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Propensity of pneumococcal carriage serotypes to infect the lower airways of children with chronic endobronchial infections

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ABSTRACT

Background: Chronic endobronchial infections in children are responsible for a high disease burden. *Streptococcus pneumoniae* is frequently isolated; however, few publications have described serotypes associated with non-invasive lower airway infection.
Methods: Paired nasopharyngeal (NP) swabs and bronchoalveolar lavage (BAL) fluids were collected from children undergoing bronchoscopy for chronic cough. NP swabs were also collected from asymptomatic children in otitis media surveillance studies (controls). Specimens were processed and lower airway infection defined ($\geqslant 10^4$ colony forming units/mL BAL) as previously described. Serotype-specific odds ratios (ORs) were calculated (as described for invasive pneumococcal disease) to indicate propensity for infection.
Results: From 2007–2015, paired specimens were processed from 435 children with protracted bacterial bronchitis (PBB), chronic suppurative lung disease (CSLD) or bronchiectasis. *S. pneumoniae* lower airway infection was detected in 95 children: 27% with PBB and 20% with CSLD/bronchiectasis. Most (91%) children were vaccinated with $\geqslant 2$ doses of 7-valent, 10-valent or 13-valent pneumococcal conjugate vaccine. Paired NP and BAL serotype distributions were very similar; prevalent serotypes (>10 isolates) were 19A (9%), 19F, 6C, 35B, 15B, 16F, 15A, 15C, 23A, 23F and 11A. For 21 serotypes found in both NP and BAL specimens, ORs for infection were low; range 0.46 (serotype 23B) to 2.15 (serotype 6A). In the 2008–2013 surveillance studies, NP swabs were collected from 1565 asymptomatic children; 74% were pneumococcal carriers. For 21 of 22 serotypes found in both control NP swabs and BAL specimens, ORs for infection were similarly low; range 0.33 (serotype 23B) to 3.29 (serotype 22F); none was significantly different from 1. The exception was serotype 7B with OR 8.84 (95% CI 1.46, 38.1).
Conclusions: Most NP carriage serotypes have a similar propensity to cause lower airway infection in children with suppurative lung diseases. Further development of pneumococcal vaccines is needed to prevent non-invasive disease caused by commonly carried serotypes.

1. Introduction

Streptococcus pneumoniae (pneumococcus) is the main cause of pneumonia worldwide, and has been studied extensively in this context. The pneumococcus is also important in chronic respiratory conditions such as protracted bacterial bronchitis (PBB) and bronchiectasis, but this has received less attention. Although nontypeable *Haemophilus influenzae* (NTHi) is the main pathogen associated with PBB and bronchiectasis, *S. pneumoniae* is commonly isolated from the lower airways of children with these conditions [1,2]. Severe and recurrent pneumonia are risk factors for chronic suppurative lung disease (CSLD) and bronchiectasis [3,4]. PBB, increasingly recognised as a cause of significant morbidity in children, is a precursor to bronchiectasis in some settings [2,5]. These conditions cause a high disease burden [6] and bronchiectasis is associated with early death in some populations [7,8].

Much research effort has been directed towards determining the serotypes that cause invasive pneumococcal disease (IPD, e.g.

Abbreviations: AOM, acute otitis media; BAL, bronchoalveolar lavage; CFU, colony forming units; CI, confidence interval; CSLD, chronic suppurative lung disease; IPD, invasive pneumococcal disease; MARS, monitoring antibiotic resistance and serotypes; NP, nasopharyngeal; NT, Northern Territory; NTHi, nontypeable *Haemophilus influenzae*; OR, odds ratio; PBB, protracted bacterial bronchitis; PCV, pneumococcal conjugate vaccine; PHiDCV, 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine; Qld, Queensland; SID, Simpson's Index of Diversity; STGGB, skim-milk tryptone glucose glycerol broth.

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bacteremic pneumonia) to inform vaccine development. The first licensed pneumococcal conjugate vaccine (PCV7) contained seven serotypes causing most IPD in children <5 years old in the United States (4, 6B, 9V, 14, 18C, 19F, 23F) [9]. The current 13-valent vaccine (PCV13) includes these plus six additional serotypes (1, 3, 5, 6A, 7F, 19A) [10]. Invasiveness can be described as the ability of an organism to reach and grow in normally sterile tissues; various indices of invasiveness, such as empirical odds ratios (ORs), have been determined to compare the prevalence of invasive strains with the prevalence of strains detected in nasopharyngeal (NP) carriage [11,12]. A study in Papua New Guinea found that the most invasive serotypes were 1, 2, 5 and serogroup 7 (likely 7F) with high ORs (>10); serogroups such as 6, 19 and 23 were less invasive but caused a high proportion of IPD because they were frequently acquired and carried for long periods [12]. Subsequent studies in the United Kingdom (UK) [13], Finland [14], Portugal [15] and Spain [16] found similar results: serotypes 1, 4, 5, 7F, 14, 18C and 19A had high ORs (most >5) indicating a propensity to cause invasive disease. Apart from serotype 2, all serotypes identified as highly invasive in these studies are included in PCV13.

Until recently there were few data available on *S. pneumoniae* serotypes associated with non-invasive lung disease. A very different serotype distribution in non-invasive pneumococcal pneumonia to that described for IPD was found for adults in Portugal [17], and two small studies in children with severe pneumonia [18] and PBB [19] found that serotypes from lower airway specimens were mostly common carriage types. A recent article reported no statistically significant difference between the prevalence of serotypes in NP and lower respiratory specimens from children in Moscow; however, serotypes from the different specimen types (617 NP isolates, 118 isolates from sputum or tracheal/bronchial aspirates) were not described separately [20]. The absence of published data on pneumococcal serotypes in the upper and lower airways of children with non-invasive lung disease is not surprising given the difficulty in obtaining lower airway specimens from young children.

Using paired NP and bronchoalveolar lavage (BAL) specimens from 435 children with chronic endobronchial infections, and NP swabs from 1565 asymptomatic children in otitis media surveillance studies [21,22] (controls), we aimed to: (1) compare pneumococcal serotype distributions in the upper and lower airways; (2) determine the propensity of pneumococcal carriage serotypes to infect the lower airways by calculating ORs as an index of 'infectiveness'; and (3) describe serotype distributions in children vaccinated with PCV7, PCV13 and 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine (PHiDCV).

2. Methods

Paired NP swabs and BAL fluids were collected from children enrolled in ongoing prospective studies of chronic cough in Queensland (Qld) and the Northern Territory (NT), Australia. NP swabs were also collected from asymptomatic NT children in studies monitoring *S. pneumoniae* antibiotic resistance and serotypes (MARS) and otitis media [21,22]; control swabs from asymptomatic Qld children were not available. PBB, CSLD and bronchiectasis were defined as previously described [23,24]. Data on pneumococcal vaccinations and antibiotic use were collected in all studies. The Human Research Ethics Committees of the NT Department of Health and Menzies School of Health Research (HREC 05/66, 07/63, 08/83) and Qld Children's Health Services (HREC 03/17) approved the studies. In the surveillance studies, each community council provided written approval to the Ethics Committee. Written informed consent was obtained from each child's parent or caregiver.

Hospital and field specimens were collected, transported and stored in skim-milk tryptone glucose glycerol broth (STGGB) at −80 °C, as previously described [25–27]. Qld specimens frozen in STGGB were transported on dry ice to our Darwin laboratory for processing. Specimens were thawed in batches and 10 μL aliquots cultured. *S. pneumoniae* was isolated and identified using standard published methods and serotyped using antisera from Statens Serum Institute (Denmark). To detect multiple serotypes, 4 colonies (including any with differing morphologies) were selected for subculture [28]. Antimicrobial susceptibilities and minimum inhibitory concentrations (MICs) were determined as described previously [29]. Macrolide resistance and beta-lactam non-susceptibility were defined as azithromycin MIC $\geqslant$2 mg/L and penicillin MIC $\geqslant$0.12 mg/L, respectively. Lower airway infection was defined as $\geqslant 10^4$ colony forming units (CFU)/mL BAL fluid [24,25] to exclude small numbers of bacteria from possible upper airway contamination during the bronchoscopy procedure which was performed transnasally.

Stata version 14.1 (StataCorp, College Station, Texas) was used for analyses. Empirical ORs, indicating the propensity of each NP carriage serotype to cause lower airway infection, were calculated as described for invasive serotypes [12,13] as follows: OR = ad/bc (a = number of infective isolates of a specific serotype, b = number of carriage isolates of the specific serotype, c = number of infective isolates of other serotypes, d = number of carriage isolates of other serotypes). ORs were calculated for serotypes with $\geqslant$5 isolates (including at least one BAL isolate) in (1) paired NP and BAL specimens from NT and Qld children, and (2) control NP swabs and BAL specimens from NT children; Fisher's exact method was used to calculate 95% confidence intervals (CIs). Serotype diversity was calculated using Simpson's Index of Diversity (SID) [30] with 95% CIs calculated according to the method of Grundmann et al. [31].

3. Results

3.1. Study populations

A total of 435 paired NP and BAL specimens was analysed; 242 from NT children (collected July 2007-July 2015) and 193 from Qld children (July 2008-May 2014) (Table 1). NT children came from 4 towns and 31 remote Indigenous communities. Control NP swabs were collected (September 2008-August 2013) from 1565 asymptomatic NT children in 26 remote communities. The median age at diagnosis for NT children with CSLD/bronchiectasis was 2.2 years, with 92% Indigenous; the median ages of Qld children with PBB and CSLD/bronchiectasis were 1.9 and 3.9 years, with 7% and 26% Indigenous, respectively. Control children were all Indigenous with a median age of 2.1 years (Table 1). Most (>90%) children had received $\geqslant$2 doses of PCV7, PHiDCV (NT only) or PCV13; >80% had received $\geqslant$3 doses. Current beta-lactam or macrolide antibiotics were recorded for <10% Qld children; however, macrolide antibiotics (mostly azithromycin) were recorded within 2-weeks preceding bronchoscopy for >50% NT children with CSLD/bronchiectasis (Table 1).

3.2. Pneumococcal carriage, lower airway infection and NP-BAL concordance

S. pneumoniae lower airway infection was detected in 95 children; 27% of Qld children with PBB, and 22% and 20% of Qld and NT children with CSLD/bronchiectasis, respectively (Table 2). Overall NP carriage was 35% in NT children and 30% in Qld children (36% in mostly younger children with PBB); however, in children with *S. pneumoniae* lower airway infection, NP carriage was 81% and 74%,

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