Evidence that pneumococcal serotype replacement in Massachusetts following conjugate vaccination is now complete

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ABSTRACT

Invasive pneumococcal disease (IPD) has been reduced in the US following conjugate vaccination (PCV7) targeting seven pneumococcal serotypes in 2000. However, increases in IPD due to other serotypes have been observed, in particular 19A. How much this "serotype replacement" will erode the benefits of vaccination and over what timescale is unknown. We used a population genetic approach to test first whether the selective impact of vaccination could be detected in a longitudinal carriage sample, and secondly how long it persisted for following introduction of vaccine in 2000. To detect the selective impact of the vaccine we compared the serotype diversity of samples from pneumococcal carriage in Massachusetts children collected in 2001, 2004, and 2007 with others collected in the pre-vaccine era in Massachusetts, the UK, and Finland. The 2004 sample was significantly ($p > 0.0001$) more diverse than pre-vaccine samples, indicating the selective pressure of vaccination. The 2007 sample showed no significant difference in diversity from the pre-vaccine period and exhibited similar population structure, but with different serotypes. In 2007 the carriage frequency of 19A was similar to that of the most common serotype in pre-vaccine samples. We suggest that serotype replacement involving 19A may be complete in Massachusetts due to similarities in population structure to pre-vaccine samples. These results suggest that the replacement phenomenon occurs rapidly with high vaccine coverage and may allay concerns about future increases in disease due to 19A. For other serotypes, the future course of replacement disease remains to be determined.

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Introduction

Conjugate vaccination against seven serotypes of *Streptococcus pneumoniae* (the pneumococcus) has had great benefits for public health. In the US between 1998–1999 and 2003, following vaccine licensure and despite vaccine shortages, the incidence of invasive pneumococcal disease (IPD) due to vaccine serotypes among children under 5 fell by 94% (Anon, 2005). Moreover, the interruption of pneumococcal transmission within this core group has led to a substantial reduction in IPD due to vaccine types among unvaccinated older adults and the population at large (Lexau et al., 2005).

Despite this success, concern has been raised that these public health benefits are being eroded by the phenomenon of serotype replacement (Hanage, 2008; Spratt and Greenwood, 2000). This refers to the fact that the vaccine targets only seven of the more than 90 known pneumococcal serotypes. As well as protecting against IPD, vaccination also prevents carriage of vaccine serotypes (Ghaffar et al., 2004) and so removes these from the population. If vaccine and non-vaccine serotypes compete in the human nasopharynx (their normal anatomical niche) then this will produce an opportunity for those serotypes not in the vaccine to benefit from the removal of any such competitors, and increase in prevalence.

Such serotype replacement has been documented in carriage (Ghaffar et al., 2004; Huang et al., 2009, 2005) and a number of disease contexts (Byington et al., 2005; Eskola et al., 2001). However replacement has, thus far, been limited in invasive disease (Hicks et al., 2007). The incidence of a small number of non-vaccine serotypes, notably 19A and serogroup 15 strains has been increasing in IPD (Hicks et al., 2007). Prior to vaccination, the incidence of IPD due to 19A was estimated at 2.5/100,000 per year in the vulnerable under 5 age group. By 2004 in the same age group it was estimated to have increased to 7.8/100,000 per year, equivalent to a relative risk for IPD due to 19A of 3.2 (95% CI: 2.3–4.4) in comparison with the pre-vaccine era (Hicks et al., 2007). In some particularly vulnerable populations, the overall 19A disease rate can be much higher (Singleton et al., 2007).

A question of pressing public health importance is whether we are only seeing the start of this phenomenon. Will the increase in IPD due to replacement serotypes continue as the pneumococcal population responds and adapts to the presence of vaccination? Or has the population already reached a new equilibrium, and so future changes will be relatively small in comparison with those that have already occurred?

Previous analyses have focussed on longitudinal trends in carriage or invasive disease rates to detect and analyze serotype replacement (Hicks et al., 2007; Huang et al., 2005). Here we present an alternative approach based on changes in the observed diversity of serotypes, hypothesizing that this is a sensitive measure of the selective effects of the vaccine on the pneumococcal population as a whole, rather than individual serotypes. We use longitudinal data from Massachusetts (MA) to show the effect of vaccination on the diversity and rank-frequency distribution of serotypes in carriage.

Methods

Samples

Pre-vaccine communities

We use three data sets. As an indicator of the baseline serotype distribution in Massachusetts we use a sample of 71 carried isolates from children between 3 months and 6 years of age with acute otitis media (AOM). These were collected in 1998 and 1999 from practices in two Massachusetts communities (Pelton et al., 2004). They are taken as representative of the serotypes in NP carriage (since there are small differences in the ability of colonizing serotypes to cause AOM Hanage et al., 2004). Serotyping was carried out in the Maxwell Finland Laboratory at Boston University School of Medicine. For further comparison we use two additional samples, comprising isolates from nasopharyngeal (NP) swabs from healthy children <2 years of age in Oxford, UK and Tampere, Finland (Brueggemann et al., 2003; Hanage et al., 2004). To ensure that a single isolate was included per episode of carriage, we used multilocus sequence typing (MLST) (Enright and Spratt, 1998) data, which was available for all three pre-vaccine data sets. Where more than one isolate with the same serotype and sequence type (ST) was retrieved from the same child, all but one was excluded (leading to the exclusion of 18 isolates from the 1998–1999 MA sample; 69 isolates from the Tampere, Finland sample; and 69 isolates from the Oxford, UK data set). For the latter two data sets the resulting samples are as described previously Fraser et al., 2005). The sizes of the data sets used in this work are Tampere, $N = 216$; Oxford, $N = 228$; MA 1998–1999, $N = 71$.

Longitudinal sampling of post-vaccine cohorts

Serial samples of children <7 years of age were collected in 2001, 2004, and 2007 (described in detail in Hanage et al., 2007; Hicks et al., 2007; Huang et al., 2005) from children seen in pediatric practices in 8 Massachusetts communities. Eight additional communities were sampled in 2001 and 2004 but have been excluded from this analysis to ensure consistency. The overall carriage prevalence of pneumococcus did not change greatly over the three study periods (Huang et al., 2009). Final sample sizes were given as follows: 2001, $N = 124$; 2004, $N = 220$; 2007, $N = 295$.

For samples to be comparable, they must be serotyped to the same level of discrimination. For example, we cannot compare data sets that pool all serogroup 19 isolates together with those that do not. Because 15B and 15C strains have been found to interconvert with high frequency (van Selm et al., 2003), these have been pooled together as 15B/C in all samples including those from post-vaccine communities (see below). For those cases in the MA 1998–1999 data set where isolates were unavailable for testing to a finer level of discrimination, the MLST data were used to determine the most likely serotype based

on the relationship of the isolate to the rest of the sample and the MLST database. For example, isolates recorded as serogroup 15 with no further discrimination, and ST 199 by MLST, were assumed to be 15B/C because in all previously recorded cases of this ST expressing serogroup 15 capsule, the subtype has been found to be 15B/C. In one case where this was not possible (ST 1910 is a singleton that does not have any close relatives in the MLST database), the isolate was removed from the data set.

Statistical analysis

The diversity of each data set was estimated using Simpson's index of diversity D (Simpson, 1949); defined here as $D = \frac{N}{N-1} \left(1 - \sum_{i=1}^{m} x_i^2\right)$ where $x_i = \frac{n_i}{N}$ is the fraction of the sample with serotype i, m is the total number of serotypes, and N is the sample size. The choice of measure was motivated by analogy with population genetics, where D is equivalent to the heterozygosity of a sample, and here is the probability that two randomly selected isolates have different serotypes. $\frac{N}{N-1}$ is a correction for sample size. Variances and 95% confidence intervals for each sample were calculated as previously described (Simpson, 1949). Since diversity did not differ between the baseline pre-vaccine era samples (see Table 1), we pooled the pre-vaccine samples by summing the number of isolates with each serotype across the three samples, and dividing by the combined sample size of 515 isolates (from the 1998–1999 MA sample combined with the Tampere and Oxford data sets) to compare diversity D between the pre- and post-vaccine samples. To test whether estimates of D were significantly different p-values were calculated by two-tailed Welch's t-test. We also tested the effect of pooling by rank (i.e., combining the numbers of isolates in the category of most common serotype, second most common serotype, etc.) and obtained similar results to those reported below (not shown).

To further explore the selective impact of vaccination, we calculated the rank-frequency distribution of each sample. Specifically, if $\{x_1, x_2, ..., x_m\}$ denotes the distribution of serotype frequencies, then we denote the permutation of this distribution reordered from largest to smallest as $\{x_{[1]}, x_{[2]}, ..., x_{[m]}\}$, which is called the rank-frequency distribution. We do this ranking in turn for each sample, and note the strong similarity between all the pre-vaccine era samples despite differences in serotype composition (Fig. 1a and b). To obtain a range of rank-frequency distributions to compare with the vaccine era samples, we simulated 1000 Monte Carlo replicates drawn from the empirically observed rank-frequency distribution of the pooled pre-vaccine samples. All procedures were carried out in Microsoft Excel 2007.

Results

Comparison of serotype diversity in pre-vaccine populations

To characterize the pneumococcal population prior to vaccination, we used three independent samples from similar age groups typed to the same level of discrimination (namely Oxford, UK Meats et al.,

Table 1
Diversity of samples used in this study.

Sample	N	No. of serotypes	D (95% CI)
Finland	216	28	0.911 (0.896–0.926)
Oxford	228	34	0.922 (0.905–0.938)
MA 1998–1999	71	20	0.918 (0.889–0.946)
Combined pre-vaccine	515	40	0.919 (0.909–0.929)
MA 2001	124	24	0.941 (0.918–0.964)
MA 2004	220	29	0.946 (0.934–0.958)
MA 2007	295	32	0.923 (0.912–0.935)

N is the total number of isolates after censoring as described in the Methods section. D is the observed diversity. 95% CIs were calculated by the method of Simpson (1949).
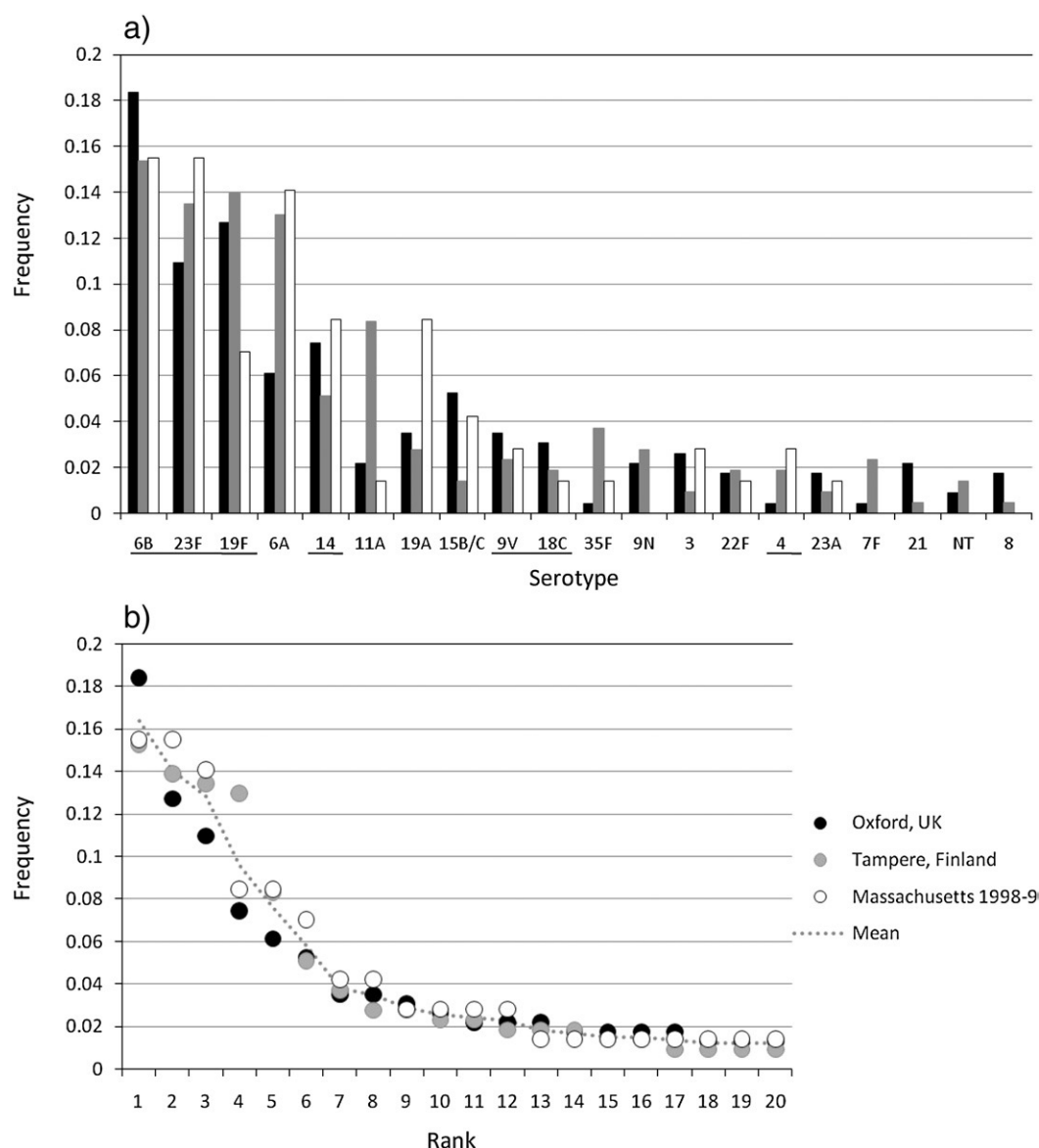
Fig. 1. (a) Frequencies of individual serotypes in the three pre-vaccine data sets. Sample details are described in the main text and Table 1. For reasons of space only the 20 most common serotypes in the combined data set are shown. Vaccine serotypes are underlined. (b) The same data arranged by rank (most–least common).

2003; Tampere, Finland Hanage et al., 2004, and Massachusetts 1998–1999). Details of all samples used in this work are provided in Table 1. Fig. 1a compares the frequency of individual serotypes in the different locations (for reasons of clarity, only the 20 most common are shown). While 6B was the most common in all three, marked differences were observed (for example in the frequencies of 19F, 6A, 11A, and 19A). However the rank-frequency distribution of serotypes, shown in Fig. 1b, is strikingly similar. In none of these samples does the most common serotype make up more than 20% of the population. Also shown in Fig. 1 is the mean rank-frequency distribution, calculated as the sum of each serotype in the combined data set, divided by the combined sample size of 515, and arranged by rank. The diversity of this combined data set was 0.919 (95% CI: 0.909–0.929), with the diversity of individual data sets shown in Table 1.

Diversity and rank-frequency serotype distributions following vaccination

All three samples collected following vaccination are more diverse than the pooled pre-vaccination data set (see Table 1), however only the 2004 sample is significantly so (p > 0.0001). While a similar increase in diversity is apparent in 2001, this is not significant, possibly due to the small size of this sample. The rank-frequency distributions for each sample, calculated as previously, are shown in Fig. 2b–d, along with that from the 1998–1999 MA sample, which is reproduced from Fig. 1b for purposes of comparison (Fig. 2a). For each sample, the median and 95% intervals of 1000 samples of the same size drawn from the empirical pre-vaccine distribution are also shown. The greater diversity of the 2004 sample is reflected by the pronounced flattening in the rank-frequency distribution (Fig. 2c). Although serotype replacement has led to changes in the specific serotypes that compose the 2007 sample (the largest single sample N = 295), its diversity is not significantly different from that of the pre-vaccine samples (see Table 1), while the frequency of the most common serotype (19A) and the rank-frequency distribution appear similar to that expected from the pre-vaccine data sets (Fig. 2d).

Replacement

Serotype replacement refers to the expansion of non-vaccine serotypes as a result of the removal from the population of vaccine types that compete with them to colonize new hosts. The major
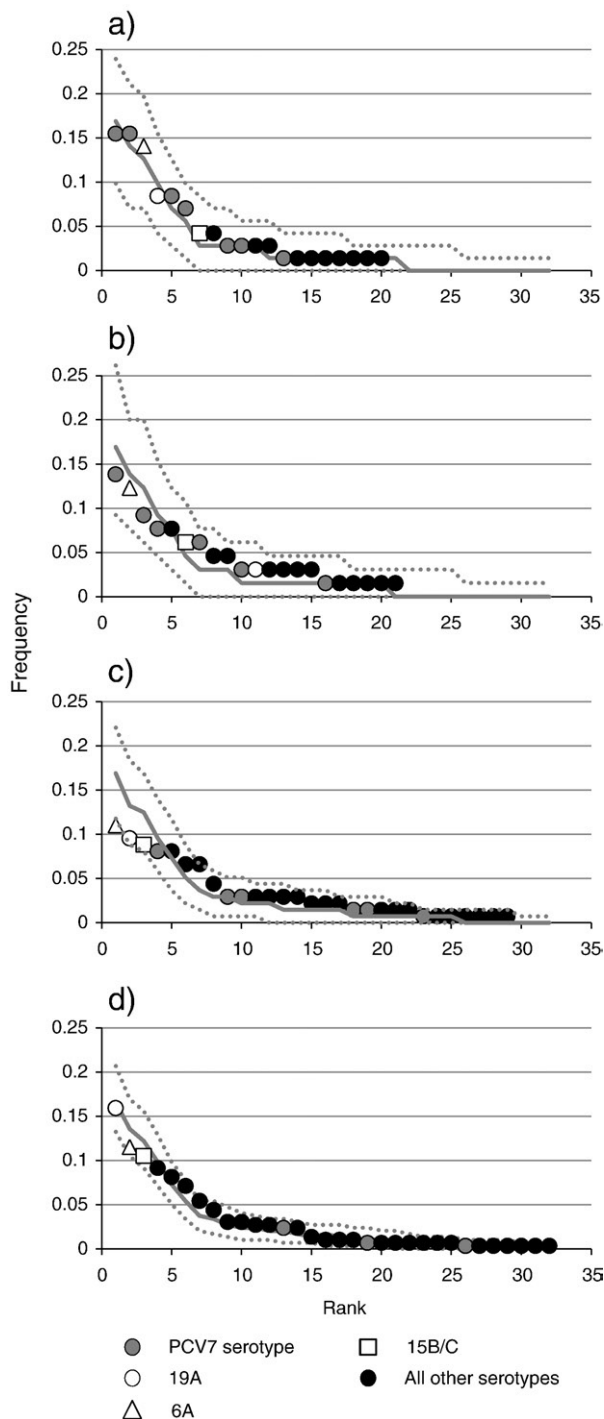
Fig. 2. Rank-frequency distributions of serotypes in the MA samples from (a) 1998–1999, (b) 2001, (c) 2004, and (d) 2007. Details of the samples are as described in the text and table. Serotypes are shown as indicated in the legend. The median values obtained from 1000 samples of the same size drawn from the combined pre-vaccine distribution (as described in the text) are shown as a solid line with dashed lines either side representing the 5th and 95th percentiles for each point. Larger numbers of serotypes in the later samples reflect larger sample size.

replacement serotypes in the carriage studies described here, thus far, have been serotypes 6A, 19A, and 15B/C. The serial increase of these has been previously documented in these communities (Huang et al., 2009, 2005), and their expansion may be clearly observed in Fig. 2. In contrast, by 2007 the pooled vaccine serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F and shown in gray) were responsible for a small fraction of carriage. Note that over the period of the study the overall carriage

prevalence did not change (1998, 21.6%; 2001, 25%; 2004, 24%; 2007, 31%; Huang et al., 2009).

Discussion

We have found that we can detect the impact of selection, due to conjugate vaccination, on the population structure of carried pneumococci by comparing three longitudinal samples collected post-vaccination with samples collected in the absence of vaccination. The samples collected from MA in 2001, 2004, and 2007 document the change produced by vaccination. The 2004 sample is significantly more diverse than the pre-vaccine samples (with a non-significant change in the same direction in 2001). The pre-vaccine samples are similar to each other in terms of serotype diversity (Table 1). Data from the National Immunisation Survey (http://www.cdc.gov/vaccines/stats-surv/imz-coverage.htm) show that vaccine coverage in MA was relatively low in 2001 and 2002, as most toddlers were born before the vaccine became available and "catch up" vaccination was incomplete. The first time point for which data are available is July 2001–June 2002, when coverage with three doses of vaccine was $35.8 \pm 5.1\%$ among children aged 19–35 months. By 2004 coverage in the same age group had reached $89.7 \pm 3.8\%$ and has not dropped below 90% since. It is reasonable therefore to suggest that the data from 2004 reflect the period at which the pneumococcal population was in the process of responding to the novel selective pressure of the vaccine, and it is this that produces the comparatively flat distribution observed as vaccine serotypes, initially common, are selected against (clearly evident in the decline of the gray points in Fig. 2) and non-vaccine serotypes experience a relative fitness advantage. The results show that the 2004 sample is more diverse than expected from samples in the absence of vaccination ($D = 0.946$; 95% CI: 0.934–0.958).

In contrast by 2007 the distribution has regained many of its pre-vaccine characteristics, in both serotype diversity and the rank-frequency distribution of serotypes (Table 1 and Fig. 2d). It should be noted however that although we did not find the 2007 sample to be significantly different from the pre-vaccine observations, this could be because of limitations in the power of the test used here. Nevertheless the similarity between the rank-frequency serotype distributions of the 2007 sample and those observed in pre-vaccine samples is pronounced (Fig. 2d). In this most recent sample, the most common serotype in 2007 is 19A, now a relatively common cause of invasive disease (Hicks et al., 2007). The vaccine serotypes, which together were responsible for the majority of isolates in all pre-vaccine samples (56% in Oxford; 53% in both Finland and MA), have dwindled to a small fraction of their previous prominence. There has been considerable concern about the future trajectory of replacement disease due to 19A strains: do we expect that in the absence of vaccination against 19A they would continue to expand? Based on these observations we suggest that, at least in MA, this is unlikely. Given that carriage rates remain similar to the pre-vaccine era (data not shown), by 2007 19A is already as common as the most common serotypes in the pre-vaccine population, making up ~20% of the carriage population. If this empirically observed limit in the frequency of the most common serotype is maintained in the post-vaccine era, further large increases in the carriage prevalence of this serotype are unlikely. Likewise in consequence, large increases, compared to current rates, in invasive disease due to 19A should not be expected in the coming years.

This study has several shortcomings. It would be preferable to have a larger sample for 2001. The relatively small sample size at this time point and 2004 is due to the exclusion of data from the 8 additional communities not sampled in 2007, in order to ensure consistency. Inclusion of these communities does not alter the results (not shown). Another concern is that the only pre-vaccine sample available for MA is from NP carriage in children with AOM. Those serotypes causing AOM may not be representative of those in carriage, but the differences are expected to be minor in the light of a study showing that the serotypes in

AOM are a relatively good reflection of NP carriage, in comparison with IPD (Hanage et al., 2004). Furthermore the children in question are not necessarily suffering from pneumococcal AOM. We hence use this sample as an approximation to the pre-vaccine carriage population in MA. It is gratifying to note that the serotype diversity D is similar to other pre-vaccine populations and the fit to the empirical rank-frequency distribution is a good one (Table 1 and Fig. 2).

In addition to this it should be noted that the clonal composition of 19A could continue to change to include larger numbers of antibiotic resistant or non-susceptible strains (Hanage et al., 2007). This could have a substantial clinical impact. It must also be emphasized that small fluctuations in the carriage prevalence of a rarely carried but highly invasive serotype (such as serotype 7F) could have a substantial impact on overall IPD rates, and that such changes would not be apparent in analyses of carriage serotype diversity such as this one. We have made no assumptions in this study regarding the reasons for the success of 19A, but it may be related to the relatively high frequency of penicillin non-susceptibility in clones of this serotype (Pelton et al., 2007). We suggest that 6A strains, which in Fig. 2 appear well positioned to expand, have not done so because of confusion with the newly discovered serotype 6C (Nahm et al., 2009). Until recently, it was not possible to distinguish these serotypes by usual quellung reaction, but they nevertheless differ markedly in terms of vaccine efficacy: 6A appears to cross react with the 6B component of PCV7 and has been declining since vaccine introduction whereas no such decline has been noted for 6C (Park et al., 2008). We suggest therefore that our data reflect a decrease in 6A, and replacement with 6C, but because the methods used here were unable to distinguish between these two serotypes 6A prevalence apparently remains reasonably constant. Finally, the data shown in Fig. 1 clearly show that even prior to vaccination considerable variation in the prevalence of individual serotypes could exist among different communities.

New vaccines with higher valency that target 19A strains (among other serotypes) will be available in the near future. We would predict that the impact of these will be similar to that which has been seen with the current conjugate vaccine, removing vaccine serotypes over a period of 4–6 years, with replacement occurring on the same timescale. The effect on IPD will depend on the invasive nature of the replacing serotypes though we propose that no serotype is likely to increase to cause >20% of carriage episodes. The precise rank order of the serotypes cannot be predicted with confidence at this stage, but it is reasonable to suppose that presently common non-vaccine serotypes will be well represented. Finally, we do not wish to suggest that once replacement has occurred, substantial secular changes are still possible in terms of the precise serotype composition of different communities, such as have given rise to the differences between unvaccinated communities seen in Fig. 1a.

In conclusion, we have used a simple test to demonstrate the selective impact of vaccination on the carried population of pneumococci. In 2004 differences in population structure are evident, but by 2007 the distribution of serotype frequencies is not significantly different from that observed in three pre-vaccination samples. We suggest that this means we are unlikely to see any great further increase in the common carriage serotypes, either in carriage or disease. Of course, this hypothesis will be tested through ongoing monitoring of IPD serotypes in Massachusetts. The impact of the next generation of vaccines, which will provide an opportunity for rarer serotypes, remains unknown.

Acknowledgments

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