Diagnostic Microbiology and Infectious Disease 74 (2012) 258–262

Contents lists available at SciVerse ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Pneumococcal carriage in children attending a hospital outpatient clinic in the era of pneumococcal conjugate vaccines in Barcelona ☆

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ARTICLE INFO

Article history:
Received 2 April 2012
Accepted 13 July 2012
Available online 24 August 2012

Keywords:
Carriage
Children
Pneumococcal conjugate vaccines
Serotype
Streptococcus pneumoniae

ABSTRACT

Between April 2004 and March 2006 an oropharyngeal swab was obtained from 502 asymptomatic children, aged 6 months to 6 years, at a tertiary children's hospital outpatient department to assess the pneumococcal colonisation rate, risk factors, serotype distribution and antimicrobial susceptibility. Only 126 (25.3%) children had received ≥ 1 dose of PCV7. The pneumococcal carriage rate was 23.5%. Carrier rates were significantly higher in children aged ≥ 24 months and children attending daycare center. Thirty six (31.0%) of the isolates were contained in PCV7, 39 (33.6%) in PCV10 and 62 (53.4%) in PCV13. Forty-four strains (37.9%) were resistant to penicillin. Vaccine serotype (VT) strains were more likely to be penicillin-nonsusceptible *S. pneumoniae* than non-PCV7 serotype (NVT) strains (66.7% vs. 21.6%; $P < 0.001$). In our pediatric population, NVT were predominant among pneumococcal carriers whereas antibiotic resistance was significantly associated with VT. PCV13 can substantially increase the serotype coverage of *S.pneumoniae* in healthy carriers.

1. Introduction

Streptococcus pneumoniae is a leading cause of invasive disease, such as meningitis and bacteraemia, and the most frequent bacterial cause of community-acquired pneumonia, otitis media and sinusitis in paediatric populations worldwide. Children aged less than two years are at greatest risk.

Pneumococcus commonly colonizes the upper respiratory tract of healthy children and is easily transmitted, usually by droplet secretions, from person to person. When the balance between host and pathogen is disturbed, *S. pneumoniae* can spread to adjacent mucosal tissues to cause mucosal infections or invade the blood-stream to cause invasive infections. Although most children are colonised at some point during the first two years of life, only a small minority will develop an invasive infection (Bogaert et al., 2004).

Several factors have been associated with increased prevalence of carriage, including overcrowding (e.g., attending daycare centres (DCC), residing in orphanages), younger age, family contacts, exposure to cigarette smoke, colder months of the year, frequent respiratory tract infections and an excessive use of antibiotics (Principi et al., 1999).

The introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in the United States in 2000 was followed by a significant reduction in invasive pneumoccocal disease (IPD) and nasopharyngeal (NP) carriage due to vaccine serotype (VT) rates (Black et al., 2000; Whitney et al., 2003). Despite the effectiveness of this vaccine, the emergences of nonvaccine serotypes (NVT) in both IPD and NP isolates and changes in antimicrobial susceptibilities have been documented around the world [Frazao et al., 2005; Gonzalez et al., 2006; Farrell et al., 2007; Muñoz-Almagro et al., 2008; Muñoz-Almagro et al., 2009; Sa-Leao et al., 2009; Huang et al., 2009). In 2010, two pneumococcal conjugate vaccines that included additional emerging serotypes were licensed: the 10-valent pneumococcal conjugate vaccine (PCV10; Synflorix®), adding serotypes 1, 5, and 7F, and the 13-valent pneumococcal conjugate vaccine (PCV13; Prevenar-13®), adding serotypes 1, 3, 5, 6A, 7F, and 19A.

PCV7 became available in Spain in June 2001, although at present in our region (Catalonia) it is not subsidized by the national public health system. A study carried out in 2005 in Catalonia found an estimated vaccination rate of 30% (Calbo et al., 2006). There are limited data on pneumococcal serotypes involved in carriage in our country, particularly since the introduction of PCV7 (García de Lomas et al., 1997; Lopez et al., 1999; Sánchez-Tatay et al., 2008).

Because NP carriage is a major factor in the transmission of pneumoccocal disease, continuing careful surveillance of colonized

☆ Conflicts of interest and source of funding. This study was supported by a grant from Caja Navarra Foundation and Agencia de Gestio d'Ajuts Universitaris i de Recerca AGAUR 2009/SGR00136. The authors declared no conflict of interest.
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http://dx.doi.org/10.1016/j.diagmicrobio.2012.07.005

children is essential to increase understanding of changes in the serotype distribution and antibiotic susceptibility of IPD isolates.

The main objectives of this study were 1) to determine the carriage rate, serotype distribution and antibiotic susceptibility patterns of *S. pneumoniae* in oropharyngeal (OP) carrier healthy children of our community, 2) to assess the potential serotype coverage of the recently licensed pneumococcal conjugate vaccines PCV10 and PCV13 against pneumococcal carriage in our population, and 3) to evaluate the reported risk factors for pneumococcal carriage.

2. Materials and methods

2.1. Study population

From April 2004 through March 2006, OP swabs from 502 children aged between 6 months and 6 years of age were obtained. The children were attended for minor surgical procedures at the Outpatient Department of Sant Joan de Déu Hospital. During the study period, this was tertiary-care children's and maternity hospital in Barcelona (Catalonia, Spain) with 345 beds and an average referral population of 210,000 children under 18 years of age (data obtained from the Statistical Institute of Catalonia [Institut d'Estadística de Catalunya], http://www.idescat.net, accessed August 2011]).

The samples were obtained uniformly during the study period except in August, when no cases were recruited because of researcher vacation. Children suffering from fever or acute upper or lower respiratory tract infection were excluded. During the first year of the study period OP swabs were also collected from the patients' mothers.

A structured interview was conducted to elicit the following data: age, gender, number of siblings, passive smoking, relatives with chronic respiratory illness, DCC attendance, and medical history, including underlying disorders, PCV7 status (not vaccinated, partially vaccinated, or vaccination up-to-date for age at the time of enrolment), hospital admissions or ear infections within the previous three months, and recent antibiotic use (defined as use at time of study or in the immediately prior month). No specific information from the patients' mothers was recorded.

Signed informed consent was obtained from the parents or legal guardians of participating children prior to OP swabbing.

2.2. Sampling

One sample from each individual (mother and child) was obtained at the same visit. OP swab specimen was taken by direct inspection of the posterior wall and tonsil regions using rigid cotton-tipped wooden applicators. The swabs were then inoculated as previously described by the WHO working group (O'Brien et al., 2003). In order to minimize possible distortion due to different collection methods, all the specimens were obtained exclusively by the researcher herself, having been trained in advance.

2.3. Bacteriology

Swabs were inoculated onto Columbia Agar with 5% sheep blood and 5.0 μg of gentamicin/ml and were incubated aerobically at 37ºC for 48h. *S. pneumoniae* isolates were identified by standard microbiological procedures. Agar dilution technique was used to determine the minimum inhibitory concentrations (MIC) of penicillin, cefotaxime, erythromycin, tetracycline, levofloxacine, and chloramphenicol. Antibiotic susceptibilities were interpreted according to the 2008 meningeal break points defined in Clinical Laboratory Standards Institute (CLSI) document M100-S18 (CLSI, 2008) so, isolates with MIC to penicillin $\geq$ 0.12μg/ml were defined as penicillin-nonsusceptible *S. pneumoniae* (PNSP). Multidrug resistance was defined as resistance to three or more classes of antimicrobial agents. Serotyping was performed with the Quellung reaction. Pneumococccal isolates were classified as PCV7 serotypes (VT), non-PCV7 serotypes (NVT) (all other serotypes), or non-typable (NT). All strains were sent to the National Pneumococcus Reference Centre (Majadahonda, Madrid, Spain) for determination of both MICs and serotypes.

2.4. Statistical analysis

Sample size calculation was performed according to an estimated carriage prevalence of 25%, an alpha level of 0.05 and a power of 90%. All clinical, epidemiological and microbiological variables analyzed were introduced into a computerized data base (Microsoft Access 97). Quantitative and qualitative variables were tabulated. Statistical studies were carried out using the SPSS program (version 17.0) for Windows. The chi-square test and Fisher's exact test were used for the analysis of qualitative variables. Univariate analysis was performed to determine the relative risk of being a carrier according to different risk factors. The confidence interval was calculated at 95%. Two-tailed tests to determine the significance of risk factors were performed at the 5% significance level. Statistical significance was defined as a P value of $\leq$ 0.05.

3. Results

3.1. Study population characteristics

OP swabs were obtained from 502 children between 6 months and 6 years of age during the study period. Mean age was 36.9 months (standard deviation: 18.8 months) and 317 (63.1%) were males. Three-hundred forty-five (68.7%) children attended DCC and 57 (11.3%) had more than one sibling. One hundred and twelve (22.3%) children had received antibiotic treatment in the previous month. Of 497 patients with available data about PCV7 immunization, 25.3% had received at least one dose; this rate increased from 20.1% (45 of 224 patients) during the first year of the study to 29.7% (81 of 273 patients) during the second year.

3.2. Prevalence of carriage and risk factors

S. pneumoniae was isolated from the oropharynx of 118 of the 502 children, representing an overall colonization rate of 23.5% (95% CI: 19.9- 27.5). This rate ranged from 5.9% (children aged between 6 and 12 months) to 32.3% (children aged between 24 and 36 months). Univariate risk factors for pneumococcal carriage are shown in Table 1. *S. pneumoniae* was isolated from the oropharynx of 6 (2.4%) of the 248 mothers included during the first year of the study; in one case it was also isolated from the child. Because of this low prevalence, no OP swabs were collected from mothers during the second year of the study.

3.3. Serotype distribution and antibiotic susceptibility of the OP isolates in healthy children

A single pneumococcal isolate was recovered from each carrier. Serotyping was performed on 116 (98.3%) of 118 isolates. A total of 31 serotypes were identified, with 6 isolates being NT. The predominant serotypes were 19F (16 cases; 13.8%), 6A (12 cases; 10.3%), 23F (8 cases; 6.9%) and 19A (7 cases; 6.0%) (Fig. 1). Overall, 36 (31.0%) of the isolates were contained in the PCV7, 39 (33.6%) in the PCV10, and 62 (53.4%) in the PCV13.

Carriage of VT pneumococci among PCV7 unvaccinated children was significantly higher than among partially and completely vaccinated children (40.5% vs. 20.0% and 10.5% respectively, $P = 0.009$).

The only mother-child pair of *S. pneumoniae* carriers was colonized with the same serotype (19F). The serotypes isolated in the other 5