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Pneumococcal diagnosis and serotypes in childhood community-acquired pneumonia[☆],[☆☆]

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ABSTRACT

The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced routinely in the UK from September 2006 and replaced by PCV13 in 2010. In a prospective study from 2009 to 2011 of 160 children aged ≤16 years with radiologically confirmed pneumonia, likely pneumococcal infections were identified in 26%. Detection of pneumococci was improved with polymerase chain reaction compared to culture (21.6% versus 6% of children tested, $P = 0.0004$). Where serotyping was possible, all ($n = 23$) were non-PCV7 but PCV13 serotypes; 1 (43.5%), 3 (21.7%), 7A/F, and 19A (17.4% each).

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1. Introduction

Streptococcus pneumoniae is the leading bacterial cause of paediatric community-acquired pneumonia (PCAP) (Rudan et al., 2008). The 7-valent pneumococcal conjugate vaccine (PCV7) (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) was added into the UK immunisation schedule from September 2006; subsequently replaced by PCV13 from April 2010 (additional serotypes 1, 3, 5, 6A, 7F, and 19A). Three vaccine doses are administered at the age of 2, 4, and 13 months. When the vaccine was introduced, a catch up programme was offered to all children born between September 2004 and September 2006. This vaccination programme would thus be expected to impact upon the pneumococcal serotypes implicated in PCAP.

Pathogens are difficult to identify in children with pneumonia, with blood culture and serological testing often negative due to minimal presence of bacteremia (Brent et al., 2006; Korppi et al., 2008). This paucity of *S. pneumoniae* isolation makes examining pneumococcal serotype distribution in PCAP difficult, with no UK, and little worldwide, data. Using non-culture techniques including pneumococcal detection by polymerase chain reaction (PCR) in blood (Resti et al., 2010) and pleural fluid (Strachan et al., 2012), pneumococcal antigen detection in urine (Charkaluk et al., 2006), and pneumococcal serotype detection by PCR (Esposito et al., 2012; Resti et al., 2010), we sought to determine the contribution of *S. pneumoniae* and specific serotypes to the aetiology of PCAP.

We included urinary pneumococcal antigen detection by Binax Now as being indicative of invasive pneumococcal infection for several reasons. It has good sensitivity (100%) and specificity (56%) in children with suspected pneumococcal disease (Charkaluk et al., 2006), but is confounded as a diagnostic test by nasopharyngeal carriage where up to 21% may be positive (Hamer et al., 2002). Urinary pneumococcal antigen may also be positive in 4% healthy nasopharyngeal culture-negative children (Hamer et al., 2002), 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 (Turner et al., 2011). Thus, a positive urinary antigen in a child with pneumonia and no pneumococcal nasopharyngeal carriage by PCR is likely to reflect invasive pneumococcal disease.

2. Materials and methods

2.1. Study population

From October 2009 to March 2011 children aged ≤16 years old with clinical and radiological features suggestive of pneumonia admitted to any of three hospitals in North East England were prospectively enrolled. Informed consent was obtained from parents and assent from older children. 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)).

Pneumonia was defined by signs and symptoms suggestive of lower respiratory tract infection and chest radiographic findings consistent with pneumonia as determined initially by the local paediatrician. Exclusions included being resident outside North East England, clinical bronchiolitis, hospitalisation in the preceding three weeks, or normal chest radiograph after review by a radiologist. Chest radiographs were first reported by local radiologists then reviewed by a second consultant cardiothoracic radiologist at the regional centre. Radiographic findings were categorised into lobar, patchy, or perihilar according to World Health Organization criteria (Cherian et al., 2005). Conjugate pneumococcal vaccination history was ascertained.

2.2. Laboratory procedures

Blood samples were collected for serum, blood culture (BacT/ALERT®, bioMérieux) and pneumococcal PCR testing. Nasopharyngeal aspirates (NPA) were obtained from infants, and placed in 0.9% sodium chloride transport solution. For older children, bacterial nasopharyngeal swab (NPS) was collected. Expectorated sputum and tracheobronchial secretions (TBS) (sampled via endotracheal tube or bronchoalveolar lavage) and pleural fluids were tested when collected. Secretions 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) into CBA plates for incubation at 37°C in 5% carbon dioxide for 48 hours. Pneumococcal isolates were identified by standard methods including latex agglutination and API 32 STREP (bioMérieux). Urine samples were tested for pneumococcal antigen using Binax NOW (Inverness Medical Innovations Ltd, Ireland). Not every child had all tests performed.

Total nucleic acid was extracted from blood, respiratory secretions, culture-negative pleural fluid, and pneumococcal antigen positive urine samples for PCR using an EasyMag automated nucleic acid extraction instrument (bioMérieux) and the resulting nucleic acid used as template in a *S. pneumoniae* specific PCR targeting the pneumolysin gene (and autolysin gene for pleural fluids) (Corless et al., 2001). Any recovered *S. pneumoniae* was serotyped using a multiplexed xMAP immunoassay for detection of serotype-specific *S. pneumoniae* antigens (Sheppard et al., 2011). This assay identifies PCV13-serotypes plus serotype 8.

2.3. Diagnostic criteria for pneumococcal infection and statistical analysis

S. pneumoniae was considered a definite or probable pathogen when detected from a sterile site, TBS or urine samples. Pneumococcal detection in NPA/NPS or sputum was considered a possible pathogen and likely to represent nasopharyngeal carriage in children (Table 1). Detection rates of pathogens are expressed as proportions of those tested. Fisher's exact test was used to compare different detection methods and odds ratios and 95% confidence intervals were calculated. Data were analysed using Epi Info™ 7.

3. Results

Of 160 children, 56% were male and 69% aged <5 years, median age 2.6 years. All received antibiotics, none died. Radiographs showed 61% lobar consolidation, and 42.5% pleural effusion. Forty (25%) children had empyema. Based on age, pneumococcal vaccination uptake among 119 eligible children was 94% (89 had PCV7, 10 PCV13, and 13 received combinations of each) (online supplement). Pneumococci were detected from any site in 64% of children (103/160). *S. pneumoniae* was identified from nasopharyngeal secretions by culture in 7% (10/141) and by PCR in 63% (76/121); and from sterile sites in 17.4% (24/138); by culture in 6% (8/132); compared with PCR in 21.6% (21/97), $P = 0.0004$.

S. pneumoniae was the likely (definite/probable) pathogen in 26% (39/149); 20.8% (21/101) and 37.5% (18/48) tested among those aged <5 and ≥5 years respectively. Of the 39 pneumococcal infections, 21 children had empyema. Pneumococcal infections among those who received PCV7 was 26.5% (22/83), compared to 10.5% (2/19) for those who either had PCV13 alone or doses of both ($P = 0.182$). A serotype was identified in 82% tested (23/28); serotypes 1 ($n = 10$), 3 ($n = 5$), 7A/F and 19A ($n = 4$ each) (Table 2). Apart from two children who

Table 1
Laboratory investigations and diagnostic criteria of pneumococcal infection.

Sample	Pathogen/antigen	Tests[a]	Diagnosis of causative pathogens	
			Definite/probable	Possible
Blood	*S. pneumoniae*	Culture/real-time PCR	Growth/positive	–
Nasopharyngeal secretions, sputum	*S. pneumoniae*	Culture/real-time PCR	–	Growth/positive
Tracheobronchial secretions (endotracheal/bronchoalveolar lavage)	*S. pneumoniae*	Culture/real-time PCR	Growth/positive	–
Pleural fluid	*S. pneumoniae*	Culture/real-time PCR	Growth/positive	–
	Pneumococcal antigen	ELISA	Positive	–
Urine	Pneumococcal antigen	Binax NOW	Positive	–

[a] ELISA, enzyme-linked immunosorbent assay.

Table 2
Proportions of detected pneumococci and serotype distribution.

Tests	Tests, n	Positive, n (%)	Serotype (n/N, types)[a]	
Overall definite/probable infections	149	39 (26.0)	23/28	1 (n = 10), 3 (n = 5), 7A/F (n = 4), 19A (n = 4)
Blood (total)	*136*	*11 (8.0)*	*6/6*	
Culture	126	5 (4.0)	3/3	1, 3, 19A
Pneumolysin real-time PCR	86	7 (8.1)	3/3	3, 19A (n = 2)
Pleural fluid (total)	*40*	*19 (47.5)*	*17/19*	
Culture	40	3 (7.5)	3/3	1, 19A (n = 2)
Pneumococcal antigen	30	7 (23.3)	6/6	1 (n = 5), 3
Pneumolysin real-time PCR	30	18 (60.0)	18/18	1 (n = 7), 3 (n = 5), 19A (n = 4), 7A/F and NT (n = 1 each)[b]
Respiratory secretions (total)	*151*	*82 (54.3)*	*1/10*	
Nasopharyngeal culture	141	10 (7.0)	5/10	23B, NT (n = 4)
Tracheobronchial culture[c]	12	1 (8.3)	–	
Pneumolysin real-time PCR	121	76 (62.8)	–	
Urinary pneumococcal antigen	106	30 (28.3)	9/17	1 and 7A/F (n = 4 each), 19A

[a] N, number of isolates assayed for serotype identification.
[b] NT, non-typeable.
[c] Collected via endotracheal tube or bronchoalveolar lavage.

had 2 doses of PCV7 and a booster with PCV13, others with these non-PCV7 serotypes were either unvaccinated (n = 9) or PCV7-vaccinated (n = 12).

Pneumococcal urinary antigen was not detected in any of the 40 children with nasopharyngeal pneumococcal carriage only (on culture and/or PCR) and no evidence of likely pneumococcal infection. Half (15/30) of those with Binax-positive samples also had *S. pneumoniae* identified in a sterile site. Of the 17 Binax-positive urines tested, nine identified pneumococcal serotypes. Four of these had concordant identification of same serotypes from pleural fluid (2 were serotype 1, one each of serotypes 7A/F and 19A).

4. Discussion

This study provides the first information on serotype distribution of pneumococcal PCAP in the UK after the routine introduction of the pneumococcal conjugate vaccine. Its timing towards the end of 3 years of PCV7 and during the first year of PCV13 gives an 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 CAP.

We detected likely pneumococcal infection in 26% of children with PCAP, more than previously described in the UK (Clements et al., 2000; Drummond et al., 2000). With no association between urinary pneumococcal antigen and nasopharyngeal pneumococcal carriage in this study, urinary pneumococcal antigen is likely to indicate a pathogen than carriage. Diagnosis of pneumococcal infection was increased considerably when pneumolysin PCR was used (Esposito et al., 2012; Strachan et al., 2012), improving the detection rate from 6% by culture alone to 23%. This is similar to the increase (3.8% to 15.4%) reported in a recent Italian study (Resti et al., 2010). It has recently been recognised that the pneumolysin gene can be detected in non-pneumococcal Viridans-group streptococci, particularly *S. pseudopneumoniae* and *S. mitis* (Carvalho Mda et al., 2007), thus introducing the possibility of false positivity with pneumococcal PCR. We feel this is unlikely given the clinical context; serotype identification would not be affected.

In England and Wales non-PCV7 serotypes are now associated with invasive pneumococcal disease (Miller et al., 2011), and an increase in pneumococcal serotype 19A is associated with complicated pneumonia with empyema (Thomas et al., 2012). 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. Although the number of serotyped pneumococcal isolates was low, with the majority being identified from pleural fluids, none were covered in PCV7, but are included in PCV13. This is reflected by data

from the USA on children with empyema, where 98% were non-PCV7 serotypes (Byington et al., 2010). This suggests that PCV13 could substantially reduce invasive pneumococcal disease (Jefferies et al., 2011).

In conclusion, non-PCV7 serotypes were the major contributor to the aetiology of pneumococcal pneumonia in UK children. Continued surveillance is required to monitor for the emergence of serotype replacement.

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