic prescriptions predate the published BTS management guidelines of pneumonia in 2002 [6]. They reflect a continued fall in the use of antibiotics despite a marginal increase in antibiotic prescription during the period between 2003 and 2006, primarily for non-specific upper respiratory tract infections, for which national guidance aimed at primary care was introduced in 2008 [14, 15]. Intravenous antibiotics were used far less frequently than oral, with a substantial increase in the use of Amoxicillin overall and orally, at the expense of IV Cefuroxime and oral cephalosporins, which decreased from one fifth to 2%. In contrast, oral macrolides remain frequently prescribed particularly to those aged under five, similar to previous data [3], although not recommended as first line treatment [6]. Evidence for the safety and efficacy of oral antibiotics even in severe pneumonia in children

accumulated over the six years period between surveys, including a Cochrane review in 2006[11] and the PIVOT trial in 2007[12].

The selection of initial antibiotic route was influenced by disease severity and lobar changes, possibly reflecting that these criteria were considered markers of bacterial infection. The fact that lobar changes were associated with high mean value of inflammatory markers may support this. Other factors that could have influenced the decision to give IV antibiotics, such as the level of training of admitting medical staff or the knowledge of the published guidelines, could not be ascertained with the data collected.

In conclusion, there has been a positive change in the management practices of PCAP reflected by reduced number of overall investigations performed and an increased preference for oral antibiotic use.

Authorship

JEC developed the survey concept and with KME and MAE were responsible for the survey logistics and facilitation of data collection. MAE managed and validated the data. All authors were involved in the interpretation of the results and writing of this article.

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Table 1 Clinical features at presentation

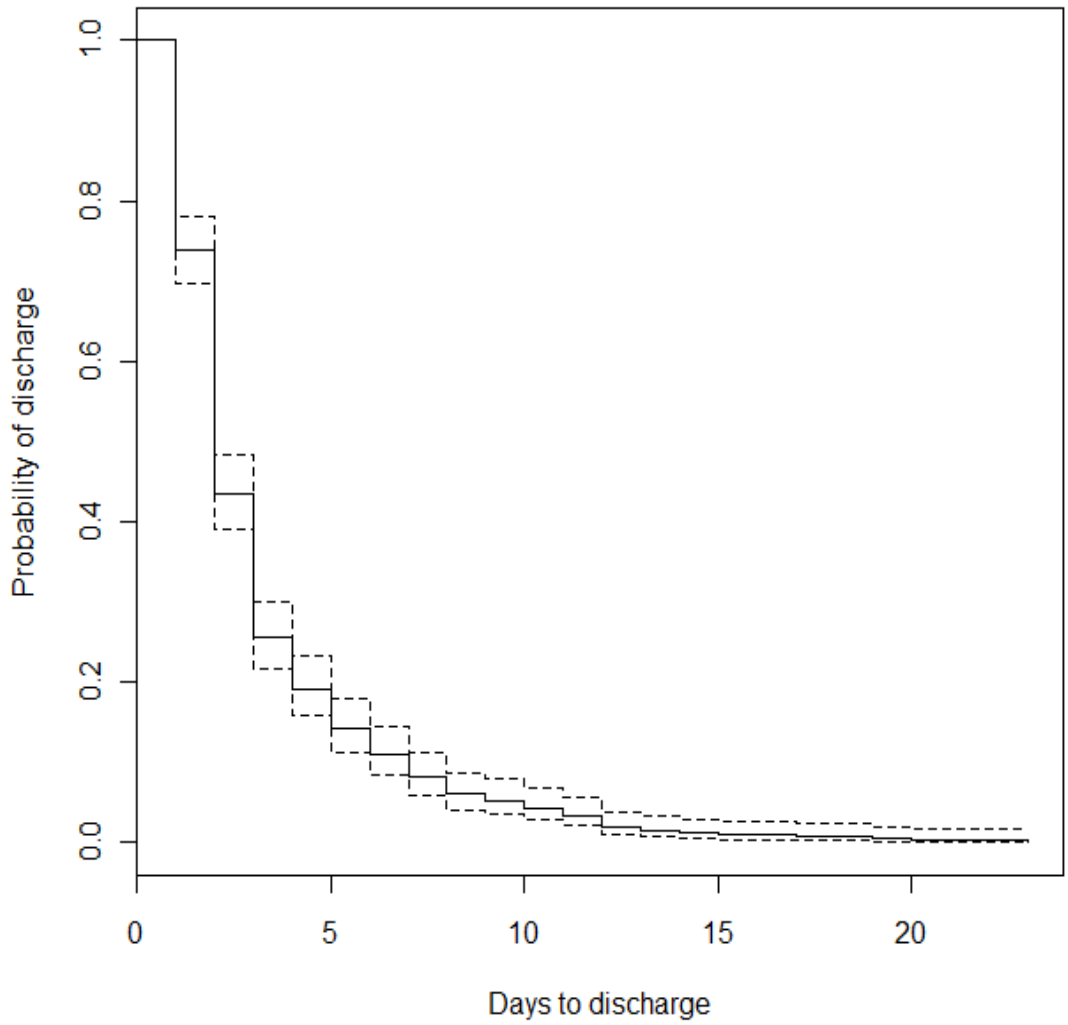
	2001 survey (n=711) [3]	**2008 survey (n=542)**		
Characteristics	**n (%)**	**n (%)**	**OR (95% CI)**	***P*-value**
Pre-admission antibiotics	214 (30.0)	119 (22.0)	0.7 (0.50 to 0.85)	0.001
Triage temperature >38°C	333/702 (47.4)	266/531 (50.0)	1.1 (0.89 to 1.39)	0.358
Oxygen saturation <93%	213/689 (31.0)	145/529 (27.4)	0.8 (0.66 to 1.08)	0.204
Disease severity				
Mild/moderate	293 (41.2)	259 (47.8)	1.3 (1.04 to 1.64)	0.022
Severe	418 (58.8)	283 (52.2)	0.8 (0.61 to 0.96)	0.023
Chest radiographic findings				
Lobar	145 (20.4)	162 (29.9)	0.6 (0.46 to 0.78)	0.0001
Patchy	436 (61.3)	296 (54.6)	0.8 (0.61 to 0.95)	0.019
Perihilar	130 (18.3)	67 (12.4)	1.6 (1.15 to 2.18)	0.006
Other infiltrates	–	17 (3.1)		–
Pleural effusion	65 (9.0)	52 (9.6)	1.1 (0.72 to 1.55)	0.845

OR, odds ratio; CI, confidence interval.

Figure Legend

FIGURE 1 Survival curve showing probability of discharge from hospital in relation to duration of admission

Abbreviations in figure 1:
Solid line, probability of discharge from hospital; broken lines, 95% CIs


Probability of discharge
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
Days to discharge

A case-control study to assess the urinary pneumococcal antigen test in childhood pneumonia

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Running head: Urinary pneumococcal antigen

Contributors
DAS and JEC developed the original study concept. MAE collected and managed the data. JDP and ADS performed the laboratory analyses. All authors were involved in the interpretation of the results and writing of this article.

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We thank the research nurses; Kerry Pollard and Pauline Singleton for the assistance with data collection. We are grateful to Dr Fiona Hampton at the James Cook University Hospital, Middlesbrough for the facilitation of recruitment; and Audrey Nicholson at the Department of Microbiology, Freeman Hospital Newcastle for overseeing the laboratory logistics.

Conflicts of interest and source of funding

The study was supported by Pfizer Vaccines UK (No: 0887X1-4479). DAS and JEC received unconditional research support from Pfizer. The sponsor had no role in the study design, data collection, analysis or interpretation, and writing of the manuscript.

Ethics approval

This study was conducted with the approval of the Newcastle and North Tyneside Research Ethics Committee (No: 08/H0906/105) and the Research Approval Board at South Tees Hospitals NHS Trust in Middlesbrough (No: 2008075). Caldicott approval was also obtained.

ABSTRACT

We investigated prospectively the association of urinary pneumococcal antigen with pneumococcal pneumonia in children aged ≤16 years). Control urine samples were collected from children undergoing investigation of urinary tract infection. Urinary antigen was detected in more cases than controls (*P*=0.00003). Among cases with identified pneumococcal infections, 75% (15/20) had positive urinary antigen (*P*=0.000008).

LETTER TO THE EDITOR

Urinary pneumococcal antigen is a rapid non-invasive test which may indicate recent invasive pneumococcal infection (Elemraid et al., 2013) or carriage (Hamer et al., 2002), and guide appropriate antibiotic therapy (Charkaluk et al., 2006; Neuman & Harper, 2003). We investigated the association of urinary pneumococcal antigen with pneumococcal infection in childhood pneumonia.

A prospective aetiological study of childhood pneumonia was conducted from October 2009 to March 2011. Enrolled cases were children aged ≤16 years with clinical and radiological features suggestive of pneumonia. Patients were resident in North East England (excluding Cumbria) who presented or were transferred to the paediatric services at the Great North Children's Hospital (GNCH), the regional cardiothoracic centre at Freeman Hospital, Newcastle or the James Cook University Hospital, Middlesbrough. Exclusion criteria included clinical diagnosis of bronchiolitis, hospitalisation in the preceding three weeks or normal chest radiograph after formal reporting by a radiologist. For cases, informed written consent was obtained from parents as well as assent from older children. Control urine samples were collected from children who attended the paediatric renal service at the GNCH during March to May 2010 for routine follow up or investigations of previous urinary tract infection. Controls had no clinical evidence of concurrent infectious illness and urine microscopy performed to exclude acute infection.

Extensive microbiological and virological testing informed the aetiology of pneumonia using defined diagnostic criteria and positive results were classified as definite/probable or possible (online supplement). Urine samples were tested for pneumococcal antigen using Binax NOW (Inverness Medical Innovations Ltd, Galway, Ireland). Investigations were performed in the Microbiology Laboratory, Newcastle Hospitals NHS Trust and

the Health Protection Agency Public Health Laboratory Newcastle. Epi Info™ 7 was used for data analysis. Fisher's exact test was used to evaluate group differences.

A total of 160 children were enrolled with a median age of 2.6 years (56% males and 69% aged <5 years). All of whom had at least one microbiological investigation performed. Control urine samples were collected from 122 children with a median age of 4.7 years (37% males and 52% aged <5 years). Urinary pneumococcal antigen was detected in 28.3% (30/106) of cases, compared to 7.4% (9/122) in controls (OR=0.2, 95% CI 0.09–0.45, *P*=0.00003). Among those aged <5 years, the urine antigen was positive in 23.5% (16/68) cases and 9.5% (6/63) in controls (OR=0.3, 95% CI 0.12–0.94, *P*=0.037). *S. pneumoniae* was the definite cause of pneumonia in 17.4% (24/138) of children; 15% (14/93) and 22.7% (10/44) tested among those aged < and ≥5 years respectively. Among those children with identified pneumococcal infections, 75% (15/20) had positive urinary pneumococcal antigen (OR=12.0, 95% CI 3.76–38.26, *P*=0.000008).

Our study has shown that in children with radiologically confirmed pneumonia the urinary pneumococcal antigen is more likely to be positive than in healthy asymptomatic children. This indicates that positive urinary Binax test could be highly suggestive of this infection in healthy children including young age group who had no recent infections. Although previous findings showed poor utility of urine antigen test in distinguishing pneumococcal pneumonia from nasopharyngeal colonisation in children (Dominguez et al., 2003; Dowell et al., 2001), our findings showed significantly positive results of urinary pneumococcal antigen between children with pneumococcal infections and those with other causes pneumonia. Urinary pneumococcal antigen testing may be a useful investigation to help establish the diagnosis of invasive pneumococcal disease, particularly in low-resource countries where expensive PCR-based assays are not readily available. Used in this way this test may also prove a useful tool in studies of the epidemiology of childhood pneumonia.

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Parental pneumococcal nasopharyngeal carriage of children with pneumonia

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Running head: Pneumococcal nasopharyngeal carriage

Authorship

DAS and JEC developed the original study concept. MAE collected and managed the data. JDP and ADS performed the laboratory analyses. All authors were involved in the interpretation of the results and writing of this article.

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We thank the research nurses; Kerry Pollard and Pauline Singleton for the assistance with data collection. We are grateful to Dr Fiona Hampton at the James Cook University Hospital, Middlesbrough for the facilitation and support of recruitment; and Audrey Nicholson at the Department of Microbiology, Freeman Hospital for overseeing the laboratory logistics. We extend our thanks to Dr Robert George and Carmen Sheppard for running the xMAP immunoassay for serotyping the pneumococcal isolates in the national Respiratory and Systemic Infection Laboratory at the Health Protection Agency in London.

Declaration of interest

The study was supported by Pfizer Vaccines UK (No: 0887X1-4479). DAS and JEC received unconditional research support from Pfizer. The sponsor had no role in the study design, data collection, analysis or interpretation, and writing of the manuscript.

Ethics approval

Ethical and Caldicott approvals were granted (Newcastle and North Tyneside Research Ethics Committee (No: 08/H0906/105), and Research Approval Board at South Tees Hospitals NHS Trust (No: 2008075)).

Abstract

Pneumococcal serotype 1 was isolated from adult's nasopharynx during a school outbreak with this infection. We therefore tested the hypothesis that parents may be involved in the transmission of this serotype in children with pneumonia. 212 parental nasopharyngeal swabs were obtained from 144 children with pneumonia. Serotype 1 was identified in the parent of one child with invasive pneumococcal serotype 1 disease. This accumulating evidence warrants the hypothesis investigation in a larger community-based study.

Letter to the Editor

Asymptomatic carriage of *Streptococcus pneumoniae* is common in the human nasopharynx, but invasion of the mucosal barrier can lead to local or systemic infections.[1] Transmission of pneumococcal carriage within the household and community is common, and is particularly prevalent in young children.[2] Paediatric pneumococcal empyema has increased dramatically in many countries in recent years, and this problem has predominantly been related to infection with serotype 1.[3] The mode of transmission of this serotype is uncertain as it does not frequently colonise the nasopharynx.[4] We previously found serotype 1 in the nasopharynx of an adult during the investigation of a school outbreak of serotype 1 disease.[5] We therefore tested the hypothesis that parents and family members may be involved in the transmission of this serotype in children with pneumonia.

A prospective aetiological study of radiologically-confirmed pneumonia in children aged ≤16 years was conducted from October 2009 to March 2011.[6] They were resident in North East England and admitted to the Great North Children's Hospital, the regional cardiothoracic centre at Freeman Hospital, Newcastle or the James Cook Hospital, Middlesbrough. Informed written consents were obtained for children and parents participation. A nasopharyngeal swab (NPS) was collected from parents. Aliquots of NPS were inoculated into plates of Columbia agar (Oxoid) supplemented with 5% horse blood (CBA) and Oxoid brain-heart infusion broth with 10% serum (Oxoid). Broths were incubated overnight at 37°C and sub-cultured (10 µL) onto CBA plates for incubation at 37°C in 5% carbon dioxide for 48 hr. Isolates of *S. pneumoniae* and group A *Streptococcus* (GAS) were identified by standard methods including latex agglutination and API 32 STREP (bioMérieux) and stored in STGG medium (skim milk-tryptone-glucose-glycerol).

At least one parental NPS was obtained from 144 children with pneumonia (57% males, 70% aged <5 years); 136 from mothers and 76 fathers. From parents, 212 NPSs were collected and four pathogens were isolated; one GAS and three *S. pneumoniae*. There were four cases of pneumonia whose parents had a positive swab culture. Firstly, a 13-month-old who had GAS grown from the blood culture as well as mother's NPS. Secondly, a 6.3-year-old who had *S. pneumoniae* serotype 1 isolated from blood culture and also mother's NPS. Thirdly, a 20-month-old whom her father's NPS grew non-typeable pneumococcus. Fourthly, a 26-month-old with father's NPS positive for *S. pneumoniae* but typing was not carried out.

This is the first published study to investigate the parental pneumococcal nasopharyngeal carriage in a selected group of children with pneumonia in North East England. The established carriage rate was low. Although the findings are limited, they still provide information for future surveillance of pneumococcal carriage in our population. Limiting testing to parents might not fully reflect the complete pattern of pneumococcal carriage within the family. The finding of serotype 1 in the parent of one child with invasive serotype 1 disease in this series adds to our previous findings,[5] and this hypothesis now warrants investigation in a larger community-based study.

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