Epidemiol. Infect. (2011), **139**, 783–790. © Cambridge University Press 2010
doi:10.1017/S0950268810001743

Serotype distribution of *Streptococcus pneumoniae* causing invasive disease in the Republic of Ireland

I. VICKERS[1,2]*, M. FITZGERALD[3], S. MURCHAN[3], S. COTTER[3], D. O'FLANAGAN[3], M. CAFFERKEY[1,2] AND H. HUMPHREYS[2,4]

[1] *Epidemiology and Molecular Biology Unit and Irish Meningococcal and Meningitis Reference Laboratory, Children's University Hospital, Dublin, Ireland*
[2] *Department of Clinical Microbiology, RCSI Education and Research Centre, Beaumont Hospital, Dublin, Ireland*
[3] *Health Protection Surveillance Centre, Dublin, Ireland*
[4] *Department of Microbiology, Beaumont Hospital, Dublin, Ireland*

(*Accepted 25 June 2010; first published online 19 July 2010*)

SUMMARY

The 7-valent pneumococcal conjugate vaccine (PCV7) was included in the routine infant immunization schedule in Ireland in September 2008. We determined the serotype of 977 *S. pneumoniae* isolates causing invasive disease between 2000–2002 and 2007–2008, assessed for the presence of the recently described serotype 6C and determined the susceptibility of isolates during 2007–2008 to penicillin and cefotaxime. Serotype 14 was the most common serotype during both periods and 7·7% of isolates previously typed as serotype 6A were serotype 6C. During 2000–2002 and 2007–2008, PCV7 could potentially have prevented 85% and 74% of invasive pneumococcal disease in the target population (i.e. children aged <2 years), respectively. The level of penicillin non-susceptibility was 17% in 2007–2008. Ongoing surveillance of serotypes is required to determine the impact of PCV7 in the Irish population and to assess the potential of new vaccines with expanded valency.

Key words: *Streptococcus pneumoniae* (pneumococcus).

INTRODUCTION

Streptococcus pneumoniae is a major cause of life-threatening infections such as meningitis, septicaemia and community-acquired pneumonia [1]. Pneumococcal pneumonia is a leading cause of death in children worldwide, with approximately 1 million deaths in children aged <5 years occurring annually. The population groups at highest risk of pneumococcal infection are young children and the elderly [2].

S. pneumoniae owes its success as a pathogen in part to the diversity of the circulating capsular serotypes. The recent identification of new serotypes, 6C and 6D, serologically cross-reactive with the 6A and 6B population, respectively [3, 4], and the identification of a new subtype (11E) within the 11A population [5], suggests that at least 92, if not 93, immunologically distinct serotypes exist, based on the chemical composition of the polysaccharide capsule [2, 4]. Immune responses elicited by current pneumococcal vaccines are directed towards the polysaccharide capsule [6].

* Author for correspondence: Dr I. Vickers, Epidemiology and Molecular Biology Unit and Irish Meningococcal and Meningitis Reference Laboratory, Children's University Hospital, Temple St, Dublin, Ireland.
(Email: imelda.vickers@cuh.ie)

The 23-valent polysaccharide vaccine (PPV23) (Pneumovax®, Merck and Co. USA), recommended for older adults and other high-risk groups aged ⩾2 years, is considered moderately effective with efficacy ranging from 50% to 80% for protection against invasive pneumococcal disease (IPD) in the elderly in developed countries [7]. However, PPV23 does not elicit a sufficient immune response in children aged <2 years to confer protection against pneumococcal infection nor does it reduce nasopharyngeal carriage and thus has no 'herd effect'. The 7-valent pneumococcal conjugate vaccine (PCV7) (Prevenar®, Pfizer Inc., USA), is immunogenic in infants despite B cell immaturity in this age group [8]. PCV7 is estimated to cover at least 50% of IPD-associated serotypes in children aged <5 years in every region of the world [9]. However, serotype distribution varies depending on population group, region and geographic location [10]. The recently licensed pneumococcal non-typable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) (Synflorix™, GlaxoSmithKline plc., UK) includes serotypes 1, 5 and 7F in addition to those already contained in PCV7. The novel carrier protein D is also considered to elicit protection against non-typable *H. influenzae* [11]. PCV13 (Prevenar 13™, Pfizer Inc.) contains the PCV7 serotypes and 1, 3, 5, 7F, 6A, and 19A.

PCV7 was introduced into the USA routine childhood immunization programme in 2000. Its effects have been well documented and PCV7 administration practically eliminated meningitis and bacteraemic pneumonia (94% decline) caused by vaccine serotypes in children aged <5 years [12, 13]. In addition, reduction in nasopharyngeal carriage of vaccine serotypes (the 'herd effect') generated by PCV7 also reduced IPD rates in adults by about 50% [14–16]. However, a potential drawback of the vaccine is an observed increase in non-vaccine-type disease [17], which seems particularly evident in the UK since the introduction of PCV7.

In Ireland, PCV7 was introduced as part of the routine infant immunization schedule in September 2008. Although PCV7 was licensed in Ireland in 2001, the vaccine was only recommended for children aged <2 years considered to be at increased risk of IPD. At that time PCV7 was not part of the universal immunization programme and the number of children vaccinated was limited (Pfizer Inc., personal communication). As a result vaccine uptake levels in the general paediatric population remained very low. In order to evaluate the impact of the introduction of this vaccine into the routine schedule in 2008, an understanding of the serotype distribution within the Irish population is essential. Here, we describe the national baseline serotype distribution of invasive *S. pneumoniae* isolates from 2000 to 2002 and from 2007 to 2008, in what can be described as the pre-PCV7 era in Ireland. In addition, all serologically confirmed serotype 6A isolates were retrospectively analysed for the presence of the recently described serotype 6C.

MATERIALS AND METHODS

Bacterial isolates

All Irish laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS) were encouraged to submit *S. pneumoniae* isolates recovered from blood and CSF for serotyping. Only the first isolate from each patient was included in the analysis. Participation in the study was on a voluntary basis. The first 30-month study period was from January 2000 to June 2002, while the second 18-month period was from April 2007 to September 2008. For the interim period between the study dates, no invasive *S. pneumoniae* isolates were received due to funding and resource constraints. All pneumococcal isolates were stored in 1:3 glycerol (nutrient broth containing 0·5% glucose and 10% glycerol) horse serum storage medium at −70 °C.

Population group

The age groups in this study were: very young children (<2 years), young children (2–5 years), older children (6–15 years), adults (16–64 years), and older adults (⩾65 years).

DNA preparation

A single colony from a fresh overnight culture grown on Columbia blood (5% sheep blood) agar plates was immersed in 20 μl Microlysis™ Plus (Microzone Ltd, UK) and processed according to the manufacturer's guidelines.

Serotyping

Multiplex PCR to detect 11 common serotypes and confirm pneumococcal identification was performed on lysed single colony extracts in duplicate. Serological co-agglutination [18] using antisera provided by the Statens Serum Institute (Denmark) was performed to

confirm the PCR results obtained and also determine the serotype of isolates not targeted by the PCR reactions.

Serotype 6C screening

All isolates identified as serotype 6A by serological methods were screened for the presence of the recently described serotype 6C using previously described primers (5'-taccatgcagggtggaatga-3′ and 5′-ccatccttc-gagtattgc-3′) [19]. Serotype 6C isolates produce a ~1·8-kb product, serotypes 6A and 6B isolates produce a ~2-kb product and serotypes 6A and 6B isolates containing the INDEL insert produce a ~2·3-kb product [19, 20]. A serotype 6C control (a kind gift from M. H. Nahm, University of Alabama at Birmingham, USA) was included in each run. The PCR reaction was performed in a 25 μl volume using the following reagents: 1x GoTaq® Flexi buffer (Promega, USA), 2·5 mM $MgCl_2$, 200 μM of each dNTP (Promega), 1 μM of each primer, 1·25 U GoTaq® Flexi DNA polymerase (Promega) and 1 μl of lysed Microlysis Plus colony extract as template. Thermocycling was performed on a PTC 200 DNA engine (MJ Research, USA) using previously described conditions [19], except the initial denaturation was at 95 °C for 2min. PCR products (10 μl) were analysed using 1% (w/v) agarose gel electrophoresis with 5 μg/ml ethidium bromide and visualized under UV transillumination. PCR product sizes were compared with a 1-kb ladder molecular size standard (New England Biolabs, USA).

Antimicrobial susceptibility

Susceptibility testing was performed using the Etest (AB bioMérieux, Sweden) method and results were interpreted following CLSI guidelines [21]. Susceptibility to penicillin was defined as follows: susceptible, MIC $\leqslant$0·06 μg/ml; intermediate, 0·12–1 μg/ml; and high-level resistant, $\geqslant$2 μg/ml; corresponding to the new oral penicillin breakpoints for comparison purposes with previous data [21]. Susceptibility to penicillin and cefotaxime were also analysed according to the current CLSI non-meningitis interpretive criteria [21]. All isolates from April 2007 to September 2008 were tested for susceptibility to penicillin and cefotaxime. *S. pneumoniae* ATCC 49619 was used as a positive control strain. The antimicrobial susceptibilities of isolates collected from January 2000 to June 2002 have been previously described [22].

Statistics

Statistical analysis was performed using Epi Info version 6 software (CDC, USA) and proportions compared using the χ^2 test and Yates' correction where appropriate.

RESULTS

Population coverage

From January 2000 to June 2002, 504/604 (83%) of IPD isolates reported through EARSS were received for serotyping and from April 2007 to September 2008, 497/593 (84%) of isolates were received. Over the years the number of laboratories reporting to EARSS have increased such that overall coverage of the Irish population has also changed (mostly increased, but occasionally laboratories failed to submit isolates for one or more quarters due to resource constraints). This affects the rates – for example, we cannot utilize the total Irish population as the denominator for the earlier years when the coverage was <50%. However, from our estimates, calculated using the appropriate census figures and adjusted based on the estimated population coverage by EARSS for each complete year, IPD incidence rates of 7·8, 8·1 and 8·8/100 000 population were observed for 2000, 2001 and 2002, respectively. IPD incidence rates of 10·5 and 10·8/100 000 population were observed for 2007 and 2008, respectively. For the first study period (January 2000–June 2002), the male:female ratio was 1·1:1 for isolates received, which increased to 1·5:1 for the second study period (April 2007–September 2008).

Serotype distribution

2000–2002

Between January 2000 and June 2002, the serotype of 480 isolates was determined (24 were non-recoverable). In total, 33 different serotypes were identified. Serotype 14 (n=68) was the most prevalent and accounted for 14% of infections, followed by serotypes 9V (9%), 1 (8·5%), 4 (8%) and 8 (6·5%) (Fig. 1). Most notably, in 2001 the proportion of serotype 1 increased significantly (χ^2=19·7, P<0·0001) to equal that of serotype 14, but there was a decrease in frequency observed in 2002 (only 6 months of the year were studied), but this was not significant (χ^2=0·51, P=0·48). In contrast, between 2000 and 2001 the frequency of serotype 12F decreased significantly (χ^2=7·03, P=0·008), and a decline in serotype 3 was also observed.

https://doi.org/10.1017/S0950268810001743 Published online by Cambridge University Press

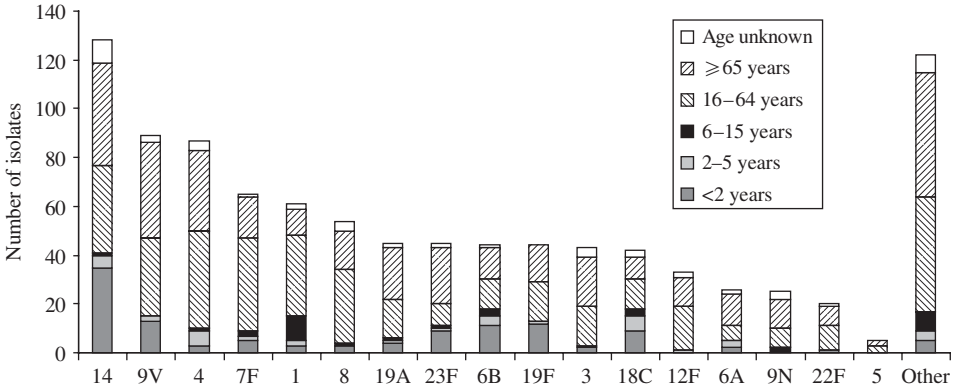

Fig. 1. Pneumococcal serotypes causing invasive disease in the Republic of Ireland prior to introduction of PCV7 vaccine. Data from 2000–2002 and 2007–2008 combined.

In addition, a significant increase ($\chi^2 = 13{\cdot}51$, $P < 0{\cdot}001$) in serotype 19F was observed in 2002, causing the most infections in older adults and as many infections as serotype 14 in very young children in that particular year.

2007–2008

From April 2007 to September 2008, 497 isolates were serotyped. Overall, 36 serotypes were identified. Serotype 14 (12%) was most common, followed by serotypes 4 (9·4%), 9V (8·8%), 7F (7·4%) and 19A (5·6%). When the two study periods were compared, an increase in the overall proportion of serotype 19A was observed, particularly in the adult population ($\chi^2 = 2{\cdot}91$, $P = 0{\cdot}08$); however, this did not reach significance. A non-significant ($\chi^2 = 1{\cdot}02$, $P = 0{\cdot}31$) overall proportional increase of serotype 7F from 5·8% to 7·4% of isolates was also observed. However, a significant increase ($\chi^2 = 4{\cdot}34$, $P = 0{\cdot}03$) in serotype 7F among the older adult population was evident. The overall proportion of serotype 1 decreased significantly ($\chi^2 = 8{\cdot}51$, $P = 0{\cdot}003$) from the first study period, similarly, the proportion of serotype 8 decreased significantly ($\chi^2 = 10{\cdot}37$, $P = 0{\cdot}001$) among older adults.

For this study, the majority of IPD isolates were from the adult population. The adult population accounted for 75% of isolates in 2000, 71% of isolates from January 2001 to June 2002 and 79% of isolates from April 2007 to September 2008. For the first study period, certain serotypes were exclusively associated with the adult population, e.g. serotypes 19A and 6A (Fig. 1). However, in the 18-month period from April 2007 to September 2008, 14% of all serotype 19A isolates were recovered from very young children. Similarly, 13% or 33% of all serotype 6A isolates were recovered from very young and young children, respectively. For the two study periods, serotype 3 ($n = 43$) predominantly infected the adult population. Only two serotype 3 infections occurred in very young children and one in an older child (aged 12 years).

Serotype 6C screening

Of the 26 IPD isolates serologically identified as serotype 6A for both study periods, 7·7% ($n = 2$) were determined to be serotype 6C. The first serotype 6C isolate was collected in October 2007 from an 85-year-old male, while the second isolate was collected in December 2007 from a 91-year-old female. Each of the serotype 6C isolates was susceptible to penicillin and cefotaxime.

Potential vaccine coverage

2000–2002

For this study period, PCV7 could, in theory, have prevented 85% of IPD in very young children ($n = 48$). For this age group, the potential coverage provided by PHiD-CV and PCV13 was 94% and 96%, respectively (Table 1). For the older adult population, coverage of serotypes contained in the conjugate vaccines during the same period was 51% for PCV7, 60% for PHiD-CV and 75% for PCV13. The coverage provided by PPV23 was 92% for older adults (Table 1). For this study period, PCV13 provided significantly more coverage (by 24%) in older adults than PCV7 ($n = 172$, $\chi^2 = 20{\cdot}98$, $P < 0{\cdot}00001$) (Table 1).

2007–2008

Between April 2007 and September 2008 (a period of 18 months), 105 cases of paediatric ($\leqslant 15$ years) IPD

https://doi.org/10.1017/S0950268810001743 Published online by Cambridge University Press

Table 1. *Number (%) of IPD isolates by age group covered by the pneumococcal conjugate and polysaccharide vaccines*

	Age group (years)											
	<2		2–5		6–15		16–64		⩾65		Overall	
Vaccine	2000–2002	2007–2008	2000–2002	2007–2008	2000–2002	2007–2008	2000–2002	2007–2008	2000–2002	2007–2008	2000–2002	2007–2008
PCV7	41 (85)	51 (74)	12 (75)	13 (59)	7 (35)	2 (14)	70 (40)	87 (42)	88 (51)	86 (47)	239 (50)	239 (48)
PHiD-CV	45 (94)	55 (80)	13 (81)	16 (73)	15 (75)	6 (43)	112 (64)	118 (57)	103 (60)	101 (55)	311 (65)	297 (60)
PCV13	46 (96)	62 (90)	13 (81)	20 (91)	16 (80)	7 (50)	127 (72)	133 (64)	129 (75)	129 (70)	362 (75)	360 (72)
PPV23			14 (88)	18 (82)	19 (95)	11 (79)	160 (91)	165 (80)	159 (92)	160 (87)	441 (92)	446 (90)

were identified. Sixty-nine of these occurred in very young children, of which 74% were covered by PCV7, 80% by PHiD-CV and 90% by PCV13. There was a small but not statistically significant decline in coverage of the conjugate vaccines for this age group compared to the first study period. For the older adult population ($n=185$), vaccine coverage was similar to that observed for the first study period, namely, 47% (PCV7), 55% (PHiD-CV), 70% (PCV13), and 87% (PPV23). Therefore, PCV13 ($\chi^2=20{\cdot}6$, $P<0{\cdot}00001$) and PPV23 ($\chi^2=66{\cdot}97$, $P<0{\cdot}00001$) provided significantly more coverage than PCV7 within this population (Table 1).

Antimicrobial susceptibility

The overall level of decreased susceptibility to penicillin from April 2007 to September 2008 was 17%. This represented an increase of 2·4% on a previous study [22]. Serotypes 9V and 14 accounted for 68% of penicillin non-susceptible (which includes both intermediate and high-level resistance) isolates. High-level penicillin resistance was evident for 7·8% ($n=39$) of isolates, and 8·7% ($n=43$) of isolates demonstrated intermediate resistance to penicillin. Eighty-one per cent of these were potentially covered by PCV7, rising to 94% for PCV13. However, according to the current CLSI non-meningitis breakpoints, 0·8% ($n=4$) and 4% ($n=19$) of isolates demonstrated reduced susceptibility to penicillin and cefotaxime, respectively; all expressing PCV7 serotypes except for one serotype 19A, covered by the PCV13 formulation.

DISCUSSION

This study represents the first comprehensive report outlining the national baseline serotype distribution of IPD in Ireland before the introduction of PCV7. For each of the two study periods, serotype 14 was the most common and accounted for 14% and 12%, respectively, of invasive isolates investigated. This is a similar finding to that of other studies and other countries [10, 23, 24]. Overall, between 2000 and 2002, serotype 14 was most common in very young children and older adults, whereas serotype 1 was most common in older children and shared the highest proportion of infections (11%) with serotype 7F in the adult population. Interestingly, serotype 1 (commonly associated with outbreaks) significantly increased in prevalence in 2001 compared to the data for 2000, and accounted for the same proportion (13·7%) of isolates as serotype 14 in that particular year. The reasons for this remain unclear. As PCV7 had yet to be introduced into the routine immunization schedule in Ireland, this increase is unlikely to be attributed to the vaccine. Similarly, in South West England the proportion of serotype 1 increased significantly between 2000 and 2005 prior to introduction of PCV7 in 2006 [25, 26]. In addition, Norway, Sweden and Denmark have reported serotype 1 as the most commonly identified IPD serotype within their respective countries [27]. In 2002, a non-significant decrease in serotype 1 was observed. This decline continued to be observed for the second study period, whereby serotype 1 was represented by 4% of isolates overall and ranked tenth in prevalence, as opposed to third for the previous study period. However, as PCV7 does not contain serotype 1, it is noteworthy that infection predominantly occurred within the adult population. Moreover, if a cyclical pattern of serotype distribution occurs (as is commonly observed with pneumococcal disease), protection would be conferred by PHiD-CV and PCV13 if future increases in serotype 1 prevalence occur.

https://doi.org/10.1017/S0950268810001743 Published online by Cambridge University Press

A significant increase in the proportion of invasive *S. pneumoniae* infections caused by serotype 19F was observed from 2001 to 2002. The main increase occurred in adults and very young children (in whom it was as common as serotype 14). However, England, Wales, Italy, and China among others, have previously identified the presence of a high proportion of serotype 19F in isolates [25, 28, 29]. An overall comparison of each study period revealed that 5·2% and 3·8% of infections, respectively, were caused by 19F. In general, 19F is one of the most commonly carried serotypes and is not considered to be particularly invasive. However, its high carriage frequency contributes to its ability to cause invasive disease [23, 30].

For the second study period, a significant increase in serotype 7F in older adults is noteworthy. Serotype 7F is highly invasive and not contained in PCV7 [9, 30]. For all other age groups, no significant changes in 7F frequency were observed. Protection against this serotype is provided by both PHiD-CV and PCV13. More recent data (2007–2008) also suggest that overall a slight increase in serotype 19A prevalence from 3·5% of infections to 5·6% of infections occurred. This increase tended to be observed within the adult (2·3–5·8%) and very young children (0–5·8%) populations, although neither increase reached statistical significance. The prevalence of 19A remained similar in the older adult population for the two study periods. Increases in serotype 19A disease following widespread use of PCV7 in the USA have been observed [31]. Concerns have been raised over 19A multidrug resistance and association with treatment failures in children with acute otitis media [32]. Many have considered the observed increase as attributable to PCV7 introduction. However, in other countries such as South Korea and Israel, increases in the prevalence of 19A were observed prior to vaccine introduction, thus, close monitoring of this serotype is warranted [33, 34].

Only two serotype 6C isolates were identified in total, both occurring in the older adult population and both susceptible to penicillin and cefotaxime. A low prevalence of serotype 6C was also observed in The Netherlands prior to vaccine introduction, although unlike Ireland these isolates were from healthy children [35]. In the USA, serotype 6C is now the predominant serogroup 6 type causing infection and is often associated with antibiotic resistance, while serotypes 6A and 6B are declining [36]. In adults, an increase in the prevalence of serotype 6C causing IPD in northern Spain was recently documented [37]. Furthermore, an increase in serotype 6C carriage has also been observed in the UK [38]. These data and that of others suggest that protection against type 6C infection is not conferred by PCV7 [39]. It is not known whether the 6A contained in PCV13 confers cross-protection against serotype 6C. Therefore, continued surveillance of this serotype is important.

This study indicates that in Ireland, the level of coverage potentially provided by PCV7 in very young children (85% and 74% in the first and second study periods, respectively) was at the higher end of the European average of 66–80% [9]. Moreover, PCV7 coverage of 85% between 2000 and 2002 is comparable to that observed in US infants in the prelicensure era [17, 40]. Furthermore, presuming herd immunity against vaccine serotypes is generated, a PCV7 effect that has been well documented [14], then additional protection against 47–51% of older adult IPD would have occurred in this country. These results are similar to those of other studies where PCV7 was estimated to cover 30–60% of isolates in the older adult population [40].

In order to compare penicillin susceptibility data with that of previous studies, results were interpreted according to the current CLSI oral penicillin breakpoints. The level of penicillin non-susceptibility (17%) observed in IPD isolates in the latest study period represents an increase of 2·4% on the previous period [22], which is moderately high compared with other European countries. A recent study in Spain revealed that the prevalence of PCV7 serotypes was the best predictor of penicillin non-susceptibility [41]. Thus, considering PCV7 serotypes accounted for 81% of penicillin non-susceptible pneumococci isolates in the present study, the rate of penicillin non-susceptibility observed in Ireland is not surprising. Thus, based on these data, one would expect antibiotic resistance levels to decline post-PCV7 vaccine introduction.

In addition, providing the efficacy of the expanded valency vaccines, PHiD-CV and PCV13 is similar to that of PCV7, a further 6–16% of IPD in very young children could have potentially been prevented based on data from the two study periods. In the older adult population, the potential protection (offered by the herd effect) for PCV13 was 75% and 70% for each study period, respectively, similar to the level observed more recently for PCV7 in infants. Although the coverage by PPV23 ranged from 80–92% in adults for each of the study periods, its protective efficacy is less than that offered by the conjugate vaccines. Additionally, vaccine effectiveness seems to diminish

with increasing age and protection against non-invasive disease is limited. Hence, the efficacy of PCV13 in the prevention of community-acquired pneumonia in the older adult population is being evaluated [42]. Despite PPV23's relative limitations, vaccination of the healthy elderly population can provide efficient protection against IPD. In Sweden, a reduction of 57% in all-cause mortality was observed in older adults participating in a combined influenza and PPV23 vaccination programme [43]. Therefore, continued surveillance is necessary to elucidate which vaccine policy is optimal at a population level.

In conclusion, it is anticipated that the introduction of PCV7 into the Irish childhood immunization programme will positively impact on IPD incidence in Ireland. Furthermore, continued surveillance of the epidemiology of *S. pneumoniae*-associated disease is a vital part of the surveillance strategy to monitor the effects of PCV7, to identify the emergence of non-vaccine serotypes and ensure adequate vaccine policy implementation.

ACKNOWLEDGEMENTS

We thank all the laboratory scientists and consultant microbiologists in Irish hospitals for forwarding pneumococcal isolates. We are also grateful for the assistance of staff in the Departments of Clinical Microbiology at the Royal College of Surgeons in Ireland and the Children's University Hospital, Dublin, Ireland. This work was supported by the Health Protection Surveillance Centre, Dublin, Ireland.

DECLARATION OF INTEREST

H.H. has or recently has been involved in research collaborations with Steris Corporation, 3M, Inov8 Science, Pfizer and Cephid. H.H. has also recently received lecture and other fees from 3M, Novartis & Astellas. M.C. has recently been in receipt of research funding from Pfizer.

REFERENCES

1. **Scott JA, *et al.*** Serogroup-specific epidemiology of *Streptococcus pneumoniae*: associations with age, sex, and geography in 7,000 episodes of invasive disease. *Clinical Infectious Diseases* 1996; **22**: 973–981.
2. **Hausdorff WP, Feikin DR, Klugman KP.** Epidemiological differences among pneumococcal serotypes. *Lancet Infectious Diseases* 2005; **5**: 83–93.
3. **Park IH, *et al.*** Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. *Journal of Clinical Microbiology* 2007; **45**: 1225–1233.
4. **Jin P, *et al.*** First report of putative *Streptococcus pneumoniae* serotype 6D among nasopharyngeal isolates from Fijian children. *Journal of Infectious Diseases* 2009; **200**: 1375–1380.
5. **Yu J, *et al.*** A rapid pneumococcal serotyping system based on monoclonal antibodies and PCR. *Journal of Medical Microbiology* 2008; **57**: 171–178.
6. **Goldblatt D, *et al.*** Antibody responses to nasopharyngeal carriage of *Streptococcus pneumoniae* in adults: a longitudinal household study. *Journal of Infectious Diseases* 2005; **192**: 387–393.
7. **European Centre for Disease Prevention and Control (ECDC).** Use of pneumococcal polysaccharide vaccine for subjects over 65 years of age during an inter-pandemic period. Technical report of the scientific panel on vaccines and immunisation, Stockholm, Sweden, 2007, pp. 1–19.
8. **Lexau CA, *et al.*** Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. *Journal of the American Medical Association* 2005; **294**: 2043–2051.
9. **Hausdorff WP, *et al.*** Pneumococcal serotype epidemiology. In: Siber G, Klugman K, Makela PH, eds. *Pneumococcal Vaccines: the Impact of Conjugate Vaccine*: Washington, DC: ASM Press, 2008, pp. 139–160.
10. **Sniadack DH, *et al.*** Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children – implications for vaccine strategies. *Pediatric Infectious Diseases Journal* 1995; **14**: 503–510.
11. **Forsgren A, Riesbeck K, Janson H.** Protein D of *Haemophilus influenzae*: a protective nontypeable *H. influenzae* antigen and a carrier for pneumococcal conjugate vaccines. *Clinical Infectious Diseases* 2008; **46**: 726–731.
12. **Black S, *et al.*** Surveillance for invasive pneumococcal disease during 2000–2005 in a population of children who received 7-valent pneumococcal conjugate vaccine. *Pediatric Infectious Diseases Journal* 2007; **26**: 771–777.
13. **Whitney CG, *et al.*** Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *New England Journal of Medicine* 2003; **348**: 1737–1746.
14. **Musher DM.** Pneumococcal vaccine – direct and indirect ('herd') effects. *New England Journal of Medicine* 2006; **354**: 1522–1524.
15. **Dagan R, *et al.*** Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. *Journal of Infectious Diseases* 1996; **174**: 1271–1278.
16. **Isaacman DJ, *et al.*** Indirect effects associated with widespread vaccination of infants with heptavalent pneumococcal conjugate vaccine (PCV7; Prevnar). *Vaccine* 2007; **25**: 2420–2427.

17. **Steenhoff AP, *et al.*** Emergence of vaccine-related pneumococcal serotypes as a cause of bacteremia. *Clinical Infectious Diseases* 2006; **42**: 907–914.
18. **Smart LE, Dougall AJ, Girdwood RW.** Pneumococcal serotyping. *Journal of Infection* 1989; **18**: 296–298.
19. **Park IH, *et al.*** Genetic basis for the new pneumococcal serotype, 6C. *Infection and Immunity* 2007; **75**: 4482–4489.
20. **Mavroidi A, *et al.*** Evolutionary genetics of the capsular locus of serogroup 6 pneumococci. *Journal of Bacteriology* 2004; **186**: 8181–8192.
21. **Clinical and Laboratory Standards Institute.** Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-S18. Wayne, PA, USA: CLSI, 2008.
22. **Clarke P, *et al.*** Antimicrobial susceptibility of invasive isolates of *Streptococcus pneumoniae* in Ireland. *Clinical Microbiology and Infection* 2004; **10**: 657–659.
23. **Brueggemann AB, *et al.*** Temporal and geographic stability of the serogroup-specific invasive disease potential of *Streptococcus pneumoniae* in children. *Journal of Infectious Diseases* 2004; **190**: 1203–1211.
24. **Bennett D, *et al.*** Penicillin susceptibility and epidemiological typing of invasive pneumococcal isolates in the Republic of Ireland. *Journal of Clinical Microbiology* 2003; **41**: 3641–3648.
25. **Sleeman K, *et al.*** Invasive pneumococcal disease in England and Wales: vaccination implications. *Journal of Infectious Diseases* 2001; **183**: 239–246.
26. **Ihekweazu CA, *et al.*** Trends in incidence of pneumococcal disease before introduction of conjugate vaccine: South West England, 1996–2005. *Epidemiology and Infection* 2008; **136**: 1096–2002.
27. **Berg S, *et al.*** Serotypes of *Streptococcus pneumoniae* isolated from blood and cerebrospinal fluid related to vaccine serotypes and to clinical characteristics. *Scandinavian Journal of Infectious Diseases* 2006; **38**: 427–432.
28. **Liu Y, *et al.*** Serotype distribution and antimicrobial resistance patterns of *Streptococcus pneumoniae* isolated from children in China younger than 5 years. *Diagnostic Microbiology and Infectious Diseases* 2008; **61**: 256–263.
29. **Marchese A, *et al.*** Antibiotic susceptibility and serotype distribution in *Streptococcus pneumoniae* circulating in Italy: results of the SEMPRE surveillance study (2000–2002). *International Journal of Antimicrobial Agents* 2005; **26**: 138–145.
30. **Brueggemann AB, *et al.*** Clonal relationships between invasive and carriage *Streptococcus pneumoniae* and serotype- and clone-specific differences in invasive disease potential. *Journal of Infectious Diseases* 2003; **187**: 1424–1432.
31. **Moore MR, *et al.*** Population snapshot of emergent *Streptococcus pneumoniae* serotype 19A in the United States, 2005. *Journal of Infectious Diseases* 2008; **197**: 1016–1027.
32. **Critchley IA, *et al.*** Prevalence of serotype 19A *Streptococcus pneumoniae* among isolates from U.S. children in 2005–2006 and activity of faropenem. *Antimicrobial Agents and Chemotherapy* 2008; **52**: 2639–2643.
33. **Hwa Choi E, *et al.*** *Streptococcus pneumoniae* serotype 19A in children, South Korea. *Emerging Infectious Diseases* 2008; **14**: 275–281.
34. **Dagan R, *et al.*** Introduction and proliferation of multidrug-resistant *Streptococcus pneumoniae* serotype 19A clones that cause acute otitis media in an unvaccinated population. *Journal of Infectious Diseases* 2009; **199**: 776–785.
35. **Hermans PW, *et al.*** Low prevalence of recently discovered pneumococcal serotype 6C isolates among healthy Dutch children in the pre-vaccination era. *Vaccine* 2008; **26**: 449–450.
36. **Carvalho Mda G, *et al.*** PCR-based quantitation and clonal diversity of the current prevalent invasive serogroup 6 pneumococcal serotype, 6C, in the United States in 1999 and 2006 to 2007. *Journal of Clinical Microbiology* 2009; **47**: 554–559.
37. **Marimon JM, *et al.*** Prevalence and molecular characterization of Streptococcus pneumoniae serotype 6C causing invasive disease in Gipuzkoa, northern Spain, 1990–2009. *European Journal of Clinical Microbiology and Infectious Diseases*. Published online: 14 May 2010. doi:10.1007/s10096-010-0947-6.
38. **Tocheva AS, *et al.*** Increase in serotype 6C pneumococcal carriage, United Kingdom. *Emerging Infectious Diseases* 2010; **16**: 154–155.
39. **Nunes S, *et al.*** Temporal trends and molecular epidemiology of recently described serotype 6C of *Streptococcus pneumoniae*. *Journal of Clinical Microbiology* 2009; **47**: 472–474.
40. **Hausdorff WP, *et al.*** Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clinical Infectious Diseases* 2000; **30**: 100–121.
41. **Fenoll A, *et al.*** Temporal trends of invasive *Streptococcus pneumoniae* serotypes and antimicrobial resistance patterns in Spain from 1979 to 2007. *Journal of Clinical Microbiology* 2009; **47**: 1012–1020.
42. **Hak E, *et al.*** Rationale and design of CAPITA: a RCT of 13-valent conjugated pneumococcal vaccine efficacy among older adults. *Netherlands Journal of Medicine* 2008; **66**: 378–383.
43. **Christenson B, *et al.*** Effects of a large-scale intervention with influenza and 23-valent pneumococcal vaccines in adults aged 65 years or older: a prospective study. *Lancet* 2001; **357**: 1008–1011.