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Differences in nasal pneumococcal carriage of vaccinated and non-vaccinated children, during the first 2 years of wide-spread PCV uptake in Hungary

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22nd ECCMID London, United Kingdom 31 March – 3 April 2012

Modified abstract

Objectives: Infections caused by *Streptococcus pneumoniae* often originate from children who carry this bacterium in the nasopharynx. Conjugate vaccines (PCVs) are available for children to prevent not just infections, but also carriage. In Hungary, Prevenar (first PCV7, later PCV13) was inserted into the national vaccination calendar as an optional but recommended and free vaccine in April 2009. The aim of the present study was to observe the effect of PCV vaccination on pneumococcal carriage of children attending DCCs in Hungary.
Methods: Nasal specimens were collected from 854 children (aged 3-6 years) from 20 DCCs in different parts of Hungary, from February 2009 to December 2010. Serotyping of the isolated pneumococci was done using antisera and a PCR-based method, antibiotic sensitivity was determined by agar dilution, and the genetic relatedness of the strains was examined by PFGE.
Results: Out of 854 children, 324 were carriers (=37.94%), with no difference in genders. The most prevalent types among the vaccinated children were: 11A, 6A/B and 15B; while among the non-vaccinated ones: 6A/B, 14, 19F and 23F. The strains (especially the new types, e.g. 11A, 15B) were generally very sensitive to antibiotics, except for macrolides (R=18%). The close genetic relatedness of the isolates from a given DCC group was obvious by the PFGE results, indicating the intensive exchange of bacteria between children.
Conclusions: During the examined period the PCV vaccination rate increased, but there were differences between nurseries. The carriage rate was nearly the same in the vaccinated and non-vaccinated population, but there was a clear difference in the serotype distribution. For example, while serotypes 14 and 23F were found almost entirely in the non-vaccinated group, rare types such as 11A or 15B emerged rapidly in the entire population. It seems so that even if only a certain percentage of children within a group is vaccinated, it helps in the elimination of the PCV types. Interestingly 19F and 6B remained frequent. The coverage of PCV13 over the non-vaccinated carriers would be 78,85%, while that of the vaccinated ones only 35.3%. This could be explained with the emergence of the new types, but it indicates the need for newer vaccine composition in Hungary.

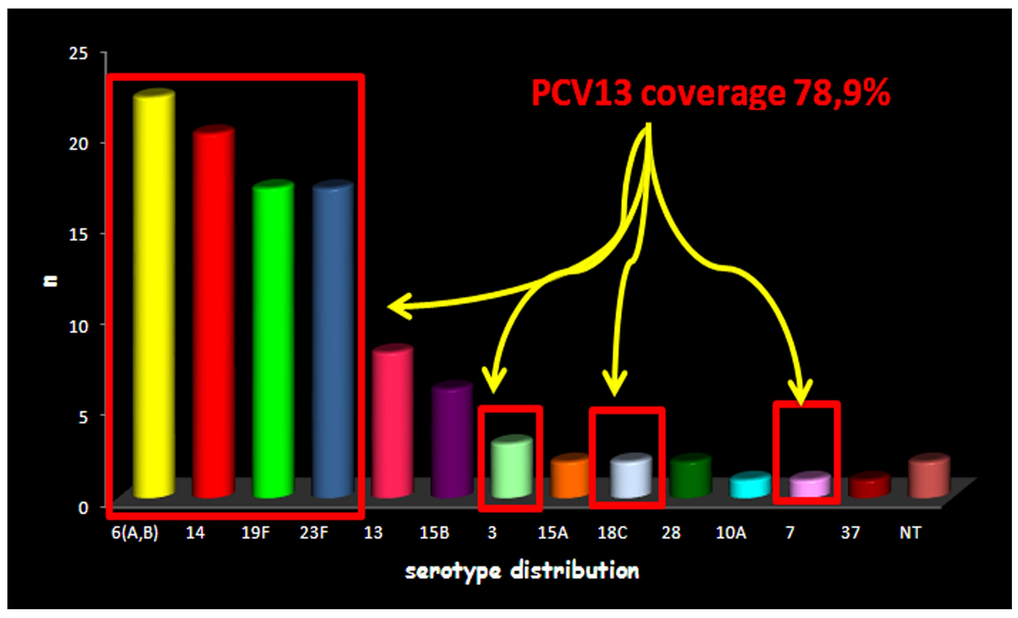
Introduction

Streptococcus pneumoniae causes a significant burden of diseases, including invasive and noninvasive infections such as pneumonia and acute otitis media. The highest rates of diseases occur in little children: according to the WHO estimation it is responsible for the death of 0,7-1 million children every year [1]. Pneumococcal diseases often originate from nasopharyngeal colonization [2]. The widespread use of 7-valent pneumococcal conjugate vaccine (PCV7) has first led to a dramatic decline in PCV7-serotype invasive pneumococcal diseases, not only in vaccinated children [3] but also in unvaccinated persons of all ages [4], but parallelly an increase of non-vaccine serotypes have been demonstrated in nasopharyngeal carriage and also in infections [5]. Therefore, surveillance of nasopharyngeal carriage of pneumococci in vaccinated and in non-vaccinated persons provides another useful tool for monitoring the effect of vaccination on the circulating pneumococcal serotypes.
In Hungary, the conjugate pneumococcal vaccine (first PCV7, than PCV13) is not obligatory but a recommended vaccine since April 2009. The aim of our study was to compare the serotype distribution before [6] and after the wide-spread use of conjugate vaccines.

Materials & Methods

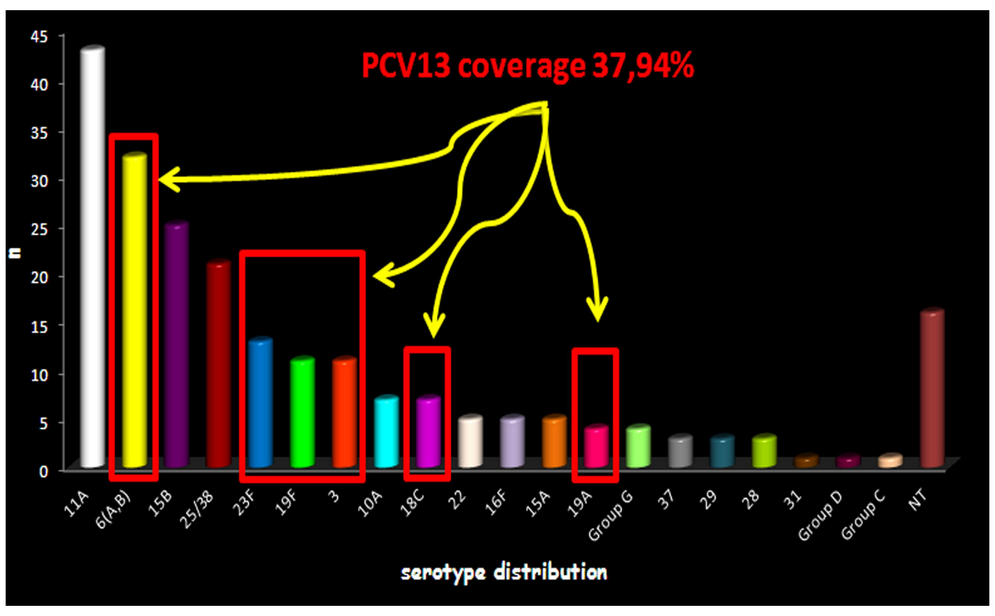
Bacterial isolates. 324 pneumococcal isolates were collected from 854 children in 18 different day-care centres in Hungary. The identity of the strains was confirmed by optochin sensitivity and the presence of the *lytA* gene [7].
Serotyping. Serotyping was done with the combination of latex agglutination and a PCR-based method [8]. Several strains were serotyped at the German National Reference Centre for Streptococci (GNRCS, Aachen).
Antibiotic sensitivity testing. The MIC of the strains to 9 antibiotics (penicillin, cefotaxim, imipenem, erythromycin, clindamycin, telithromycin, levofloxacin, moxifloxacin, vancomycin) was determined by agar dilution method, using the EUCAST breakpoints [9].
Pulsed-field gel electrophoresis (PFGE). Preparation of chromosomal DNA was done as described previously [10]. The embedded and purified genome was digested with 20U *Sma*I for 4 hours at 25°C.

Figure 1. Serotype distribution of the carried pneumococci before the wide-spread vaccination with PCV7 (n=104)



PCV13 contains the following serotypes: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F. NT: Non-typeable.

Figure 2. Serotype distribution of the carried pneumococci following the wide-spread vaccination with PCV7 (n=221)



Group G: serotypes 29/34/35F/35B/47, Group D: serotypes 16/36, Group C: serotypes 24/31/40. NT: Non-typeable.

Result-1: Carriage rate

Out of 854 children, 324 were carriers, with no difference in genders. So the overall carriage rate was 37.94%. In the pre-PCV group the rate was 39,0%, in the PCV group 37,7%.

Result-2: Serotype distribution

The serotype distribution in the non-vaccinated population is shown in Figure 1. It is clear that before the widespread use of the vaccine the so-called pediatric serotypes were dominant in the carriers. Though, we could detect some rare serotypes, and non-typable strains.
Figure 2 shows the serotype distribution in the vaccinated population. We can see, that on one side formerly prevalent serotypes, such as 14, vanished; on the other side previously rare serotypes, namely 11A, 10A and 16F emerged in a large number.

Result-3: Antibiotic susceptibility

Although strains from the non-vaccinated population were generally very sensitive to antibiotics (except for macrolides, R=26%), strains from the vaccinated population showed even higher sensitivity rates. Their macrolide resistance decreased from 26% to 14%. None of them were resistant to penicillin, the highest MICs (0.5-2 mg/L) were detected in serotypes 14, 23F, 19F and 18C.

Results-4: Genetic relatedness

Based on the PFGE results, we could identify clear clonality among the isolates within a given group. This indicates a very intensive exchange of the carried strains between children.

Conclusions

- The overall carriage rate was 37,94%.
- As a result of the wide-spread use of conjugate vaccines it is proved, that new, previously rare serotypes replaced the formerly prevalent pediatric serotypes also in Hungary.
- Therefore the coverage of the conjugate vaccines became reduced, e.g. in case of PCV-13 from the original 78,9% to 35.3%.
- On the one hand, it verifies the effectiveness of the vaccines, as multiresistant serotypes causing life threatening invasive infections are disappearing. On the other hand, vaccines select for the non-vaccine serotypes, and let them colonize the nasopharynx of carriers.
- These new serotypes, however, are rarely the causative agents of invasive infections. Additionally, they are (at the moment) very sensitive to antibiotics, also their macrolide resistance is lower than that of the older types, only 14%.
- Strains show strong clonality within the groups, which mirrors the high bacterium exchange between children.

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Acknowledgements

This work was financially supported by the Hungarian Research Fundation (OTKA), grant no. PD75660. The number of the ethical permit (at the Semmelweis University) is TUKEB 4/2009.

The participation of the first author at the congress was financed by TÁMOP-4.2.1/B-09/KMR-2010-0001.

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