# **Using pneumococcal carriage data to monitor post vaccination changes in pneumococcal otitis media incidence.**

Running title: Monitoring pneumococcal OM via carriage

Authors: Stefan Flasche\*1, Noga Givon-Lavi2,3 , Ron Dagan2,4

Affiliations: 1 London School of Hygiene and Tropical Medicine, Department of Infectious Disease Epidemiology, London, UK; 2 Soroka University Medical Center, Beer Sheva, Israel; 3 Ben-Gurion University of Negev, Beer Sheva, Israel; 4 Ben-Gurion University of Negev, Beer Sheva, Israel

Corresponding Author\*: Dr. Stefan Flasche, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, WC1E 7HT, London, UK, Stefan.Flasche@lshtm.ac.uk

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Abbreviations*:*

IPD Invasive pneumococcal disease

MEF Middle ear fluid

NP Nasopharyngeal

NVT Non-vaccine serotype

OM Otitis Media

PCV“x” Pneumococcal conjugate vaccine containing antigens against “x” serotypes

VT Vaccine serotype

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**Abstract**

Pneumococcal conjugate vaccines (PCVs) have substantially reduced the burden of pneumococcal disease including otitis media (OM). However, in most countries no pneumococcal OM surveillance exists to monitor the change in OM incidence after introduction of PCVs. We explore whether pneumococcal carriage is a useful surrogate to monitor post vaccination pneumococcal OM changes. PCV7 was introduced to the Israeli national immunisation programme in July 2009 and gradually replaced by PCV13 from November 2010. Since 2009 nasopharyngeal swabs have been obtained on a daily basis from the first 4 Bedouin and 4 Jewish children <5 years old who attended a paediatric emergency room in Southern Israel. During the same time OM surveillance in Southern Israel included all children <2y who were diagnosed with OM resulting in middle-ear fluid (MEF) culture. The relative change in vaccine serotype group (VT) carriage prevalence was predictive of the relative change in VT OM incidence. However, the serotype replacement observed in non-VT carriage is not paralleled in OM. This could hint at more complex mechanisms of the immune response to prevent initial and consecutive episodes of OM which has been changed through declining prevalence of the most virulent serotypes as a result of vaccination.

INTRODUCTION

Pneumococcal conjugate vaccines (PCVs) have substantially reduced the global burden of pneumococcal disease which is mainly composed of invasive pneumococcal disease (IPD), non-bacteraemic pneumococcal pneumonia and pneumococcal otitis media (OM) (1–3). While OM is the least severe of the pneumococcal diseases, PCVs have prevented a disproportionally high amount of OM disease episodes if compared with non-bacteraemic pneumonia or IPD. For example, in the US Prevenar 7TM (Pfizer, New York City, New York) (PCV7) has prevented 129 hospitalisations due to IPD per 100,000 children per annum in the 3 years after PCV introduction and about 500 hospitalisations due to non-bacteraemic pneumonia per 100,000 children per annum (4) while preventing 92,900 ambulatory visits attributable to OM per 100,000 person-years in children 4 years after PCV7 introduction (5).

Post-PCV implementation surveillance on pathogens causing OM, particularly *S. pneumoniae* serotypes is only rarely attempted, due to the paucity of microbiological samples from middle ear fluid (MEF). This raises the need for identification of indirect means to monitor OM pathogens. It was previously demonstrated that for paediatric IPD, post-PCV7 changes in epidemiology could be inferred to a certain extent, by monitoring nasopharyngeal carriage dynamics (6,7). It is plausible that with OM, similar link exists, but the feasibility of such approach has not been studied.

PCV7 was licensed in Israel in 2007, with sporadic use until 2009. The vaccine was introduced into the Israeli National Immunization Plan in July 2009 (administered at age 2, 4 and 12 months) with a catch up campaign in all children <2 years. In November 2010, PCV13 replaced PCV7 in the Israeli NIP, with a further catch-up.

Active surveillance of pneumococcal OM via cases enriched with recurrent, non-responsive, spontaneously draining cases and OM with effusion (collaborately termed here complex OM) from whom MEF culture was obtained, conducted in southern Israel, showed that the sequential inclusion of PCV7 and PCV13 into the routine vaccination programme has rapidly led to a 77% and 60% reduction in pneumococcal and all-cause OM episodes in children <2 years old (2). By combining this data with nasopharyngeal carriage surveillance in children from the same population, we investigated if pneumococcal nasopharyngeal carriage can be used as a surrogate to monitor post vaccination dynamics in pneumococcal OM.

METHODS

**Setting:**

In southern Israel (the Negev region), Jewish and Bedouin populations live side by side. The socioeconomic conditions and the lifestyles of the 2 groups differ, but both have access to the same medical services. The Jewish population is mainly urban, resembling developed populations, whereas the Bedouin population, formerly desert nomads, is in transition to a western lifestyle and resembling developing populations (2,8,9). Total annual births are ~15,000 (~50% Bedouins and ~50 Jewish children). Almost all (>95%) of the children in the Negev region are born and receive medical services at the only medical centre in the Negev, the Soroka University Medical Center (SUMC).

**Study population:**

*Otitis media*: The study population consisted of children <2 years of age from southern Israel who had an OM episode resulting in MEF culture. No change in any of the indication for referral to the pediatric ER, tympanocentesis or obtaining MEF culture has occurred in the past 10 years. The diagnosis of OM was made by a paediatrician, a family physician, or an otolaryngologist, as previously described, and culture specimens were obtained by tympanocentesis or collection of pus from draining ears (of <7 day duration) (2). Demographic and clinical information was prospectively obtained from children with positive cultures (2) For each episode, we collected information on the culture date, patient’s age, ethnicity. Data were obtained from the medical charts, the child’s physician, or parents, as appropriate. For this study the annual pneumococcal OM incidence in children <2 years of age between July 2010 and June 2014 was used to provide a benchmark for the prediction models. All episodes of OM in which *Streptococcus pneumoniae* was identified from MEF were included in the analysis.

*Nasopharyngeal carriage:* The detailed methodology and design of the study on nasopharyngeal carriage was described elsewhere (10). In brief, each working day, nasopharyngeal (NP) culture was obtained from the first 4 Jewish and 4 Bedouin children <5 years old, resident of the Negev region, seen at the PER for any reason, whose parents agreed to their enrolment. After obtaining an informed consent and NP swab, PCV vaccination dates and the nature of the vaccine (PCV7 or PCV13) were recorded by the study team from data obtained directly from the vaccination center, together with selected ethnic and demographic data, obtained from the parents. Children not residing in the Negev region were excluded. At most one nasopharyngeal swab per month was obtained for each participating child. Approximately 75% of the patients offered enrolment consented.

*Bacteriology:* Swabs of MEF aspirates and NP specimens were placed in MW173 Amies transport medicum (Transwab; Medical Wire and Equipment, Potley, UK) and were processed as previously described (2,10). Material from swabs was plated on Columbia agar with 5% sheep blood and 5.0 μg/mL gentamicin, and incubated aerobically at 35oC in a CO2-enriched atmosphere for 48h. This method was used in our previous studies and yielded a high rate of positive cultures (10) *Streptococcus pneumoniae* was identified bacteriologically as previously described (2,10). Pneumococcal serogrouping and serotyping were performed by means of the Quellung reaction using antisera provides by Statens Seruminstitut of Copenhagen, Denmark. Only one colony was serotyped from each NP swab. This typically represents the most dominant serotype in the NP. Typing 2-5 colonies does not increase significantly the yield. For multiple colonisations detection molecular techniques are more appropriate (11,12)

The annual NP carriage prevalence of the seven serotypes in PCV7 (“PCV7”), the additional 6 serotypes in PCV13 (“PCV13-7”) and the non-PCV13 serotypes (“NVT”) between July 2009 and June 2014 was included in this analysis as a proxy for the exposure of children <2 years old and susceptible to the development of OM. As part of the sensitivity analysis we also present model predictions where the annual OM carriage incidence in children <2 years old was used as a proxy for exposure (13,14).

**Model:**
The prediction model was based on pneumococcal OM incidence in 2009/10 and nasopharyngeal carriage prevalence between 2009/10 and 2013/14 to predict the pneumococcal OM incidence between 2010/11 and 2013/14. All analyses were stratified by ethnicity (Jews and Bedouins). We used a model that predicts the change in vaccine-type and non-vaccine type OM incidence based on the observed change in carriage similar to previously described approaches with paediatric IPD as an endpoint (6,7).

Let $C\_{T, Y}$ be the prevalence of serotype-group $T\in \{PCV7, PCV13-7, NVT\}$ carriage in year $Y$. Similarly let $O\_{T,Y}$ be the incidence of OM of serotype group $T$ in year $Y$. By assuming that any change in exposure, i.e. carriage prevalence, is identically reflected in OM incidence the OM incidence at any point in time ($Y\_{2}$) is predicted by the incidence at a baseline year ($Y\_{1}$) multiplied with the change in carriage from that baseline:

$$O\_{T,Y\_{2}}= O\_{T,Y\_{1}}\frac{C\_{T,Y\_{2}}}{C\_{T,Y\_{1}}}$$

The predicted overall incidence of OM in year $Y\_{2}$ was then calculated as the sum of predicted incidence of all serotype groups:

$$O\_{Y\_{2}}=\sum\_{T}^{}O\_{T,Y\_{2}}$$

OM incidence was calculated by using census data on the annual number of Jewish and Bedouin children in catchment area of the hospital. Assuming that the distribution of annual NP carriage and OM isolates into “PCV7 types”, PCV13-7 types”, “NVT”, “others” (including non typeable and untested) and “no pneumococcus” are samples of respective multinomial distributions, bootstrapping techniques were used to infer confidence intervals on all outcomes. Only one serotype per isolate was identified during the study period. All analyses were performed with R version 3.1 (15)

RESULTS

Overall, 667 episodes of pneumococcal OM (80% of which were obtained by tympanocentesis) and 6788 nasopharyngeal samples were included in the study. Forty nine percent of all pneumococcal OM episodes and 40% of all nasopharyngeal samples were from the Jewish population and the remainder from Bedouins. After the year 2009/10 the incidence of pneumococcal OM in children <2 years that was caused by serotypes included in PCV7 or the types in PCV13-7 declined similarly to the respective carriage prevalence in children younger than 5 years **(Figure 1 and Web Table 1).** In contrast, while NVT serotype carriage prevalence in children < 5 years old steadily increased after 2009/10, the incidence of NVT OM slightly increased in the first two years but declined thereafter, with no overall increase compared to the baseline year. As a result, the model appropriately predicted the post 2009/10 incidence of pneumococcal OM among the two vaccine serotype groups (**Figure 2, Web Table 2 and Table 1**) but systematically overestimates the increase in NVT OM incidence for both the Bedouin and the Jewish population after 2012. The forecasts were insensitive to inclusion of 6A in the PCV7 serotype group due to cross protection rather than in the PCV13-7 serotype group and the use of carriage incidence in children <2 years old rather than carriage prevalence in children <5 years old as the proxy for exposure that is used in the prediction model (**Figure S1 and Figure S2).**

We looked separately at serotype 19A. After introduction of PCV7, serotype 19A has been the major replacing serotype among IPD isolates globally including Israel and was of increased concern due to increasing drug resistance, especially in the Bedouin population (16). In the Bedouin population 17 and 18 isolates of serotype 19A were detected in middle ear fluid in 2009/10 and 2010/11 respectively and 3 and 4 isolates in 2012/13 and 2014/15, respectively. This decrease in serotype 19A OM incidence was paralleled among NP carriers. In the Jewish population, serotype 19A became very prevalent among children with OM episodes in 2010/11 but this was not observed to the same extent in NP samples: In 2009/10 there were 9 serotype 19A isolates from MEF sharply increasing to 43 (4.8 fold) in 2010/11 and then sharply decreasing to 2 and 3 in the two subsequent years. Among carriers, the increase in 2010/11 was not as steep, increasing from 32 in 2009/10 to 56 (1.8 fold) and then sharply decreasing to 7 isolates each in 2012/13 and 2013/14.

Between 2009 and 2014 all of the serotypes in PCV13-7 but 6A were more likely to be found among pneumococcal OM episodes in children <2 years old than among the <5 years old carriers (**Figure 3, Web Figure 3 and Web Table 3**). This was similar for serotypes 9V, 18C and 19F of PCV7 but among the PCV7 serotypes significance was found only for serotype 19F. Within the NVT group the odds ratio for isolates among OM versus NP carriage controls was 1.44 (0.68 to 3.07) for serotype 22F and 1.22 (0.66 to 2.28) for serotype 33F, the additional serotypes in a currently developed 15-valent formulation.

DISCUSSION

We used the extensive surveillance of OM and pneumococcal carriage in southern Israel to study if, similarly to invasive disease, pneumococcal carriage could be used to monitor post vaccination changes in paediatric pneumococcal OM. We found that predictions for changes in vaccine serotype OM incidence match the observed changes for both the Bedouin and the Jewish population. However, serotype replacement among pneumococcal carriage overestimated the change in NVT OM incidence in the post vaccination era. The serotypes included in PCV13-7 had a very high propensity to cause OM. The two additional serotypes in a potential 15-valent PCV were found to be only marginally more likely to cause an OM episode given their rate of exposure than the average NVT.

A disproportionate increase in OM caused by the PCV13-7 serotypes compared to that in NP carriage was seen during the years 2010-2011. This was mostly observed among the Jewish children and was predominated by the dynamics of serotype 19A, which constituted the majority of both carriage and OM among the PCV13-7 serotypes. It is plausible that OM caused by serotype 19A, which was largely highly antibiotic resistant and multi-resistant, often presented as treatment failures, resulting more frequently in tympanocentesis, overemphasizing the incidence of OM caused by this serotype. Starting in 2012-2013, the reduction in carriage of this serotype resulted in reduced serotype 19A OM diminishing this potential artefact.

In principle, one could anticipate an increase in NVT OM in the presence of a clear increase in NVT carriage post PCV introduction, because of the replacement of VT by NVT in NP carriage. A change in surveillance sensitivity is among the hypothesis that would explain the absence of such increase in NVT OM incidence in the post-vaccination era. However, we could not detect a similarly pronounced over-estimation of PCV7 and PCV13-7 serotype group incidence which one would expect to find if surveillance sensitivity declined (17). Similar to our findings, no increase in NVT OM incidence was reported in the PCV7 era between 2006 and 2011 in New York while in the same time NVT carriage tripled (18). An additional multicentre study of 8 children hospitals, showed that OM caused by serotype 19A rapidly decreased in the immediate 3 years post PCV13 introduction in the US (2011-2012) but NVT OM did not increase (19). Anticipation for an increase in NVT OM post vaccination is based on the finding that NVT NP carriage increased and therefore exposure to NVT strains. Inference of an increase in NVT OM implicitly assumed that the susceptibility of the host’s middle ear to NVT pneumococci in the post vaccination era remains unchanged. However, in the PCV era the lower rate of early acute OM occurrence (which was predominantly caused by vaccine serotypes) may reduce early tissue damage in turn decreasing susceptibility to NVT pneumococci as well as to other potentially secondary pathogens (such as NTHi and *M. catarrhalis*, often presenting as polymicrobial etiology, often with biofilm formation). Thus, widespread implementation of PCV may have resulted in a lower susceptibility of the host to NVT pneumococci OM, if NVT are less virulent than the VT strains as has been observed for bacteremic pneumococcal infections but also OM (20). This in turn may explain the non-increase or even the eventual reduction of NVT OM that was observed in southern Israel. Indeed the findings of first increase and then a decrease of NVT OM, the latter occurring mainly after the year 2011-2012 when coverage of PCV13, which includes the serotypes with the highest propensity for OM, increased, supports our hypothesis.

Previous studies have based the idea to monitor paediatric IPD via pneumococcal carriage on the notion that pneumococcal carriage is a pre-requisite for disease. As IPD is believed to follow shortly after acquisition they used changes in the epidemiology of new asymptomatic infections (carriage incidence) as means to predict changes in IPD in the same age-group. In contrast to that we hypothesize that a change in exposure to pneumococcal serotypes drives changes in pneumococcal disease. Hence we use the carriage prevalence among children up to 5 years old to predict changes in OM incidence in children younger than 2 years. We show, however, as part of the sensitivity analysis that model predictions based on carriage incidence in children younger than 2 years of age show similar predictive abilities (**Figure S1**). Furthermore, we derive pneumococcal carriage prevalence from children that attend the paediatric emergency room. This assumes that pneumococcal carriage among those children is representative for pneumococcal carriage among otherwise healthy children. Consistent surveillance for nasopharyngeal carriage of pneumococcus in Israel as described earlier only started after introduction of PCV7. Hence in this study we were only able to assess changes using the first year of observation as a baseline but not to predict the impact of PCV which would require pre-vaccination data on OM and carriage.

We here present a study that uses a simple model representing only one of the most fundamental features of pneumococcal epidemiology (21,22), i.e. a serotype group dependent risk of infected individuals to develop pneumococcal disease, to attempt predicting OM incidence based on NP carriage data. Pooling serotypes into groups ignores any heterogeneity within that group which may hinder predictions for example if serotype replacement in carriage or OM is driven predominantly by specific and a-typical serotypes. However, given the complexity of *S. pneumoniae* such pooling is often necessary and has been used frequently to demonstrate properties of pneumococcal epidemiology (23–25). We calculate the serotype specific odds ratio for isolates among OM versus NP carriage controls, as done for pneumonia by Greenberg et al. (26), however, our estimates are based on samples obtained in the changing epidemiology of the post-vaccination period. In particular, the difference in change in NVT carriage and OM incidence may introduce a bias towards underestimating the propensity of NVTs to cause OM.

NP carriage has been established as a surrogate for monitoring changes in invasive pneumococcal disease. We showed that changes in VT NP carriage have been predictive of changes in VT OM incidence in southern Israel. However, the increase in NVT carriage together with a decline in NVT OM incidence may suggest a decline in susceptibility to OM in young children as a result of vaccination that is targeting the most virulent serotypes. We furthermore predict that an extension of PCV serotype coverage to include type 22F and 33F may only result in a modest additional reduction in OM incidence in southern Israel.

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Affiliations: 1 London School of Hygiene and Tropical Medicine, London, UK; 2 Soroka University Medical Center, Beer Sheva, Israel; 3 Ben-Gurion University of Negev, Beer Sheva, Israel; 4 Ben-Gurion University of Negev, Beer Sheva, Israel

Corresponding Author\*: Stefan Flasche, Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, WC1E 7HT, London, UK, Stefan.Flasche@lshtm.ac.uk

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**Conflicts of interest**

SF and NGL have no conflicts of interests. In the past 5 years, RD has received grants/research support from Berna/Crucell, MSD and Pfizer. He has been a scientific consultant to Berna/Crucell, Genocea, GlaxoSmithKline, MeMed, MSD, Novartis and Pfizer and has received speaker’s fee from Berna/Crucell, GlaxoSmithKline and Pfizer.

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**Figures & Tables**

Table 1: Comparison of observed and predicted OM incidence per 1,000 population in children less than 2 years old in southern Israel in 2013/14.

|  |  |  |
| --- | --- | --- |
|  | Jews | Bedouins |
|  | Observed OM incidencea, Median (95% CI) | Predicted OM incidencea, Median (95% CI)  | Observed OM incidencea, Median (95% CI)  | Predicted OM incidencea, Median (95% CI) |
| NVT  | 2.15 (1.82, 2.48) | 2.86 (1.97, 3.97) | 0.88 (0.65, 1.11) | 2.53 (1.76, 3.43) |
| PCV7 | 0.26 (0.07, 0.52) | 0.29 (0.11, 0.52) | 0 | 0.39 (0.22, 0.63) |
| PCV13-7 | 0.33 (0.07, 0.59) | 0.29 (0.12, 0.57) | 0.23 (0.06, 0.47) | 0.53 (0.32, 0.86) |

a per 1,000 population.
OM: Otitis media

Figure 1: The change in pneumococcal carriage prevalence in under 5 year old children from 2009/10 onwards, in comparison with the change in pneumococcal incidence of otitis media (OM) in under 2 year old children. Point estimates are presented in conjunction with respective 95% CI error bars. Southern Israel, 2009 – 2014. PCV7: The 7 serotypes that are included in 7-valent pneumococcal conjugate vaccine. PCV13-7: The 6 serotypes that are included in the 13-valent pneumococcal conjugate vaccine but not in the 7-valent formulation; NVT: the serotypes that are not included in the 13-valent formulation.

Figure 2: Time and ethnicity stratified observed (black dots with 95% CI error bars) and predicted annual incidence of pneumococcal otitis media (OM). The Epi-year 2009/2010 served as a baseline for the predictions. Carriage prevalence in children less than 5 years of age was used as a proxy for exposure of children less than 2 years of age to the pneumococcus and their risk to contract OM. Southern Israel, 2009 – 2014. PCV7: The 7 serotypes that are included in 7-valent pneumococcal conjugate vaccine. PCV13-7: The 6 serotypes that are included in the 13-valent pneumococcal conjugate vaccine but not in the 7-valent formulation; NVT: the serotypes that are not included in the 13-valent formulation.

Figure 3: The proportion of isolates from middle ear fluid among pneumococcal otits media (OM) that contain a specific serotype versus the respective proportion among nasopharyngeal (NP) carriage of controls. Southern Israel, 2009 – 2014. PCV7: The 7 serotypes that are included in 7-valent pneumococcal conjugate vaccine. PCV13-7: The 6 serotypes that are included in the 13-valent pneumococcal conjugate vaccine but not in the 7-valent formulation; NVT: the serotypes that are not included in the 13-valent formulation.