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Bacterial Bronchitis Caused by *Streptococcus pneumoniae* and Nontypable *Haemophilus influenzae* in Children

The Impact of Vaccination

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Background: **Protracted bacterial bronchitis is a major cause of persistent cough in childhood. The organisms most commonly isolated are nontypable *Haemophilus influenzae* and *Streptococcus pneumoniae*. There are no studies addressing typing of these organisms when recovered from the lower airways.**

Methods: **Isolates of these two organisms (identified in BAL samples from children undergoing routine investigation of a chronic cough thought to be attributable to a protracted bacterial bronchitis) were subject to typing. Samples were collected in Sheffield, England, and Athens, Greece. The majority of the children from Sheffield had received pneumococcal-conjugate vaccines 7 or 13 (PCV-7 or PCV-13) conjugate vaccine but only a minority of Greek children had received PCV-7.**

Results: **All 18 *S pneumoniae* isolates from Greek BAL samples are serotypes contained in PCV-13 while 10 are contained in PCV-7. In contrast, 28 of the 39 samples from Sheffield contained serotypes that are not included in PCV-13. All 26 of the nontypable *H influenzae* samples obtained in Sheffield produced distinct multilocus variable-number tandem repeat analysis profiles. There was a significant difference between children from Athens and Sheffield in the distribution of serotypes contained or not contained in the pneumococcal vaccine ($P = .04$). More specifically, immunization with pneumococcal vaccine was related with isolation of *S pneumoniae* serotypes not included in the vaccine (OR, 0.021; CI, 0.003-0.115; $P < .001$).**

Conclusions: **The data suggest that both vaccine and nonvaccine *S pneumoniae* serotypes may play a role in protracted bacterial bronchitis and provide some hints that serotype replacement may occur in response to the introduction of conjugate vaccines.** ***CHEST 2013; 143(1):152–157***

Abbreviations: CF = cystic fibrosis; MLVA = multilocus variable-number tandem repeat analysis; NTHi = nontypable *Haemophilus influenzae*; PBB = protracted bacterial bronchitis; PVC = pneumococcal-conjugate vaccine

Lower respiratory tract bacterial infection may manifest as one of two discrete clinical syndromes. The first, classic bacterial pneumonia, which affects the respiratory zone of the lung, is a generally acute and potentially life-threatening illness. The second, persistent infection of the conducting airways, is quite a different form of pulmonary infection causing a chronic disease referred to as protracted bacterial bronchitis (PBB).[1-6] In PBB (defined as the presence of chronic wet/moist cough for > 4 weeks), in the absence of any alternative diagnoses (and resolution after appropriate antibiotic treatment),[1] the causative bacteria do not appear to be in a rapidly dividing planktonic state as in an acute pneumonia. Instead, the bacteria appear to exist in biofilms similar to those seen in conditions such as chronic otitis media,[7-10] in which the organisms are replicating at a low level within structures that provide protection against both host responses and antibiotic therapy. Hence, the resulting symptoms tend to be persistent with intermittent exacerbations. While not usually life threatening in the short term, colonization of the conducting airways is responsible for considerable and often unrecognized or underestimated morbidity.[11,12] Impairment of host

defenses appears to be necessary for the bacteria to become established. Impaired mucociliary clearance, especially after viral infections, seems to be a significant risk factor for the establishment of bacterial infection, whereas in some patients, varying degrees of immunodeficiency may play a role. This form of disease characterizes the bacterial lower respiratory tract infections in both cystic fibrosis (CF),[13,14] in which there is impaired mucociliary clearance probably due to collapse of the periciliary fluid layer, and primary ciliary dyskinesia. In young children much more commonly, it appears to be secondary to a significant viral lower respiratory tract infection, which is most common in the first 2 years of life and following which it is known that cilia may take many weeks to recover.[15] Other risk factors include tracheomalacia, impaired clearance associated with neuromuscular conditions, poorly controlled asthma, and aspiration.[16,17]

The organisms most commonly identified in chronic endobronchial infection are generally those also responsible for acute pneumonia in previously healthy individuals, namely *Haemophilus influenzae* (particularly nontypable *Haemophilus influenzae* [NTHi] strains) and *Streptococcus pneumoniae*.[2-6,18,19] It is known that certain serotypes of *S pneumoniae* are associated with both invasive disease and pneumonia, and these have been targeted in the 7, 10, and 13 valent pneumococcal-conjugate vaccines (PCVs) on the market. Their introduction has had a significant impact on the incidence of invasive disease and pneumococcal pneumonia, though estimates of the impact of the vaccines on the incidence of pneumonia vary from 7% to 35% depending on the rigor of the diagnostic criteria used.[20-22] Although the overall incidence of pneumococcal disease has been reduced by the introduction of these vaccines, one of the consequences of vaccination has been serotype replacement with an increase in the proportion of pneumococcal pneumonia caused by some of the many dozens of pneumococcal serotypes not covered by the conjugate vaccine.[23]

To our knowledge, there have been no studies, to date, looking at the serotypes of *S pneumoniae* associated with bacterial bronchitis. As the nature of the condition is quite different to that of pneumonia, it is possible that different serotypes to those causing pneumonia are more commonly associated with PBB. It may also be possible that the introduction of PCV will favor nonvaccine serotypes in niches such as the conducting airways, resulting in alterations in the serotypes responsible for this chronic disease. Similarly, for NTHi, there may be organism-related factors that determine the likelihood of colonization of the lower airway.

The aim of our study was to determine specific serotypes of *S pneumoniae* and NTHi in BAL samples in a childhood population with PBB in England and in Greece, and to compare the *S pneumoniae* serotypes between the two countries and between children with and without pneumococcal vaccination. These questions were explored independently in one center in Greece and one center in England. Both countries have introduced PCV. In England, PCV-7 was introduced for all children aged 0-2 years in June 2006, while in Greece coverage was not universal at the time of the study with parents having the option to purchase the vaccine.[24]

Materials and Methods

To explore the potential contribution of serotype-related factors to the likelihood of colonizing the lower airways, *S pneumoniae* cultured from BAL undertaken on children with bacterial bronchitis were sent to reference laboratories for typing. Samples of NTHi from Sheffield, England, were also typed using multilocus variable-number tandem repeat analysis (MLVA). This study involved retrospective reviews of data generated from children being investigated for chronic cough thought to be due to a PBB who had undergone bronchoscopy to confirm the diagnosis. Because of the difficulty of identifying pathogens within a few weeks of completing a course of antibiotics, the usual practice in both centers is to perform bronchoscopy on children only if they are at least 4-6 weeks free from antibiotics. The Ethics Committees of the 'Penteli' Children's Hospital and the University General Hospital Attikon (Athens, Greece), where all bronchoscopies were performed, approved the study as part of a larger research protocol (decision no. 7/03-08-07).

Data from Greek children who had PBB (according to the definition given earlier in this section) and a positive BAL culture during the period June 2005 to February 2008 were included in the study. Children with CF, immunodeficiency, neuromuscular disorder, foreign body inhalation, and primary ciliary dyskinesia were excluded from the study. Quantitative cultures for common aerobic and anaerobic bacteria, fungi, and mycobacteria were

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performed. Samples were considered to be positive for a particular bacterial species if they yielded growth of $\geq 10^5$ colony-forming units/mL or growth of $\geq 10^4$ colony-forming units/mL if only one pathogen was isolated. When *S pneumoniae* were identified, they were sent for further characterization to reference laboratories. The vaccination status for PCV-7 of all children prior to bronchoscopy was assessed. For the Sheffield leg of the study, an ethics committee approval was not necessary since this was a retrospective study, and no personal data were used.

BAL samples from children undergoing routine bronchoscopy for a clinical diagnosis of PBB at the Sheffield Children's NHS Foundation Trust in which *S pneumoniae* and/or NTHi were cultured were subsequently sent to the Respiratory and Systemic Infection Laboratory, Health Protection Agency Colindale, for further typing. Clinical diagnosis of PBB was set based on the aforementioned definition. Children with conditions such as CF, immunodeficiency, neuromuscular disorders, foreign body inhalation, and primary ciliary dyskinesia were excluded from the study. Samples were obtained during the period December 2008 to December 2010. *S pneumoniae* isolates were identified using standard methods of colonial morphology, optochin susceptibility, and bile solubility, and serotyped by slide agglutination using type-specific pneumococcal rabbit antisera (SSI Diagnostica, Statens Serum Institut). NTHi were identified and biotyped using standard methods,[25] and their species were confirmed using an *ompP2*-specific polymerase chain reaction.[26] Serotyping was performed using standard slide agglutination and confirmed by polymerase chain reaction-based capsular genotyping.[27] NTHi were typed by MLVA using the four-locus scheme of Schouls et al,[28] and types were assigned using the international reference database.[29]

For statistical analysis, comparison between groups was performed with the use of the χ^2 test. For the exploration of relations between vaccination status and pneumococci serotype isolation from BAL, we used binomial logistic regression.

Results

Sixty-five Greek children (36 boys) with PBB and a positive BAL culture were included in the study. The median age at the time of bronchoscopy was 4.8 years (range, 0.9-14.4 years). The most prevalent isolated bacterial pathogens among the Greek BAL samples were NTHi (61% of samples) followed by *Moraxella catarrhalis* (32%), *S pneumonia* (27.6%), and *Staphylococcus aureus* (6%). Polymicrobial growth was seen in 23 samples (35.4%) mostly with two species isolated; in two patients, three species were identified. Of the 39 samples from patients in Sheffield in which *S pneumoniae* was isolated, 17 contained this organism alone. NTHi was also cultured in 19 samples: one sample grew *S pneumoniae* and *M catarrhalis*, while in two cases, both *M catarrhalis* and NTHi were cultured in addition to *S pneumoniae*. Of the 26 samples harboring NTHi, this species alone was identified in 10 samples; it was cocultured with *S pneumoniae* in eight samples, with *M catarrhalis* in three samples, with group A *Streptococcus* in 2 samples, and once each with *S aureus*, group C *Streptococcus*, and group G *Streptococcus*.

Distribution of pneumococcal serotypes from the two centers is presented in Table 1. Among the 18 *S pneumoniae* typed isolates from Athens, the most prevalent pneumococcal serotypes were 6A and ^{19}F. Ten of 18 isolates were PCV-7 strains with ^{19}F accounting for one-half of these; all are included in PCV-13. Only two of the 18 *S pneumoniae*-positive children (11%) had been vaccinated with PCV-7. These two children isolated serotypes 1 and 19A in their BAL cultures. The relevant vaccination coverage among the 47 *S pneumoniae*-negative children was 34% ($P < .01$).

The samples from Sheffield included *S pneumoniae* isolated from 39 children aged 6-154 months (median, 38 months), and NTHi isolates from 26 children aged 7-145 months (median, 44 months). Only 11 of 39 samples obtained in Sheffield contained pneumococcal samples covered by the serotypes included in PCV-7 and PCV-13 which was introduced during the later period of the collection of samples. Of these 11 subjects, nine were too old to have received the relevant PCV as part of their routine immunizations. The rest of the 39 children had all received PCV vaccination.

The comparison of our data showed that there was a significant difference between the two cities in the distribution of serotypes contained or not in the pneumococcal vaccine ($P = .04$). This was due to the

Table 1—Streptococcus pneumoniae *Serotypes Isolated From BAL From Children With a Clinical Diagnosis of PBB*

Serotype	Athens (n = 18)	Sheffield (n = 39)
1[a]	2	…
3[a]	…	…
4[b]	…	…
5[a]	…	…
6A[a]	5	3
6B[b]	2	2
6C	…	3
7F[a]	…	1
9V[b]	…	…
10A	…	1
11A	…	1
14[b]	2	…
15A	…	3
15B	…	1
18C[b]	1	…
19A[a]	1	3
^{19}F[b]	5	1
21	…	2
22F	…	1
23A	…	5
23B	…	7
23F[b]	0	1
33F	…	1
34	…	1
35B	…	1
35F	…	1

PBB = protracted bacterial bronchitis.
[a]Additional serotypes in 13 valent conjugate vaccine.
[b]Serotypes in 7 valent conjugate vaccine.

different immunization rate between these two groups of children. More specifically, immunization with pneumococcal vaccine was related with isolation of *S pneumoniae* serotypes not included in the vaccine (OR, 0.021; CI, 0.003-0.115; $P < .001$).

Of the 26 NTHi isolates, eight were identified as biotype 1, nine were biotype 2, three were biotype 3, two were biotype 5, three were biotype 6, and one was biotype 7. Genetic typing using MLVA produced 26 distinct types, of which 22 have not been previously described.

Discussion

To our knowledge, this is the first study to examine serotypes of *S pneumoniae* and genetic types of NTHi isolated from the lower respiratory tract of young children with a PBB. A wide range of pneumococcal serotypes was identified in both cohorts. In England, the vast majority of serotypes were ones not contained in the available conjugate vaccines, and a minority of samples containing PCV serotypes were identified in children too old to have received the relevant vaccine. In contrast, all samples from Greece contained vaccine serotypes. During the period of sample collection, PCV-7 was available but was not part of the universal schedule; hence, only a minority of children had been immunized at that time as reflected in the levels of immunization noted from the children included in the study among whom only 18 of 65 had received PCV-7. These results suggest that the introduction of PCV-7, and subsequently PCV-13, has contributed to a change in the pattern of pneumococcal serotypes contributing to this disease through serotype replacement.[23]

Our data indicate that it is about 50 times less likely to isolate PVC serotypes from the BAL of immunized children. However, we cannot estimate from our data whether this effect is only a consequence of individual immunity or whether it is due at the same time to a concurrent change of serotype circulation in immunized populations. PCV-7 programs have had a significant impact on nonvaccinated members of the community with, for example, a significant fall in pneumococcal disease in the elderly following the vaccination programs targeting young children.[23] Unlike the impact of PCV vaccines on pneumonia and invasive disease, anecdotally, the introduction of PCVs has not impacted on the incidence of PBB, with a steady increase in referrals over the past decade continuing in Sheffield despite the introduction of PCV-7. Indeed, over a 10-year period there has been an eightfold increase in the diagnosis in Sheffield (M. L. Everard, MBChB, DM, unpublished data, March 2012). It is possible that limiting the ability of more virulent organisms to colonize the host provides a niche for less virulent organisms to occupy, with no impact on the overall incidence of *S pneumoniae* colonization or with an increase in prevalence. Virulence factors carried by the PVC serotypes often carry a biologic penalty, and it is possible that less virulent serotypes are better suited to a biofilm existence. It is also possible that it may be more difficult to eradicate nonvaccine serotypes, the more virulent and previously more successful serotypes.

The NTHi biotype and MLVA results argue against a hypothesis that a limited subset of the organism is particularly adept at colonizing the conducting airways. This is an extraordinarily adaptable bacterium, able to modify its behavior and gene expression in different environments.[30-33] On the other hand, the NTHi isolates from these patients may share a key unknown virulence factor that is carried in the bacterial population independent of genetic type.

It should be noted that, in line with conventional microbiology, a single colony from each sample was identified. It is probable that there may be more than one type of each organism within the airways of symptomatic subjects at any one time. Indeed, multiple different types of particular organisms have been found in the upper airways when protocols designed to identify a range of types have been adopted.[34-37] Furthermore, the complexity of the interplay of potential pathogens with the resident flora (other potential pathogens and within species) is just starting to be unraveled.[38-45] Traditional concepts of organisms briefly colonizing the upper airways and then disappearing without any significant impact and infection by a single serotype (or genetic type) appear to be oversimplistic and are based on findings in acute severe disease states such as pneumonia and septicemia rather than an understanding of what may prove to be a chronic biofilm-type disease such as PBB, COPD, and chronic otitis media.[7]

The data presented in the study represent data from two sources. Unfortunately, we did not have the opportunity to look at serotype replacement in populations in which vaccination has been introduced. To our knowledge, there is no published data on serotypes in this condition, and this is the first publication that attempts to address the issue. It is possible that subdata can be collected from Athens in a few years' time to compare information but, at present, there are no means of addressing the issue. Our study raises the potential importance of serotype replacement in response to conjugated vaccine development; additionally, the current PCV approach may not be appropriate for more chronic conditions.

We did not specifically look at the antimicrobial susceptibility of the isolated bacteria. Although, anecdotally, we have not seen any laboratory evidence of a change in antimicrobial susceptibility, and in our

experience these organisms continue to be susceptible to the standard antibiotics. A specifically designed study would be needed to address this issue.

In summary, the data from two populations suggest that a wide range of *S pneumoniae* serotypes and NTHi genotypes are capable of colonizing the conducting airways. Our data suggest that children with PPB immunized with PVC are highly unlikely to isolate vaccine serotypes in their BAL cultures, although it is not clear whether this is due to individual immunity or to the concurrent change of serotype circulation in immunized populations. Our results also provide a hint that serotype replacement is occurring following the introduction of conjugate pneumococcal vaccines. Whether this impacts on the overall incidence of PBB and the difficulty of treating the condition is unclear.

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Dr Priftis: contributed to the writing of the manuscript, designed and coordinated the study, performed fiber-optic bronchoscopies, and wrote and approved the final version of the manuscript.
Dr Litt: contributed to the writing of the manuscript, carried out the serotyping of isolated bacteria, and approved the final version of the manuscript.
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Dr Vogiatzi: contributed to the writing of the manuscript, carried out the BAL cultures, and approved the final version of the manuscript.
Dr Douros: contributed to the writing of the manuscript, performed fiber-optic bronchoscopies as well as data collection and analysis, and approved the final version of the manuscript.
Dr Slack: contributed to the writing of the manuscript, carried out the serotyping of isolated bacteria, and approved the final version of the manuscript.
Dr Everard: contributed by designing and coordinating the study, performing fiber-optic bronchoscopies, and writing and approving the final version of the manuscript.
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