Vaccine 34 (2016) 1648–1656

Contents lists available at ScienceDirect

Vaccine

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

The impact of private use of PCV7 in 2009 and 2010 on serotypes and antimicrobial resistance of *Streptococcus pneumoniae* carried by young children in Portugal: Comparison with data obtained since 1996 generating a 15-year study prior to PCV13 introduction

Sónia Nunes[a,b,1], Sofia Félix[a,b,1], Carina Valente[a,b,1], Alexandra S. Simões[a,b], Débora A. Tavares[a,b], Sónia T. Almeida[a,b], Ana C. Paulo[a,b], António Brito-Avô[c], Hermínia de Lencastre[b,d], Raquel Sá-Leão[a,b,*]

[a] *Laboratory of Molecular Microbiology of Human Pathogens, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB/UNL), Oeiras, Portugal*
[b] *Laboratory of Molecular Genetics, ITQB/UNL, Oeiras, Portugal*
[c] *Private Pediatric Clinic, Lisbon, Portugal*
[d] *Laboratory of Microbiology and Infectious Diseases, The Rockefeller University, New York, NY, USA*

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 11 February 2016
Accepted 16 February 2016
Available online 23 February 2016

Keywords:
Streptococcus pneumoniae
Carriage
Children
Pneumococcal conjugate vaccine
Serotype
Antimicrobial resistance

ABSTRACT

In Portugal, the 7-valent pneumococcal conjugate vaccine (PCV7) was not introduced in the national immunization plan but was commercially available between 2001 and 2010. We studied serotype distribution and antibiotic susceptibility of *Streptococcus pneumoniae* carried by children in 2009 and 2010. Vaccination with PCV7 was extracted from children's immunization bulletins and information on recent antimicrobial consumption was obtained through a questionnaire. For comparison, we included data from previous studies conducted since 1996: 1996–1999, 2001–2003, 2006–2007. Pneumococci were isolated from nasopharyngeal samples of 1092 children up to six years old attending day-care in an urban area. Among these, 76% (819/1070) were vaccinated and 62% (677/1092) carried pneumococci. In 2009–2010, serotype replacement was extensive. Carriage of PCV7 serotypes was 4.9% and 5.8%, in 2009 and 2010, respectively, with the majority being of serotype 19F (carried by 4.3% and 4.6% of all participants, respectively). Colonization by serotype 19F was associated with vaccine status (7.7% (19/248) of non-vaccinees vs. 3.5% (29/818) of PCV7-vaccinees, $p = 0.010$). Carriage of serotype 19A was high in 2009 and 2010 (8.6% of all participants) consistent with values already observed in 2007; carriage of serotype 6A was <1% (10/1092), indicating a major decline after 2007 (5.8% or 31/538, $p < 0.001$). Non-vaccine serotypes increased and serotype 6C became the most frequently carried serotype in 2010 (11.2% (54/481)). High-level resistance to penicillin (MIC ≥2 mg/L) showed a decreasing trend ($p < 0.001$), whereas resistance to both penicillin and erythromycin increased ($p < 0.001$) and was detected in 15–20% of all isolates in 2009–2010, most of which were non-vaccine serotypes. Antimicrobial use decreased over time ($p < 0.001$). In conclusion, widespread private use of PCV7 has impacted on colonization leading to near elimination of all PCV7 serotypes except for serotype 19F. Antimicrobial consumption declined but it may be too soon to observe generalized changes in antimicrobial resistance rates.

1. Introduction

Streptococcus pneumoniae (pneumococcus) often colonizes asymptomatically the nasopharynx of young children. However, the pneumococcus can cross biological barriers to cause several diseases such as otitis media, pneumonia, bacteremia and meningitis [1]. Children attending day-care centers (DCC) are considered to be the major reservoirs of pneumococci and cross-transmission in these settings can be extremely high [2].

* Corresponding author at: Laboratory of Molecular Microbiology of Human Pathogens, Instituto de Tecnologia Química e Biológica António Xavier, Av. República, 2780-157 Oeiras, Portugal. Tel.: +351 214469872.
E-mail address: rsaleao@itqb.unl.pt (R. Sá-Leão).
[1] S.N., S.F. and C.V. are first co-authors.

http://dx.doi.org/10.1016/j.vaccine.2016.02.045
0264-410X/

Although over 95 pneumococcal serotypes have been described to date, only a few are responsible for the majority of disease [3]. In 2000, a seven-valent pneumococcal conjugate vaccine (PCV7), targeting serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, became available in the USA and soon after in several other countries [4]. More recently, 10-valent (targeting PCV7 serotypes plus serotypes 1, 5, and 7F) and 13-valent (targeting PCV7 serotypes plus serotypes 1, 3, 5, 6A, 7F, and 19A) PCVs have also become available (PCV13 replaced PCV7, both commercialized by the same manufacturer). PCVs not only impact on invasive disease but they also reduce colonization by vaccine serotypes [5–7].

In Portugal, PCVs were not introduced in the National Immunization Plan (NIP) until 2015 but were commercially available with no reimbursement by the state for many years. Specifically, PCV7 became available in June 2001 and was replaced by PCV13 in January 2010. PCV10 became available in April 2009. PCV13 was introduced in the NIP in August 2015. Prior to introduction of PCV in the NIP, the Portuguese Pediatric Society had recommended vaccination of young children with 4 doses of PCV7, starting vaccination at the second month of life [8]. The estimated usage of these vaccines, based on national sales and yearly birth cohorts, is widespread. In particular, among children <1 year, these estimates indicate a gradual increase in PCV7 usage since 2001, reaching 56% in 2003 and 79% in 2007. In 2008 and 2009, PCV7 coverage estimates were 75% and 62%, respectively. PCV10 coverage estimates for 2009 and 2010 were 13% and 7%, respectively. PCV13 coverage estimate for 2010 was 58% (data from IMS and INE/National Statistics Institute).

Since 1996 that pneumococcal carriage studies among children attending day-care in the region of Lisbon have been regularly conducted [9–11]. We have previously shown that by 2006–2007, PCV7 use had led to serotype replacement in colonization [11,12] and that no significant changes on antimicrobial resistance rates had occurred [11,12]. The latter phenomenon was mainly due to the expansion of antimicrobial resistant lineages expressing non-vaccine serotypes [11].

In this study we report serotype distribution and antimicrobial resistance rates in 2009 and 2010, at a time when vaccine coverage among the birth cohort was sustained at 75–80% for nearly 5 years. We also describe for the first time the serotype distribution for all isolates obtained since 1996 expanding on previous studies that focused only on serotypes associated with antimicrobial resistance or specific serotypes [9–15]. Together, the data generated a comparable 15-year-long series, covering the pre- and PCV7-era, allowing the study of the impact of PCV7, antimicrobial use, and secular trends as drivers of changes occurring overtime.

2. Methods

2.1. Ethics statement

The study was registered and approved at the Health Care Center of Oeiras that reports to Administração Regional de Saúde (ARS, "Regional Health Administration") of Lisboa e Vale do Tejo from the Ministry of Health. The directors of day-care centers approved the study. Written signed informed consent was obtained from parents or guardians of participating children. A numeric code was attributed to each sample upon collection and processed anonymously thereafter.

2.2. Study design

A common study design has been used since 1996. Briefly, the studies were cross-sectional and were carried out during the winter months of January to March of each year (1996–1999, 2001–2003, 2006–2007, 2009–2010), in the Lisbon district (an urban area). Day-care centers covering different socio-economic strata (to reflect the diversity within the population) were invited to participate in the study. Nasopharyngeal samples of children up to 6 years old attending day-care were obtained (one per child) by a trained nurse, essentially following WHO recommendations [16]. A flexible swab (BBL Culture Swab: Becton-Dickinson, Sparks, MD) was inserted through one nostril, parallel to the base of the nasopharyngeal passage until reaching the nasopharynx, turned and removed. If resistance occurred, the swab was gently deviated to enable reaching the base of the nasopharyngeal passage. The swabs were transported to the laboratory at ambient temperature in Stuart medium and plated within four hours of collection. A questionnaire was filled in by the child's parents or guardian with information regarding demographic data and antibiotic consumption. Information on vaccination with PCVs was extracted from the child's immunization bulletin.

2.3. Isolation and identification of pneumococci

Samples were plated onto tryptic soy agar supplemented with 5% sheep blood and 5 μg/mL of gentamicin (one plate per sample). Plates were incubated overnight in anaerobic jars at 37 °C. Pneumococci were identified based on colony morphology, occurrence of α-hemolysis and optochin susceptibility. Bile solubility test was performed to optochin resistant isolates. Suspected colonies, one of each morphology, were picked and streaked onto tryptic soy agar plates. In most samples only one colony morphology was detected by naked eye. Pure cultures were frozen at −80 °C in Mueller–Hinton broth containing 15% (v/v) of glycerol. For this study, only the pure culture of the most abundant colony morphology was characterized as described below and included in the analysis.

2.4. Capsular typing

Capsular type was determined by sequential multiplex PCR using primers previously described ([17] and http://www.cdc.gov/streplab/pcr.html). Six group reactions were assigned to detect serotypes 1, 3, 5, 7A/F, 9N/L, 11A, 14, 15A, 15B/C, 16F, 17F, 19A, 19F, 21, 22F, 24A/F, 31, 34, 33A/33F/37, 35F, 38 and serogroups 6, 18 and 23. Primers for the *cpsA* gene were included in each reaction as positive control. Whenever the results were negative or incomplete (at the serogroup level), the Quellung reaction was performed using specific antisera (Statens Serum Institute, Copenhagen, Denmark) [18]. Suspected non-capsulated pneumococci (NT, non-typeable) were identified using a multiplex PCR-based strategy previously described [19].

2.5. Antimicrobial susceptibility testing

Susceptibility to erythromycin, clindamycin, tetracycline, chloramphenicol and sulfamethoxazole-trimethoprim (SXT) was tested by the disk diffusion method (Oxoid, Hampshire, England) according to recommendations and interpretative criteria of the Clinical Laboratory Standards Institute (CLSI) [20]. Minimum inhibitory concentrations (MIC) to penicillin, ceftriaxone and amoxicillin were determined by Etest (Biomérieux, Marcy-l'Etoile, France) and interpreted according to the CLSI guidelines [20]. The breakpoints used for penicillin (oral penicillin V) were ≤0.06, 0.12–1 and ≥2 (mg/L) for susceptible, intermediately resistant and resistant, respectively. Corresponding values for amoxicillin and ceftriaxone (both non-meningitis) were ≤2, 4, ≥8 and ≤1, 2, ≥4 (mg/L), respectively. Multidrug resistance (MDR) was defined as resistance to three or more classes of antibiotics.