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Carriage of *Streptoccoccus pneumoniae* 7 years after implementation of vaccination program in a population with very high and long-lasting coverage, Italy

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ABSTRACT

To evaluate how the 7-valent pneumococcal vaccine (PCV7) programme and the very high vaccination coverage reached for over 4 years affected the prevalence of *Streptoccoccus pneumoniae* serotypes in the paediatric population and to evaluate demographic, behavioural and risk factors for carriage in the post-vaccination era, a cross-sectional study on nasopharyngeal carriage was performed. Six hundred sixty-nine children under the age of 5, representative of the open population, were enrolled by cluster sampling. High sensitive techniques for detection of multi-serotype carriage, i.e. broth enrichment and real-time PCR and sequential PCRs for detection and typing, respectively, were used. Of the 669 enrolled children, 97.8% were compliant with the recommended PCV7 vaccination schedule. Post-stratification adjustment for age was applied considering the Ligurian population as standard population. Age-weighted carriage rate was 50.1% and 78% of carriers were colonized by more than one serotype. The prevalence of carriage increased with age from 22% in the first year of life, to 48.6% in the second year of life and to 60% in the 25–59 month age group.

Age-weighted prevalence of any of the PCV7, PCV10 or PCV13 serotypes was 10.3%, 20.3% and 27.5%, respectively. PCV7 serotypes were mainly represented by serotype 4 that was carried since the 3rd year of life and was responsible for invasive pneumococcal disease (IPD) and non-IPD in adults, but not in children confirming the high vaccine effectiveness. Among the serotypes included in recently available vaccines, serotypes 5 and 19A showed a higher prevalence, being carried by 15.2% and 8.8% of the population, respectively.

A multivariate analysis showed that age, the presence of child siblings at home and day care attendance covariates were strongly associated with *S. pneumoniae* carriage.

In conclusion, over 7 years of vaccination with PCV7 and very high coverage in the last 4 years has led to low carriage prevalence in the first year of life rapidly increasing in the following years and high prevalence of non-PCV7 serotypes carriage.

1. Introduction

Invasive and non-invasive pneumococcal diseases are preceded by nasopharyngeal colonization, representing the key moment to understand bacterial epidemiology and assess the effects of preventive strategies. As well as being obligatory for invasive diseases (IPD), colonization also provides the basis for horizontal spread in families and the community [1,2].

In the United States, Canada and some European Countries, widespread use of the 7-valent pneumococcal conjugate vaccine (PCV7) (Prevenar, Pfizer) in children has led to a dramatic decline in PCV7-serotype invasive pneumococcal disease (IPD), but also to an increase of non-PCV7 carriage and non-PCV7 IPD incidence [3–5]. The replacement of the serotypes previously carried in the nasopharynx of young children and higher prevalence of colonization by serotypes not included in PCV7 is only partially reducing the effect of vaccination, but it is a phenomenon that must be carefully evaluated [4,6]. The difficulty in identifying causative agents in respiratory diseases such as pneumonia or in otitis media and the relatively low incidence of IPD make difficult to evaluate the early effect of the immunological pressure induced by the vaccine and make carriage a useful tool for monitoring how vaccination affects circulating pneumococcal serotypes.

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Since May 2003, a large-scale programme of vaccination against *Streptoccoccus pneumoniae* was started in Liguria, Italy. All newborns were invited to receive PCV7 according to a 3–5–11 month schedule. PCV7 uptake reached a coverage of >80% and >90% in all districts since 2004 and 2007, respectively, determining an unusual epidemiological picture for Europe [7]. In fact, the majority of European Countries have experienced PCV7 implementation since 2006 and only a few countries, i.e. Belgium, United Kingdom and France, reached a potential coverage of over 70% for 2–3 years [8]. In Spain, universal programme was limited to the Madrid region since 2006, while a risk-based PCV7 vaccination programme has started in 2001 [8].

Ten-valent pneumococcal (PCV10) (Synflorix, GSK) was approved first in Canada in December 2008 and then approved by the European Medicines Agency in March 2009, while 13-valent pneumococcal CRM197 protein conjugate vaccine (PCV13) (Prevenar 13, Pfizer) was approved first in Chile in July 2009 and then in the USA in February 2010.

In summer 2010, PCV7 was replaced by a 13-valent pneumococcal conjugate vaccine (PCV13) in the regional vaccination plan and it was widely administered since autumn 2010.

Considering the importance of pneumococcal colonization in relation to pneumococcal disease and its prevention, a cross-sectional study of nasopharyngeal carriage among pre-school children was conducted in autumn 2010 with the aim to describe circulating *S. pneumoniae* in the paediatric reservoir following implementation of PCV7 and immediately before the introduction of PCV13. The cross-sectional study was specifically addressed (i) to evaluate how the PCV7 vaccination programme and the very high and long-lasting vaccination coverage effected prevalence of serotypes carried in the naso-pharynx and (ii) to evaluate demographic, behavioural and risk factors for carriage in this specific population in the post-vaccination era.

2. Methods

2.1. Study design

The study is population-based cross-sectional analysis of nasopharyngeal pneumococcal carriage amongst children <5 years of age.

Surveillance sampling was performed in clusters: each cluster was made up of subjects monitored by a Family Paediatrician (FP). In Italy, primary health care is provided by community paediatricians who monitor physical and psychosocial growth and development, promote age-appropriate screening, establish the first contact with the patient for diagnosis and treatment of acute and chronic disorders, and coordinate the management of health problems requiring multiple professional services. Each FP surveys ~800 children who range in age from newborns to 14 years. Ten FPs in the Genoa metropolitan area were randomly selected, each of which sampled 75 children, 25 for each of the 0–12 months, 13–24 months, and 25–59 months age groups. Children were consecutively sampled by FPs during routine survey required in the first years of life or during examinations due to signs and symptoms not potentially related to *S. pneumoniae* infection. Exclusion criteria included immune-suppression, cancer and participation in clinical trials or other epidemiological studies. The research protocol was approved by the Ethics Committee of "San Martino" University Hospital in Genoa.

2.2. Data source and variables

Parents and children were asked to go to the Department of Health Sciences, University of Genoa, where a physician (i) checked the inclusion and exclusion criteria, (ii) administered a standardized questionnaire to collect demographic data, information on household size, number of siblings, co-morbidities, respiratory tract infections in the last month, antimicrobial and steroid use in the previous 3 months, admission to hospital in the last year and data on possible behaviour risk factors for colonization with *S. pneumoniae*, i.e. second-hand smoke, and vaccination history, and (iii) collected the biological specimen for detection and typing of *S. pneumoniae*. Questionnaires were signed by the parents and were anonymous but labelled to match the respective sample per child.

2.3. Participants and study size

The study protocol was expected to enrol 750 children <5 years of age, stratified by age in three groups (0–12 months, 13–24 months, and 25–59 months) of equal numbers (250 units). The period of recruitment was limited to between October 18th and December 18th, 2010 to avoid that changes during this period due to climate change, circulation of influenza virus or respiratory syncytial virus or PCV13 administration affected the epidemiological picture. Survey sample size was estimated based on the results for expected carriage rate of 33.3%, considering the design effect of the study due to cluster sampling and weighting. The estimates of design effect for sample size calculation were 2.9 for cluster sampling and 1.3 for effect of weighting. Overall sample size necessary to achieve a precision for carriage prevalence, the main outcome measure of the study, of ±6.5% for the 95% confidence interval were calculated as 745 children; the final sample size chosen was 750 children. The software used for sample size calculation was Open Epi, Version 2.0, freely available on http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm.

2.4. Nasopharyngeal swabs and laboratory assays

Carriage samples were collected by trained physicians by using calcium alginate swab specimens (Fisherbrand, catalog number 14-959-78; Fisher Scientific, Pittsburg, PA) and transported in 1.0 ml skim milk–tryptone–glucose–glycerol (STGG) medium. These nasopharyngeal swabs in STGG medium were preserved on wet ice and frozen at −70 °C within 4 h. Before freezing them at −70 °C, the NP-STGG specimens were vortexed for 10–20 s to disperse the organisms from the swab. The detection of specific sequences of *S. pneumoniae* in biological specimens was performed using Real Time PCR preceded by broth enrichment for enhanced pneumococcal growth, as recommended by CDC [9]. For broth enrichment, after a brief complete thawing and vigorous vortexing of the specimens, 200-μl aliquots were added to 5 ml Supplemented Todd-Hewitt broth (STHB) and the mixture was incubated for 4 h at 37 °C in a CO2 incubator [9]. STHB consisted of 5 ml of Todd-Hewitt broth containing 0.5% yeast extract combined with 1 ml of rabbit serum. The STHB culture was frozen for further extraction of the DNA by QIAamp DNA Minikit (Qiagen); the extracted genetic material was used in *lytA* gene-specific realtime PCR for *S. pneumoniae* detection and in sequential multiplex PCR assay for serotype deduction [9].

A *S. pneumoniae*-specific real-time PCR targeting the *lytA* gene was performed (Fast set Streptococcus pneumoniae, Arrow Diagnostics) with DNA extracts prepared from enriched STHB cultures. Negative samples were defined as those with cycle threshold (C_t) values greater than 35. A human gene sequence for β-globin was detected by the test as a positive extraction control.

Molecular typing was performed using a sequential algorithm with 8 PCR multiplexes, each comprising an amplification reaction with 4 pairs of type-specific primers, modified for the detection and typing of the bacteria in the colonized subject. The algorithm enables 40 of the most common types to be identified. Another pair