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Nasopharyngeal *s. pneumoniae* carriage and density in Belgian infants after 9 years of pneumococcal conjugate vaccine programme

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ABSTRACT

Background: In Belgium, the infant pneumococcal conjugate vaccine (PCV) programme changed from PCV7 (2007–2011) to PCV13 (2011–2015) and to PCV10 (2015–2016). A 3-year nasopharyngeal carriage study was initiated during the programme switch in 2016. Main objective of the year 1 assessment was to obtain a baseline measurement of pneumococcal carriage prevalence, carriage density, serotype distribution and antibiotic resistance.

Materials/methods: Two infant populations aged 6–30 months and without use of antibiotics in the seven days prior to sampling were approached: (1) attending one of 85 randomly selected day-care centres (DCC); (2) presenting with AOM at study-trained general practitioners and paediatricians. Demographic and clinical characteristics were documented and a single nasopharyngeal swab was taken. *S. pneumoniae* were cultured, screened for antibiotic resistance and serotyped, and quantitative Taqman real-time PCR (qRT-PCR) targeting *LytA* was performed.

Results: Culture-based (DCC: 462/760; 60.8% – AOM: 27/39; 69.2%) and *LytA*-based (DCC: 603/753; 80.1% – AOM: 32/39; 82.1%) carriage prevalence was high. Average pneumococcal DNA load in *LytA*-positive day-care samples was 6.5×10^6 copies/µl (95%CI = 3.9–9.2 $\times 10^6$, median = 3.5×10^5); DNA load was positively associated with signs of common cold and negatively with previous antibiotic use. Culture-based frequency of 13 pneumococcal vaccine (PCV) serotypes was 5.4% in DCC and 7.7% in AOM, with 19F and 14 being most frequent, and frequencies below 0.5% for serotypes 3, 6A, 19A in both populations. Predominant non-PCV serotypes were 23B and 23A in day-care and 11A in infants with AOM. In day-care, resistance to penicillin was rare (<0.5%) and absent against levofloxacin; 32.7% and 16.9% isolates were cotrimoxazole- and erythromycin-resistant respectively.

Conclusion: Four years after PCV13 introduction in the vaccination programme, PCV13 serotype carriage was rare in infants throughout Belgium and penicillin resistance was rare. Continued surveillance in the context of a PCV programme switch is necessary.

Abbreviations: AOM, acute otitis media; CRO, clinical research organisation; Chi^2, Chi-Square test; DCC, day-care centre; GP, general practitioner; IPD, invasive pneumococcal disease; MWU, Mann-Whitney *U* test; NP, nasopharyngeal; PCV, pneumococcal conjugate vaccine; STGG, skim milk-tryptone-glucose-glycerol.

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1. Introduction

More than 90 *S. pneumoniae* serotypes exist and reside in the human upper respiratory tract [1]. Pneumococcal carriage is a highly dynamic process with subsequent episodes of acquisition, carriage and clearance [2]. Reported asymptomatic carriage is between 30% and 62% in infants under two years of age in Western countries [3]. Higher carriage rates (up to 93% in Gambian babies < 1 month) were reported in non-Western countries [4]. In circumstances such as host-pathogen imbalance, pneumococci may spread and reach respiratory organs or even the bloodstream to cause infections, including otitis media, pneumonia, sepsis and meningitis [3,5,6].

Infants under two years of age constitute the major reservoir and source of transmission [7]. Furthermore, they are at high risk of pneumococcal disease of which the most common is acute otitis media (AOM) [2]. Therefore, this age group is an interesting population to be studied for nasopharyngeal (NP) carriage.

Pneumococcal disease risk increases if infants reside in day-care centres (DCC), a setting associated with enhanced pneumococcal carriage and antimicrobial resistance [8,9]. Also, the crowded DCC environment facilitates transmission from one infant to another.

With an overall incidence of 299/1000 person-years in infants up to two years of age as reported from five European countries [10], AOM is the major reason for paediatric consultation and antibiotic prescription [11,12]. Worldwide, *S. pneumoniae* causes 28–55% of AOM episodes, making it the dominant bacterial pathogen implicated in the development of AOM [13–16]. The extensive prescription and use of antibiotics promotes antimicrobial resistance, compromising the ability to effectively treat pneumococcal infection and emphasising the importance of preventing pneumococcal disease via vaccines [17].

In Belgium, a PCV7 programme with free of charge vaccine was started in 2007 (2+1 schedule). It quickly reached high 3-dose coverage in infants: from 89.1% in 2008 to 96.5% in 2012 in Flanders (Northern part of Belgium) and from 80.7% in 2009 to 89.2% in 2012 in Wallonia (Southern part of Belgium) [18–21]. Four years later, PCV13 was introduced and recently replaced by PCV10, with regional difference in timing: (1) Flemish infants were PCV13 vaccinated up to the end of June 2015 and thereafter received PCV10 for their further schedule; (2) Walloon infants were PCV13 vaccinated until the end of April 2016 and thereafter received PCV10; (3) In Brussels-Capital-Region, the individual vaccine offer depended on the particular general practitioner (GP) or paediatrician consulted (Flemish or Walloon programme). This change in the Belgian universal vaccination programme was an outcome of the regular tender processes for all vaccines (after expiry of a previous contract), during which the Belgian regions decide autonomously, based on different vaccine characteristics, including price [22].

The introduction of PCV10, lacking PCV13 serotypes 3, 6A, 19A, in European countries such as Austria and Finland resulted in a decreased incidence of invasive pneumococcal disease (IPD), but with a high proportion (70–86% in children) of PCV13-preventable cases among remaining invasive IPD, dominated by serotypes 19A and 3 [23–26]. Although case-control studies in several countries have shown early effectiveness of PCV10 against 19A-IPD, continued surveillance data suggest waning of this cross-protection [27]. In Belgium (up to 2016), remaining IPD in infants after PCV13 introduction was dominated by non-PCV13 serotypes [28].

Pneumococcal conjugate vaccines (PCV) do not only generate direct protection, but also reduce carriage of pneumococcal serotypes present in the vaccine. Such indirect effects of PCV vaccination in infants have been suggested to protect older age groups too and several IPD-surveillance data from other countries are supportive, but the effect size varies and is being downgraded through replacement by non-vaccine serotypes causing IPD [29–31]. Carriage data are essential to understand pneumococcal biology and transmission and to anticipate changes in IPD. The unique Belgian situation of a PCV programme that altered from PCV7 to PCV13 and then to PCV10 and reached high coverage in infants, created the opportunity for an observational follow-up study on NP carriage of *S. pneumoniae* in infants. The baseline results of this three-year carriage study which started in 2016 are presented here. Specific objectives were: to obtain a baseline measurement of pneumococcal carriage prevalence, carriage density, serotype distribution and antibiotic resistance; to compare pneumococcal carriage between regions and between infants with AOM and healthy infants in day-care; and to identify other predictors of carriage and density.

2. Materials and methods

The current cross-sectional study investigates carriage in two infant populations with high reported carriage of *S. pneumoniae*: those attending DCC and those with AOM. The protocol of the AOM part was based on that of a similar 13-year survey by Cohen et al. [32]. Since pneumococcal carriage and AOM incidence are both lower in summer, recruitment is restricted to non-summer seasons (October to June) from 2016 to 2018 at least. The first sampling period was between January and June 2016, i.e. during and shortly after the switch in vaccination programme (Fig. 1). Protocol details are added in the Supplement and summarised here.

2.1. Study population

After ethics approval of the study, recruitment started in: (1) healthy infants residing in one of 85 DCC randomly selected over the three Belgian regions and (2) infants with AOM visiting one of 55 trained GP's or paediatric outpatient services (private or hospital) throughout Belgium.

Age limits for inclusion were $\geq$6 months (to limit inclusion of unvaccinated infants) and $\leq$30 months and for the baseline assessment in the first recruitment season, region-specific age criteria were added to include only infants who were offered PCV13 for both primary vaccine doses in the first year of life.

AOM was defined by the acute onset (i.e. within the preceding seven days) of symptoms and specified otoscopic criteria.

Exclusion criteria were: (1) second inclusion in the same winter season, (2) use of oral antibiotics in the past seven days, (3) presence of a chronic and severe pathology.

2.2. Nasopharyngeal samples and questionnaire

After parents gave written informed consent, a trained nurse or a GP/paediatrician collected a single NP swab and a parent questionnaire about demographic and clinical characteristics and pneumococcal vaccination status. Signs of common cold were defined as coughing and/or running nose.

2.3. Cultures – Serotyping – Antibiotic susceptibility

At the Reference Centre for Pneumococci at the University Hospitals in Leuven, NP samples were cultured, *S. pneumoniae* strains were serotyped and antibiotic susceptibility to penicillin, tetracycline, erythromycin, levofloxacin and cotrimoxazole was determined following CLSI 2016 guidelines [33].

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