Trends in serotypes and sequence types among cases of invasive pneumococcal disease in Scotland, 1999-2010

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Abstract

Introduction

The 7-valent pneumococcal conjugate vaccine (Prevenar®, Wyeth; PCV7) was introduced to the UK paediatric immunisation schedule in 2006. This study investigates trends in serotypes and multi locus sequence types (STs) among cases of invasive pneumococcal disease (IPD) in Scotland prior to, and following, the introduction of PCV7.

Methods

Scottish Invasive Pneumococcal Disease Enhanced Surveillance has records of all cases of invasive pneumococcal disease in Scotland since 1999. Cases diagnosed from blood or cerebrospinal fluid isolates until 2010 were analysed. Logistic and poisson regression modelling was used to assess trends prior to and following the introduction of PCV7.

Results

Prior to PCV7 use, on average 650 cases of IPD were reported each year; 12% occurred in those aged <5 years and 35% affected those aged over 65 years. Serotypes in PCV7 represented 47% of cases (68% in <5 year olds). The serotype and ST distribution was relatively stable with only serotype 1 and associated ST 306 showing an increasing trend. PCV7 introduction was associated with a 69% (95% CI: 50%, 80%) reduction in the incidence of IPD among those aged <5 years, a 57% (95% CI: 47%, 66%) reduction among those aged 5-64 years but no significant change among those aged 65 years and over where increases in non-PCV7 serotypes were observed. Serotypes which became more prevalent post-PCV7 are those which were associated with STs related to the PCV7 serotypes.

Conclusions

Routine serotyping and sequence typing in Scotland allowed the assessment of the relationship between the capsule and the clones in the post vaccination era. Changes in the distribution of serotypes post PCV7 introduction appear to be driven by associations between serotypes and STs prior to –PCV7 introduction. This has implications for the possible effects of the introduction of higher valency vaccines and could aid in predicting replacement serotypes in IPD

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is responsible for a substantial burden of disease, accounting for an estimated 1.6 million deaths annually worldwide [1]. In developed countries, the observed incidence of invasive pneumococcal disease (IPD) is between 8 and 75 cases per 100,000 individuals [2], with studies showing that the burden of disease is predominantly attributable to only around 20 or 30 of the 94~~1~~ circulating pneumococcal serotypes [3]. Furthermore, it has been observed that approximately two thirds of adult pneumococcal disease and 80% of disease in children is attributable to between only around 8 to 10 serotypes [4].

Many recent studies of serotypes involved in IPD concern the comparison of a pre-conjugate vaccination period to a post-vaccination period to examine any changes in serotype distribution likely to be related to the use of the 7-valent pneumococcal conjugate vaccine (PCV7). In the USA, and other countries subsequently, great reductions in IPD were documented which were not limited to the vaccine targeted age group [5]. However, increases in IPD caused by non-PCV7 serotypes, in particular serotype 19A, following PCV7 use have been documented [5-11].

The capsule is thought to be the main determinant for carriage prevalence and invasiveness of the pneumococcus and hence the determinant of prevalence amongst invasive disease isolates before and after vaccination [12, 13]. However, concerns have been raised that the increase in serotype 19A IPD in particular is perhaps attributable to a capsular switch event after being found associated with a sequence type (ST), ST695, which was previously only linked with vaccine serotype 4 [14, 15].

Other studies have documented increases due to the expansion of multi-drug resistant STs such as ST276 and ST320 [16, 17]. Thus, it is becoming increasingly important to examine both the STs and serotypes involved in IPD in order to determine the potential effectiveness of serotype-specific pneumococcal vaccinations.

In September 2006, PCV7 was introduced to the routine childhood immunisation schedule in the United Kingdom in a three dose program at age 2, 4, and 13 months, with a catch-up for those aged up to 2 years. This study examines the trends in serotype and ST distributions prior to the introduction of PCV7 in Scotland, adding to existing reports on the pre-vaccine period in Scotland which describe increases in serotype 1 and ST 306 [18, 19]; the effect of PCV7 on the incidence of IPD; trends in the serotype and ST distribution post-vaccination; and the association between serotype and ST pre- and post-vaccination.

Methods

Data

Data were obtained from the Scottish Invasive Pneumococcal Disease Enhanced Surveillance (SPIDER) database on all cases of IPD, identified by blood or cerebrospinal fluid, in Scotland between 1999 and 2010. The serogroup responsible for each case of disease was available for all years, whilst serotype and ST information was only available from 2002.

Clinical isolates (grown from blood or cerebrospinal fluid) of *S. pneumoniae* were sent to Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory (SHLMPRL) after identification at diagnostic microbiology laboratories in Scotland. At SHLMPRL, these isolates were grown on Columbia blood agar (Oxoid, United Kingdom) at 37^{o}C under anaerobic conditions by use of an anaerobic pack (Oxoid, United Kingdom) and after a single subculture were stored at -80^{o}C on Protect beads (M-Tech Diagnostics, United Kingdom). Isolates were serotyped by a coagglutination method described elsewhere [20]. MLST was performed as described previously [21-23].

Epidemiological years ranging from winter of one year to the end of autumn of the following year were used to ensure winter seasons were grouped together since IPD predominantly occurs during this season.

Serotypes and STs were classified according to their joint occurrence prior to the introduction of PCV7 (1999-2005) and their emergence post-PCV7 (2006-2010). STs were classified as associated with PCV7 serotypes if they occurred at least once in conjunction with one of the seven PCV7 serotypes (labelled PCV7-ST); otherwise they were classified as not associated with PCV7 serotypes (NonPCV7-ST). STs which only occurred following the introduction of PCV7 were classified separately as PostPCV7-ST. The PCV7-ST group was subdivided into two groups: a group of 12 STs (9, 36, 113, 124, 138, 156, 162, 176, 205, 206, 246, 311) with a high frequency of co-occurrence with the PCV7 serotypes (labelled HF PCV7-ST), and a larger group with low frequency co-occurrence (LF PCV7-ST). Serotypes were categorised in four different groups: PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F); serotypes not in

PCV7 but associated with STs linked through co-occurrence ~~prior~~ to the PCV7 serotypes, denoted PCV7-ST serotypes; serotypes not in PCV7 and not associated with the STs linked to PCV7 serotypes, denoted NonPCV7-ST serotypes; and serotypes which only occurred post-PCV7 vaccination, denoted PostPCV7 serotypes.

Statistical analysis

Logistic regression models were used to examine changes and linear trends in the serogroup, serotype and ST distributions, with year treated as a continuous variable ranging from 1999 to 2005. Only serogroups, serotypes and STs responsible for at least 1% of cases of IPD were considered. Analyses were carried out for the serogroups for age groups 0-4, 5-64, and ≥65 years separately. Bonferroni adjusted confidence intervals were calculated and the Benjamini and Hochberg adjustment for multiple testing used in the assessment of the significance of the linear trend [24].

Comment [KL1]: Should I add further comment that 1999 refers to year 1999/00 ("winter year") and 2005 is 2005/06?

Poisson regression models were used to assess changes in IPD incidence. The percentage change in the incidence of PCV7 serotypes and NonPCV7 serotypes from the pre-vaccine period to the post-vaccine period was assessed by predicting the post-vaccination incidence allowing for a trend in the pre-vaccination years and comparing the observed cases with the predicted as suggested elsewhere [25, 26]; 95% confidence intervals were used. Cases with missing age (27, 0.4%) were omitted. For 637 cases (10.1%), no information on the serotype or serogroup was available. The number of vaccine type (VT) or non-vaccine type (NVT) serotypes was imputed, separately by year and age group, using the observed proportions of VT serotypes. Imputation of serotype, from serogroup, was also carried out when serotype

information was not available prior to 2002. This was based upon the observed proportions of serotypes within serogroups in the period 2002-2006, separately by age group. All analysis was carried out using R versions 2.8-2.12 [27].

Results

Trends in the serotype and sequence type distributions prior to the introduction on PCV7

Between 1999/00 and 2005/06, on average approximately 650 cases of IPD per year were reported in Scotland, rising from 538 in 1999/00 to 743 in 2002/03. A subsequent drop occurred, primarily amongst those aged ≥65 years, following the introduction of the 23-valent pneumococcal polysaccharide vaccine (PPV23) in the UK for those aged at least 65 years in 2003, with a coverage of ?. This was followed by a rise to 739 in 2005/06. IPD was most common amongst the elderly during this period, with 44% of all cases of IPD identified in those aged ≥65 years. 12% of cases affected those aged <5 years.

Comment [KL2]: Stefan/Chris- any idea of coverage?

Serogroup analysis

In total, 36 different serogroups were identified in IPD between 1999/00 and 2005/06. Serogroup 14 was the most common, accounting for approximately 17% of all cases. Serogroups 9 and 1 were also common, causing around 9% and 8% of cases, respectively. Serogroup 1 replaced serogroup 14 as the most common serogroup in

2005/06. The proportion of IPD cases associated with serogroup 1 increased steadily over the pre-PCV7 study period.

There wa~~i~~s significant evidence of an increasing trend for serogroup 1 (p<0.001), (Table 1, Part A). Serogroup 14 wa~~i~~s borderline significant in the analysis for all age groups after adjustment for multiple testing, with a decreasing trend between 1999/00 and 2005/06.

Serotype analysis

Between 2003/04 and 2005/06, 42 different serotypes were identified as causing IPD. PCV7 serotypes accounted for 47% of cases in this period and were responsible for 68% of cases in those <5 years, 40% in those aged 5-64 years and 48% in those ≥65 years. The most common serotypes, 14 (15%), 1 (13%), 4 (7%), 9V (7%), 8 (6%), 3 (6%), 23F (5%), 6B (4%), 7F (4%) and 19F (4%), together account for 71% of IPD.

A statistically significant increasing trend in the distribution of IPD isolates was found in the unadjusted test for serotype 1 IPD (p=0.029). However, no other serotypes were found to have significant increasing or decreasing trends.

Sequence type analysis

The most common STs in IPD between 2003/04 and 2005/06 were 9 (9%), 306 (9%), 162 (6%), 53 (5%), 180 (4%), 191 (4%), 124 (4%), 218 (3%), 199 (3%) and 227 (3%). ST9 is commonly associated with serotype 14, with approximately 60% of serotype 14 IPD during this period identified as ST9 whilst ST306 is commonly

associated with serotype 1. There were 158 STs ~~were~~ found in IPD in 2003/04, 140 in 2004/05 and only 115 in 2005/06, showing a reduction in the diversity of STs over time.

ST306 was found to have a significant increasing trend in the distribution of IPD isolates between 2003/04 and 2005/06, comparing to the unadjusted significance level of 0.05 (Table 1, Part A). No other STs showed a significant increasing or decreasing trend.

Comment [KL3]: Remove unadjusted?

The effect of PCV7 on the incidence of IPD

Following PCV7 use, the incidence of IPD caused by PCV7 serotypes declined by 97.4% in children aged <5 years (Table 2). Among those aged 5-64 years and ≥65 years, a significant reduction of VT IPD of 86.3% and 80.4%, respectively, was observed. In those <5 years and those aged 5-64 years, there was no significant increase in NVT notifications in 2008/09 compared to the predicted incidence (Figure 1). In the population aged ≥65 years, a significant increase in NVT disease of 46.5% was observed. The reduction in VT incidence and increase in NVT incidence resulted in no change in all-type incidence in this age group.

Almost all NVT serotypes exhibited an increase in disease incidence from the last two pre-vaccination years to 2008/09 (7F: 153.6%, 3: 26.2%, 8: 42.5%, 19A: 78.7%, 22F: 151.6%, 6A: 31.8%, 12F: 2.3%, 11A: 73.9%, 9N: 33.3%). The exception is serotype 1 which showed a decrease despite the previously reported increasing trend pre-PCV7. However, only increases in 7F (128.5%; 95% CI (30%, 308.8%)) and 22F (126.7%;

95% CI (15%, 356.6%)) were found to be significant when allowing for pre-vaccination trends. The significant decrease of serotype 1 after vaccination was mainly driven by the age groups <5 years and 5-65 years.

Trends in the serotype and sequence type distribution post vaccination

Post-PCV7, seven serotypes not previously reported in Scotland were noted- 23B (12 times), 28 (6), 6C (5), 12 (1), 16A (1), 17A (1), and 35C (1), accounting for 27 of 2213 isolates typed. 164 STs which had not previously been reported were noted, amounting to 222 reports of 2203 isolates sequenced. 10% of the isolates sequenced were new STs whilst only 1% of the isolates typed gave rise to new serotypes.

Amongst the 14 serotypes each accounting for at least 1% of IPD cases post-PCV7 (Table 1, Part B), there were significant increasing trends in the distribution amongst IPD isolates for 19A and 22F and decreasing trends for serotypes 1 and 20. Serotype 1 decreased at a rate of 29% per year and serotype 20 decreased at a rate of 36% per year. Serotype 19A increased at a rate of 40% per year while 22F increased at a rate of 34% per year.

There were 11 STs which accounted for more than 1% of all STs reported among IPD cases post-PCV7. ST306 decreased significantly at a rate of 37% per year, comparable with the decrease in its associated serotype 1. ST199 and ST433 both exhibit significant increases post-PCV7 with 25% and 51% increases per year, respectively. ST199 is principally associated with serotype 19A and, to a lesser

extent, with 15B whilst ST433 is almost universally associated with serotype 22F. Serotype 20 is principally associated with ST235.

The association between serotypes and STs pre- and post-vaccination

The association between serotypes and STs in the period prior to the introduction of PCV7 is shown in Table 3. PCV7 serotypes are associated with a total of 166 STs, however only 12 STs (9, 36, 113, 124, 138, 156, 162, 176, 205, 206, 246, 311) account for the vast majority (74.3%) of the IPD cases. The PCV7 serotypes, associated with these 12 STs (labelled PCV7-HF PCV7-ST), were responsible for 779 IPD cases. A further 269 cases of IPD were caused by PCV7 serotypes associated with the remaining 154 STs (labelled PCV7-LF PCV7-ST). ~~In total, 25 serotypes (named PCV7-ST serotypes) not present in PCV7, were associated with the 166 STs linked to PCV7 serotypes.~~

Comment [KL4]: Check number

~~There are 708 entries in Table 3, of which only 25 are linked with HF PCV7-ST, 12 are high frequency STs associated with PCV7,~~ Regarding serotypes not present in PCV7, but associated with the 166 STs linked to PCV7, 25 different serotypes were responsible for 708 IPD cases, of which only 25 were linked with HF PCV7-ST. The ~~and the~~ other 683 were associated with the remaining 154 low frequency STs (cross-classification of PCV7-ST serotypes and LF PCV7-ST). The 25 PCV7-ST serotypes had~~ve~~ associations (353 cases) with 151 STs which were not directly associated with PCV7 (cross-classification of PCV7 ST serotypes and NonPCV7-ST). Finally these 151 NonPCV7-STs were associated with 22 NonPCV7-ST serotypes (145 cases) which had~~ve~~ no direct link with any ST linked to PCV7.

Trends in the distribution of groups of serotypes and STs are presented in Figure 2 and Figure 3, respectively. Both graphs show a relatively stable distribution in the pre-PCV7 period. The serotype distribution has changed in favour of those serotypes which were associated with STs shown to have had an association with serotypes in the PCV7 vaccine– the PCV7-ST serotypes. Prior to 2006/07, these serotypes formed ~40% of all serotypes but in 2009/10 they formed 80%. The NonPCV7-ST serotypes formed 6% of serotypes prior to 2006/07 and rose to 8% in 2008/09 and 11% in 2009/10. The ratio of the percentage of NonPCV7-ST serotypes to the percentage of PCV7-ST serotypes has remained relatively constant over the whole period. The ST distribution has not changed as dramatically but the 12 high frequency STs associated with the PCV7 serotypes are decreasing while the remaining low frequency STs associated with PCV7 and the STs not associated with PCV7 have increased by about 10% each. New post PCV7 STs account for ~10% of STs in 2009-10.

Field Code Changed

Discussion

Prior to the introduction of PCV7, the distribution of serotypes and STs among Scottish IPD cases was fairly static, only the proportion of serotype 1 was found to significantly increase, along with a corresponding increase in ST306, among IPD cases. Introduction of routine vaccination with PCV7 drastically reduced the burden of VT IPD in Scotland not only among children targeted for vaccination but also for the rest of the population. Little evidence of serotype replacement was found except for the elderly where the increase in NVT IPD outbalanced the decrease in VT IPD. The major replacement serotypes were 19A and 22F along with the STs 199 and 433.

The routine collection of information for both the genetic background and the expressed capsular serotype further allowed an analysis of the relationship in response to vaccine implementation. Interestingly, the proportional increase of serotypes after vaccination was greatly attributable ~~mostly confined~~ to those serotypes which are associated with PCV7 STs.

One of the key strengths of this study is that the IPD data for Scotland can be considered as a complete national data set as more than 90% of pneumococci isolated from IPD patients in Scotland are sent to the SHLMPRL [28]. Our use of logistic and poisson regression to model linear trends in the serotype and ST distribution enables the identification of changes in the serotype epidemiology. Our findings on pre and post-vaccination trends of specific serotypes and STs mainly correspond to existing literature. In particular the distribution of serotypes and STs in Scotland prior to the introduction of PCV7 has similarly been reported by Jefferies et al. [18]. Also serotype 1 bacteraemia was found to increase over time in the UK and Ireland [29] as well as serotype 1 associated IPD in England and Wales [25]. Furthermore, the increase we observe amongst the proportion of serotype 19A has been widely observed [14-17, 30-32].

Comment [KL5]: This is a bit clumsy. Suggest better phrasing?

Within four years following PCV7 use, VT serotypes were almost eliminated from IPD cases in those aged <5 years, providing clear evidence of a strong vaccine effect in the targeted age group, as has been documented in other countries [33-35]. In addition, there appears to be evidence of herd protection in those aged 5-64 years, as well as those aged ≥65 years. This corresponds with herd protection observed elsewhere, with sustained benefits of PCV7 use in preventing VT serotypes recently

documented [36]. However, among those aged ≥65 years, there is evidence of serotype replacement with an increase in NVT incidence, as was also shown in the United States and elsewhere [37, 38]. It is possible that serotype replacement in those aged over 65 years could be attributable to the introduction of PPV23 in this age group, however, it does not appear that the timing of the observed decline in VT IPD corresponds with the introduction of PPV. Among those aged <5 years and 5-64 years, the impact of serotype replacement is less clear and is masked by the effect of serotype 1 which was increasing prior to the introduction of PCV7 and then decreased. However, even accounting for this, serotype replacement in these age groups has been less pronounced in Scotland than reported in England and Wales [25] and elsewhere [39, 40]. It is not clear why the pattern in Scotland is different from that in England and Wales. A possible reason could be the replacement in the nasopharynx of Scottish residents by mainly opportunistic NVTs which predominantly cause invasive disease in those ≥65 years of age. Studying changes in nasopharyngeal carriage before and after introduction of PCV7 as done elsewhere [41, 42] could shed more light on this.

Amongst the non-PCV7 serotypes and the STs not primarily associated with these serotypes, there is some evidence of a change in the distribution. In particular, as mentioned, serotype 1 decreased following intervention and was mirrored with a decrease in the incidence of IPD attributable to ST306. The NVT serotypes, 19A and 22F were both observed to increase in IPD, whilst serotype 20 showed a significant decreasing trend. Serotypes 19A and 22F are in the group of serotypes (PCV7-ST serotypes) linked to the low frequency STs associated with PCV7 (LF PCV7-ST) and this is the group of serotypes which were shown to increase. Serotype 20 is in the

group of serotypes not linked to PCV7 by STs (Non PCV7-ST serotypes) and as a whole this group of serotypes is relatively static in comparison with PCV7-ST serotypes. This implies that there is a possible role of the ST in determining the fitness of a pneumococcus and that it may be possible to predict the serotypes which are likely to increase the most as a result of the introduction of increased valency vaccines. In interpreting these results, however, it is important to note that the STs linked to the common disease causing serotypes in the developing world do not necessarily correspond with those in the developed world (for example, outbreaks attributable to serotype 1 in sub-Saharan Africa have been found to be associated with ST 618 and 217, not 306 and 227 as in the developed world) [43]. Therefore, the results presented here may not be applicable worldwide.

The 13-valent PCV contains the seven serotypes found in PCV7, as well as serotypes 1, 3, 5, 6A, 7F and 19A. This vaccine was introduced to the paediatric vaccination schedule in the United Kingdom in 2010 and should aid in the prevention of further IPD in Scotland, however as there will be serotypes linked to those in PCV13 through STs associated with PCV13 serotypes, a change in the serotype distribution can perhaps be anticipated due to an increase in those linked serotypes. It is therefore of clear importance to continue to monitor the STs, as well as the serotypes, associated with cases of IPD to aid in determining the potential long-term effectiveness of serotype-specific vaccine interventions and to guide the development of future vaccine~~ation~~s.

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Table 1: Results from A) the logistic regression models of serogroups and STs responsible for at least 1% of IPD between 1999/00 and 2005/06 and between 2003/04 and 2005/06, respectively; B) the logistic regression models of serotypes and STs responsible for at least 1% of IPD between 2006/2007 and 2009/2010, examining evidence of significant trends in the proportion of IPD attributable to each serogroup, serotype and ST.

Part A:

Serogroup	Count	OR	95% CI	*p*-value	ST	Count	OR	95% CI	*p*-value
14	**673**	**0.94**	**(0.883, 0.997)**	**0.003**	9	213	1.06	(0.804, 1.402)	0.539
9	364	0.97	(0.892, 1.047)	0.230	**306**	**174**	**1.40**	**(1.042, 1.869)**	**0.001**
1	**331**	**1.36**	**(1.238, 1.493)**	**<0.001**	162	145	1.03	(0.741, 1.432)	0.797
6	301	0.97	(0.891, 1.057)	0.328	53	126	1.01	(0.700, 1.464)	0.924
19	290	0.98	(0.900, 1.074)	0.595	180	96	1.01	(0.678, 1.508)	0.939
4	273	1.04	(0.946, 1.134)	0.284	191	95	1.24	(0.823, 1.875)	0.134
8	247	0.95	(0.865, 1.044)	0.135	124	85	1.00	(0.658, 1.515)	0.987
23	242	0.94	(0.858, 1.035)	0.084	218	74	1.24	(0.784, 1.956)	0.183
3	220	1.00	(0.902, 1.100)	0.917	199	68	0.72	(0.444, 1.151)	0.045
7	162	1.04	(0.926, 1.167)	0.357	227	63	1.22	(0.750, 1.990)	0.244
18	158	0.98	(0.876, 1.104)	0.685	311	63	0.92	(0.561, 1.498)	0.615
12	131	1.04	(0.914, 1.183)	0.400	246	58	0.97	(0.593, 1.601)	0.883
22	107	0.98	(0.849, 1.125)	0.648	433	48	0.60	(0.314, 1.132)	0.022
20	83	0.95	(0.808, 1.113)	0.360	205	41	1.21	(0.654, 2.228)	0.386
33	71	0.94	(0.789, 1.110)	0.285	176	41	1.18	(0.620, 2.237)	0.468
11	65	0.97	(0.811, 1.160)	0.638	206	40	1.22	(0.652, 2.271)	0.372
15	51	1.01	(0.829, 1.239)	0.864	113	38	1.02	(0.530, 1.965)	0.930
					235	35	0.77	(0.390, 1.531)	0.284
					36	32	1.19	(0.572, 2.475)	0.499
					138	30	1.16	(0.581, 2.295)	0.552
					62	30	1.14	(0.531, 2.429)	0.636
					65	25	1.63	(0.691, 3.838)	0.106

Part B:

Serotype	Count	OR	95% CI	*p*-value	ST	Count	OR	95% CI	*p*-value
1	**210**	**0.71**	**(0.58, 0.86)**	**<0.001**	**306**	**139**	**0.73**	**(0.58, 0.92)**	**<0.001**
8	162	0.85	(0.68, 1.05)	0.026	191	217	1.16	(0.96, 1.39)	0.026
7F	240	1.11	(0.93, 1.34)	0.091	53	123	0.90	(0.71, 1.14)	0.195
3	173	1.00	(0.81, 1.23)	0.997	180	135	1.08	(0.86, 1.35)	0.343
19A	**165**	**1.40**	**(1.11, 1.75)**	**<0.001**	**199**	**128**	**1.25**	**(0.98, 1.58)**	**0.008**
22F	**130**	**1.34**	**(1.04, 1.72)**	**<0.001**	**433**	**88**	**1.51**	**(1.12, 2.04)**	**<0.001**
12F	79	1.10	(0.81, 1.49)	0.372	218	59	1.00	(0.72, 1.40)	0.995
6A	74	0.86	(0.63, 1.17)	0.161	227	46	0.85	(0.58, 1.25)	0.238
9N	48	0.90	(0.62, 1.32)	0.434	62	45	1.15	(0.78, 1.69)	0.306
11A	54	1.08	(0.75, 1.56)	0.514	235	23	0.80	(0.47, 1.36)	0.232

20	**35**	**0.64**	**(0.40, 1.02)**	**0.005**	65	23	0.74	(0.43, 1.28)	0.119
33F	43	1.13	(0.75, 1.70)	0.397					
15B	31	1.16	(0.72, 1.89)	0.362					
23A	28	0.96	(0.58, 1.57)	0.800					

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594 Note: Count is the number of serogroups and STs among IPD cases in the pre-PCV7
595 period in Part A and serotypes and STs among IPD cases in the post-PCV7 period in
596 Part B; OR – Odds Ratio associated with a one year change; CI- 95% Bonferroni
597 adjusted confidence interval; *p*-value is the unadjusted *p*-value. The entries in bold
598 typeface are those with *p*-values below the Benjamini and Hochberg adjusted *p*-value.

Table 2: Incidence rates of the most common non-vaccine type (NVT) IPD serotypes and vaccine type (VT) serotypes in Scotland from 2004/05 to 2009/10.

	Incidence 2004/05	Incidence 2005/06	Incidence 2009/10	Change 2009/10 predicted compared to observed	Change 2009/10 predicted compared to observed (serotype 1 excluded)
0-4 years					
All	38.23	27.35	14.18	-68.5% (-80.4, -50.0)	-69.8% (-81.7, -50.8)
NVT	12.87	7.12	13.49	-39.6% (-71.4, 28.6)	-13.4% (-62.4, 102.7)
VT	25.36	20.24	0.69	-97.4% (-99.6, -91.3)	-97.5% (-99.6, -91.4)
5-64 years					
All	8.11	9.52	6.59	-57.2% (-65.5, -46.9)	-42.1% (-54.1, -26.9)
NVT	4.73	6.22	5.92	-45.6% (-58.3, -29.1)	3.4% (-23.7, 40.3)
VT	3.38	3.30	0.67	-86.3% (-91.6, -78.4)	-87.0% (-92.0, -79.5)
65+ years					
All	31.09	32.08	26.48	-4.9% (-24.4, 19.5)	0.0% (-20.7, 26.1)
NVT	15.06	17.00	24.12	+46.5% (9.0, 97.4)	64.7% (21.4, 124.0)
VT	16.03	15.08	2.30	-80.4% (-88.6, -67.9)	-80.5% (-88.6, -68.0)

Notes: The percentage change is a comparison of the predicted incidence in 2009/10 to the observed incidence in 2009/10 adjusting for the temporal trend pre-vaccination (see Methods). 95% confidence intervals for the percentage changes are derived from the Poisson regression model. When serotype 1 is excluded, the percentage changes for VT differ slightly because inflation in this case is assumed to distribute over all types.

Table 3: The association between ST and serotype among IPD cases in Scotland in the period prior to the introduction of PCV7 in September 2006.

		STs associated with PCV7 Serotypes												STs not associated with PCV7 Serotypes
		HF PCV7-ST [12 STs]											LF PCV7-ST	Non PCV7-ST
		9	36	113	124	138	156	162	176	205	206	246	311	[154 STs]	[151 STs]
PCV7 Serotypes	PNE004	0	0	0	0	0	0	0	0	40	37	56	0	269 entries	0
	PNE06B	0	0	0	0	25	1	0	37	0	0	0	0		
	PNE09V	0	0	0	1	0	13	102	0	0	1	0	0		
	PNE014	208	0	0	84	0	4	1	0	0	0	0	1		
	PNE18C	0	0	36	0	0	0	0	1	0	0	0	0		
	PNE19F	1	0	0	0	0	0	35	0	0	0	0	0		
	PNE23F	0	32	0	0	0	0	0	1	0	0	0	62		
PCV7-ST serotypes	Serotypes not in PCV7 but associated with the STs linked to PCV7	25 entries over all serotypes and all 12 sequence types												683 entries	353 entries
NonPCV7-ST serotypes	Serotypes not associated with any ST linked to PCV7	0												0	145 entries

Serotypes not in PCV7 but associated with the STs linked to PCV7:

PNE001 PNE003 PNE005 PNE008 PNE020 PNE034 PNE038 PNE06A PNE07A PNE07C PNE07F PNE09A PNE09N PNE11A PNE15A PNE15B PNE15C PNE16F PNE17F PNE18B PNE18F PNE19A PNE22F PNE33C PNE33F

Serotypes not associated with any ST linked to PCV7:

PNE002 PNE013 PNE021 PNE024 PNE027 PNE029 PNE031 PNE037 PNE041 PNE042 PNE10A PNE10F PNE12A PNE12B PNE12F PNE18A PNE22A PNE23A PNE24F PNE28F PNE35B PNE35F

STs in the HF PCV7-ST group had more than 10 reports of co-occurrence with PCV7 serotypes. There are 779 entries for these 12 STs. In the full matrix there are 1048 reports among 166 STs associated with PCV7.

		STs associated with PCV7 Serotypes												STs not associated with PCV7 Serotypes
		HF PCV7-ST [12 STs]												LF PCV7-ST	Non PCV7-ST
		9	36	113	124	138	156	162	176	205	206	246	311	[154 STs]	[151 STs]
PCV7 Serotypes	PNE004	0	0	0	0	0	0	0	0	40	37	56	0		
	PNE06B	0	0	0	0	25	1	0	37	0	0	0	0		
	PNE09V	0	0	0	1	0	13	102	0	0	1	0	0		
	PNE014	208	0	0	84	0	4	1	0	0	0	0	1	269 entries	0
	PNE18C	0	0	36	0	0	0	0	1	0	0	0	0		
	PNE19F	1	0	0	0	0	0	35	0	0	0	0	0		
	PNE23F	0	32	0	0	0	0	0	1	0	0	0	62		
PCV7-ST serotypes	25 Serotypes not in PCV7 but associated with the STs linked to PCV7	25 entries over all 25 serotypes and all 12 sequence types												683 entries	353 entries
NonPCV7-ST serotypes	22 different Serotypes not associated with any ST linked to PCV7	0												0	145 entries

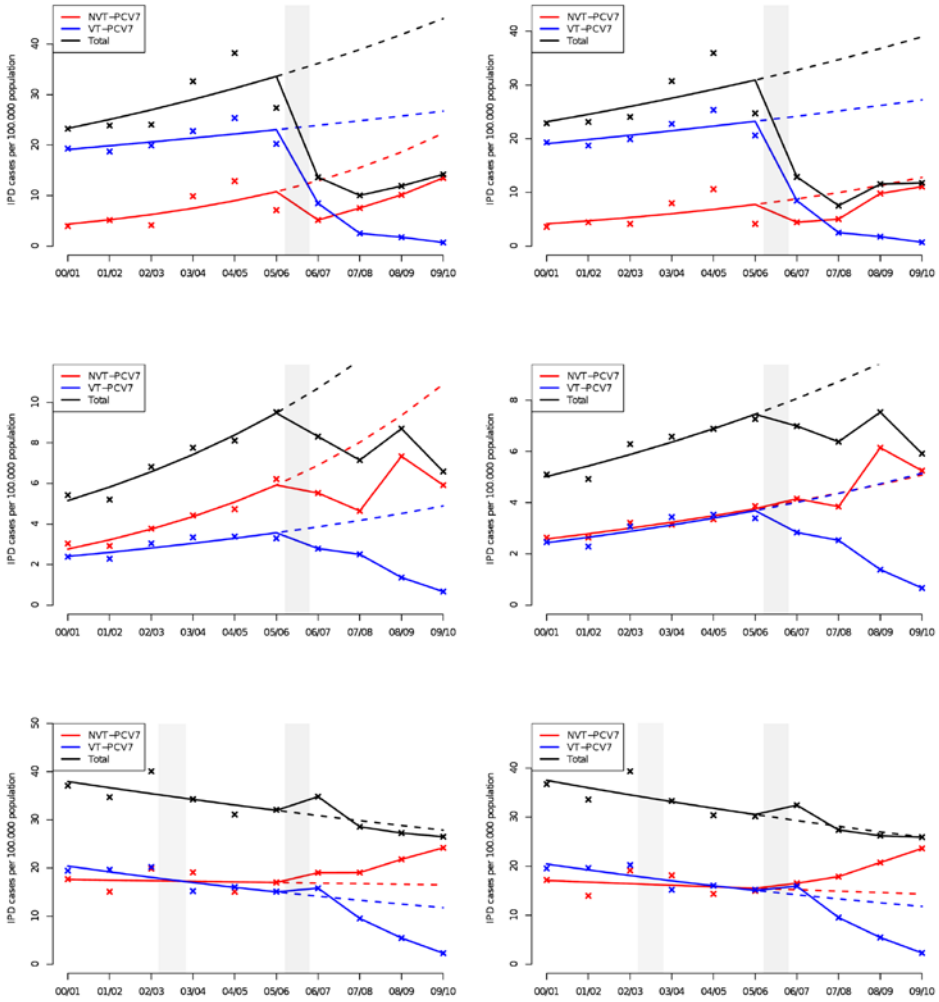
Serotypes not in PCV7 but associated with the STs linked to PCV7:
PNE001 PNE003 PNE005 PNE008 PNE020 PNE034 PNE038 PNE06A PNE07A PNE07C PNE07F PNE09A PNE09N PNE11A PNE15A PNE15B PNE15C PNE16F PNE17F PNE18B PNE18F PNE19A PNE22F PNE33C PNE33F

Serotypes not associated with any ST linked to PCV7:
PNE002 PNE013 PNE021 PNE024 PNE027 PNE029 PNE031 PNE037 PNE041 PNE042 PNE10A PNE10F PNE12A PNE12B PNE12F PNE18A PNE22A PNE23A PNE24F PNE28F PNE35B PNE35F

STs in the HF PCV7-ST group had more than 10 reports of co-occurrence with PCV7 serotypes. There are 779 entries for these 12 STs. In the full matrix there are 1048 reports among 166 STs associated with PCV7.

628 Figure 1: Incidence rates of vaccine type (VT) and non-vaccine type (NVT) IPD in

629 Scotland, by age group.



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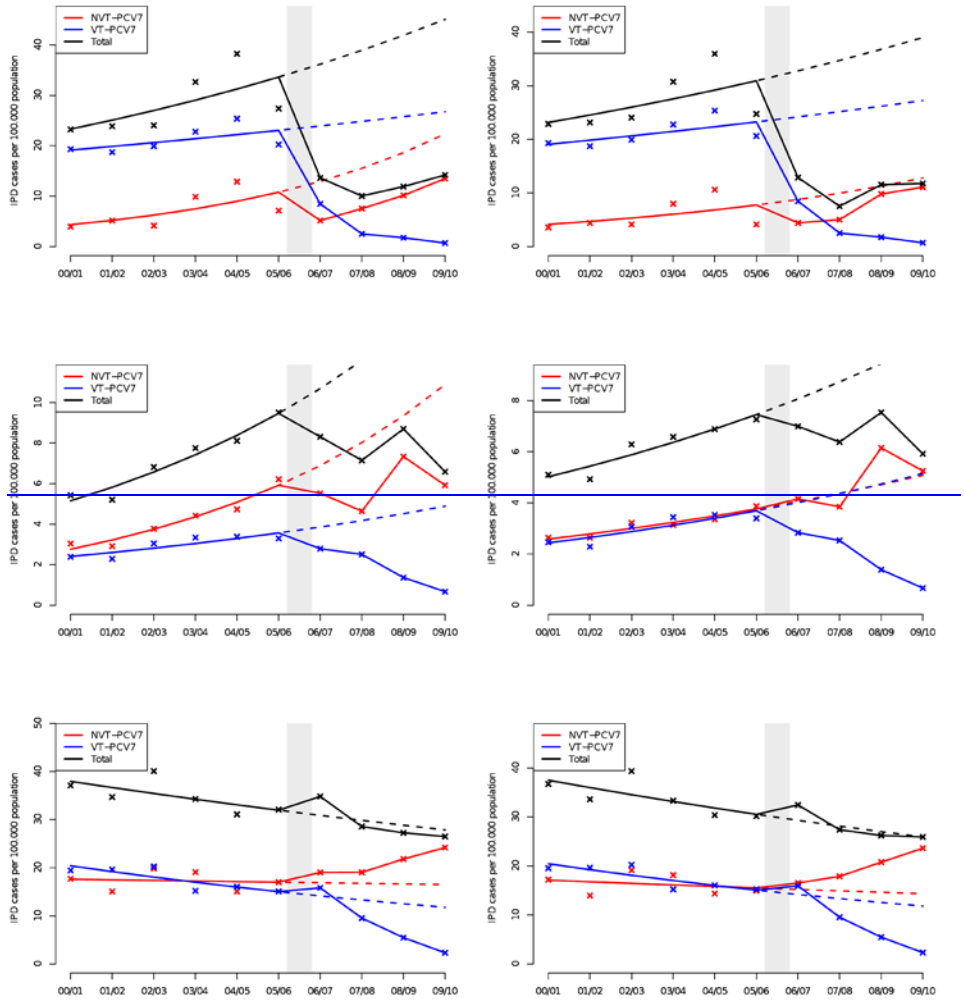
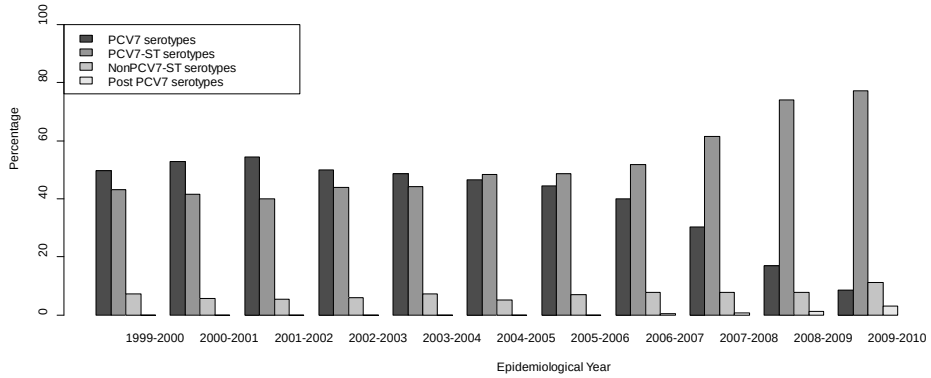


Note: All serotypes are included in the left hand column of graphs while serotype 1 is excluded from the right hand column of graphs. The top row are for those aged 0-4 years, the middle row for those aged 5-64 years and the bottom row for those aged 65+ years. The grey vertical bar denotes the introduction of PCV7. For those aged 65+ years, the first grey vertical bar denotes the introduction of PPV23. The data are plotted as crosses, the dashed lines show the predicted post vaccination incidence based on pre vaccination trends and the predicted values from the poisson regression model are the points joined by the lines.

Figure 2: Trends in the serotype distribution from 1999/2000 to 2009/2010.



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665 Note: In the period 1999-2002 when only serogroup was available, the serotypes of
666 PCV7 serogroups were imputed based upon the distribution of serotypes within
667 serogroups in the period 2003-2006. A sensitivity analysis showed that this had
668 minimal impact on the distributions presented.

669 PCV7 – serotypes in the PCV7 vaccine.

670 PCV7 serotypes – serotypes in the PCV7 vaccine.

671 PCV7-ST serotypes – serotypes not in the PCV7 vaccine but which are associated
672 with STs associated with the PCV7 serotypes.

673 NonPCV7-ST serotypes – the remaining serotypes present among IPD cases prior to
674 the introduction of PCV7. These serotypes are not associated with any ST connected
675 with the 7 PCV7 serotypes.

676 Post PCV7 serotypes – serotypes which have emerged post PCV7 (post September
677 2006).

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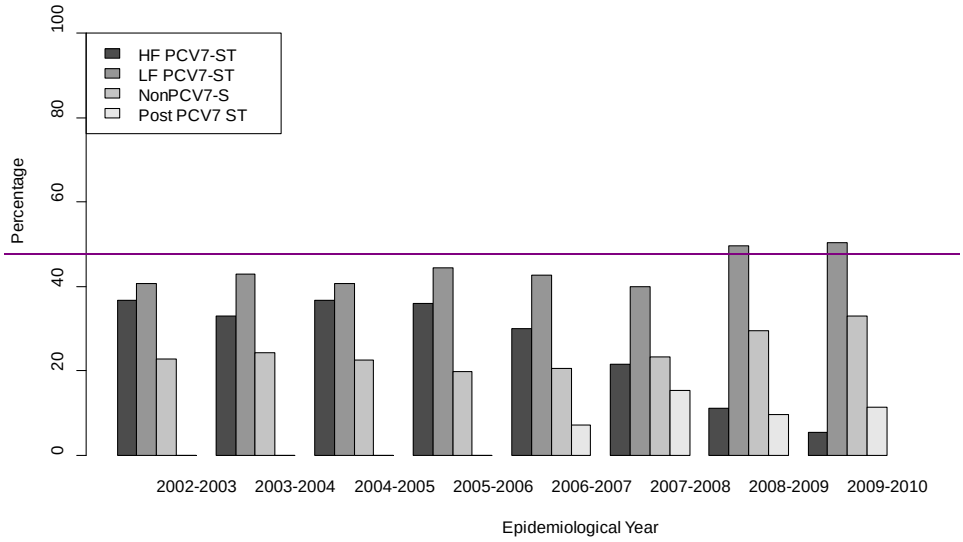
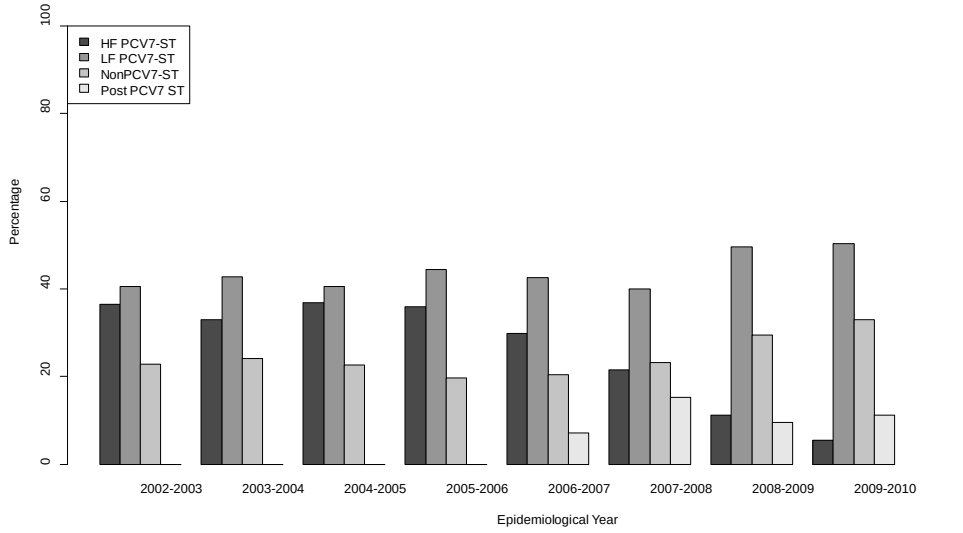
682 Figure 3: Trends in the ST distribution from 2002/2003 to 2009/2010.





Note: HF PCV7-ST~~Main PCV7~~ – The 12 STs with a strong association with the PCV7 serotypes in the PCV7 vaccine. LF PCV7-ST~~Rest PCV7~~ – The remaining STs associated with the PCV7 vaccine serotypes. NonPCV7-ST - STs not associated with any serotype in the PCV7 vaccine.

Post PCV7 – STs which have emerged post PCV7 (post September 2006).

CONFLICTS OF INTEREST:

KEL was funded through an EPSRC CASE studentship with Wyeth Pharmaceuticals. SF and MD have no conflicts to declare. DG has received funding to support a PhD studentship from Wyeth Pharmaceuticals. SCC currently receives unrestricted research funding from Pfizer Vaccines (previously Wyeth Vaccines). JMJ and SCC have received consulting fees from GlaxoSmithKline and have received financial assistance from vaccine manufacturers to attend conferences. All grants and honoraria are paid into accounts within the respective NHS Trusts or Universities, or to independent charities. JMJ, TJM, SCC, AS and GFSE previously received funding from Wyeth Pharmaceuticals for a collaborative project with the Institute of Biological Sciences, University of Glasgow and the Scottish Meningococcal and Pneumococcal Reference Laboratory (2005-2007). BD, JM and EM have no conflicts to declare. CR has received research funding from and has acted as a consultant for Wyeth Pharmaceuticals.