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Dynamics of pneumococcal carriage among day-care center attendees during the transition from the 7-valent to the higher-valent pneumococcal conjugate vaccines in Greece

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ABSTRACT

Background: In Greece recently, higher-valent pneumococcal conjugate vaccines (PCVs) replaced the 7-valent (PCV7); the 10-valent (PCV10) became available in May 2009 and the 13-valent (PCV13) in June 2010.
Methods: We investigated the nasopharyngeal colonization with *Streptococcus pneumoniae* in day-care center attendees in Athens and the prefecture of Viotia. Between December 2010 and June 2011, nasopharyngeal cultures were obtained 4 times, at enrollment and then every 6 to 8 weeks.
Results: Among the 233 children, 225 (96.6%) had been vaccinated with ≥1 dose of PCV7. One tenth of the PCV7 vaccinated attendees had also received ≥1 dose of PCV13 or PCV10. During the 4 samplings, 358 isolates were recovered from a total of 874 samples. Of the 233 children, 183 (78.5%) were found to carry *S. pneumoniae* at least once. The overall serotype distribution among carriers was similar regardless of the time lapsed since the last PCV7 dose. A high frequency of 19A (17.1%) coincided with a low frequency of 19F (1.4%). Non-PCV13 serotypes accounted for 73.1% of the isolates; 23B, 15B/C, 16F, 21, 11A, 15A, 6C, 10A, 22F and 23A were the most common. Among attendees aged 24–59 months (median age 42 months), prolonged carriage of a non-PCV13 serotype was relatively common, mainly for 21 and 16F. One out of 4 cases of colonization with the prevalent non-PCV13 serotypes was followed by persistent carriage for 5 to 14 weeks.
Conclusions: During this period of transition to the higher-valent PCVs in the day-care center setting, non-PCV13 serotypes dominated and exhibited prolonged colonization. The frequency and the duration of prolonged carriage tends to be increased, if sampling frequency increases and the carriage time before and after positive cultures is taken into consideration. Further studies regarding the fitness of the colonizing non-PCV13 serotypes will likely to be seen in the future.

1. Introduction

Streptococcus pneumoniae causes infections that range in severity from acute otitis media and sinusitis to pneumonia, septicemia, and meningitis [1,2]. Pneumococcal disease is preceded by colonization of the nasopharynx, the dominant natural reservoir of *S. pneumoniae* [3,4]. Nasopharyngeal carriage is a highly dynamic process, with pneumococci being acquired, carried for a period of weeks or months, and then cleared [4].

Day-care centers are unique settings where young children are at increased risk for colonization with pneumococci [5]. The nasopharynx of the attendees represents a good environment for the amplification of the fittest pneumococcal lineages and has been a target for the pneumococcal conjugate vaccines (PCVs), the 7-valent (PCV7) as well as the newer ones [6]. Through this process these settings act as powerful amplifiers contributing to the spread of disease-causing clones [5]. Even though point prevalence studies in day-care centers have been conducted in several countries, in order to monitor colonization with *S. pneumoniae* [7,8], longitudinal studies in this particular epidemiological setting are less frequent [9–11], especially in the post-PCV7 period. Longitudinal studies at day-care centers may provide additional information

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regarding the fitness capacities of emerging serotypes, not present in the higher-valent PCVs.

In Greece, PCV7 (capsular antigens of *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) became available in October 2004, it was incorporated into the national immunization program (NIP) in January 2006 and was 75% reimbursed by the national health insurance in the end of the 1st semester of 2006 and 100% in the spring of 2008. More recently, the higher-valent PCVs were introduced to our country. The 10-valent (PCV10; serotypes 1, 5, 7F in addition to the serotypes included in PCV7) became available in May 2009 and the 13-valent (PCV13; serotypes 1, 3, 5, 6A, 7F, and 19A in addition to the serotypes included in PCV7) in June 2010. Both were directly included in the NIP and 100% reimbursed.

We have been conducting cross-sectional surveillances of pneumococcal carriage among young children in various areas of Greece since 1995 [12–15]. In December 2010, we initiated a 6-month longitudinal study among children attending day-care centers in Athens and the nearby prefecture of Viotia. The aim of the study was to evaluate the dynamics of colonization with *S. pneumoniae* during the transition from PCV7 to higher-valent PCVs. As a secondary objective, we compared the serotype distribution observed in this study to the one found in our latest cross-sectional surveillance, which we conducted among day-care center attendees of similar age in Central Greece in 2009 [15].

2. Methods

2.1. Study population

In a 6-month longitudinal study, initiated in December 2010, we investigated the trends in pneumococcal colonization of the nasopharynx among children attending day-care centers in Athens (4 day-care centers, 113 children) and the nearby prefecture of Viotia (6 day-care centers, 120 children), Greece. Children of any age attending the studied day-care centers were eligible for enrollment in the present investigation. Nasopharyngeal samples were obtained from each participant at 4 different time points across the study period; at enrollment, and then every 6–8 weeks. Samples were taken by a physician using calcium alginate swabs, as previously described [14].

Parents responded to an interviewer-administered questionnaire, in which data on demographic characteristics as well as information regarding immunization with PCV7, PCV10 or PCV13 were collected. In addition, the interviewing pediatrician recorded the exact dates and type of immunization with PCV(s) from each child's health booklet. A PCV dose was counted if it had been received at least 21 days before the sampling date. Responses were recorded on structured case report forms. The research protocol was approved by the Ethics Committee of the Attikon General University Hospital. Written informed consent was obtained from one of the parents of each participant.

2.2. Vaccine schedule

In Greece since January 2006, the NIP has recommended the routine administration of a PCV, i.e. PCV7, PCV10 or PCV13, depending on the year, as a 4-dose series for infants at 2, 4, 6, and 12 to 18 months of age [16]. Catch-up immunization was recommended for all children up to 59 months of age. In January 2011, extra doses of PCV13 were recommended by the NIP for those who were aged less than 60 months and had been previously vaccinated with PCV7. Specifically, two doses of PCV13 were recommended for ages 12–23 months, whereas one dose for ages 24–59 months.

2.3. Laboratory procedures

Specimens of nasopharyngeal secretions were obtained pernasally using sterile swabs on flexible shafts with calcium alginate fiber tips (Fisher Scientific, Pittsburgh, Philadelphia, USA). Swabs were placed in Amies transport medium (TGV, Sanofi Diagnostic Pasteur, Marne la Coquette, France) after sampling and were transferred to the Laboratory of the Division of Pediatric Infectious Disease of the University of Thessaly, where isolation, identification and susceptibility testing of the *S. pneumoniae* isolates were performed as previously described [14].

Susceptibility testing was performed on Mueller-Hinton agar supplemented with 5% defibrinated horse blood, as follows. Isolates were screened for penicillin resistance using 1 μg oxacillin disks. If oxacillin inhibition zones were <20 mm, MICs to penicillin and cefotaxime were determined by the Etest method (AB Biodisk, Solna, Sweden). *S. pneumoniae* isolates were tested for susceptibility to erythromycin and clindamycin by both the disk diffusion method and the Etest method. Susceptibility to trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline was determined by the disk diffusion method. The 2008 susceptibility breakpoints of the Clinical and Laboratory Standards Institute (CLSI) [17] were used to classify organisms as susceptible, intermediate, or resistant to penicillin, cefotaxime, erythromycin, clindamycin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole. MICs were interpreted as follows: penicillin (oral penicillin V) ≤0.06 μg/ml, susceptible; 0.12–1 μg/ml, intermediate; and ≥2 μg/ml, resistant; penicillin (parenteral, non-meningitis) ≤2.0 μg/ml, susceptible; 4 μg/ml, intermediate; and ≥8 μg/ml, resistant; cefotaxime (non-meningitis), ≤1 μg/ml, susceptible; 2 μg/ml, intermediate; and ≥4 μg/ml, resistant. For susceptibility testing, plates with the antibiotic disks and Etest strips were incubated in 5% CO_2.

2.4. Capsule serotyping

Serotype determination of pneumococci was performed at the Laboratory of the Division of Pediatric Infectious Disease in Larissa by using Pneumotest-Latex and by the capsular swelling method using pneumococcal type/group and/or factor antisera from Statens Serum Institut (SSI, Copenhagen, Denmark). We followed the SSI guidelines for serotyping.

2.5. Definitions

"Constantly colonized" was defined a carrier who had all 4 samples positive. "Frequently colonized" was the carrier who had 2 or 3 positive samples. Finally, a carrier was considered as "infrequently colonized", when 3 or 4 samples had been obtained and only 1 of these samples yielded *S. pneumoniae*.

If isolates of the same serotype and susceptibility pattern to the antimicrobial agents tested were recovered in ≥2 consecutive samples from a certain child, it was considered an episode of persistent carriage. Duration of carriage was calculated from the date of the first isolation of a certain serotype to the date of its last isolation.

Serotype reacquisition was considered when S. *pneumoniae* isolates belonging to a specific serotype were isolated in the 1st and the 4th sample, while being undetected in the 2nd and 3rd sample.

2.6. Comparison to the serotype distribution in our previous cross-sectional study

The serotype distribution of *S. pneumoniae* isolates recovered in this investigation was compared to the one found in a cross-sectional study we conducted among 820 day-care center attendees of similar age (median age: 48 months; range 13–72 months;