Published in final edited form as:

Pediatr Infect Dis J. 2016 August ; 35(8): 901–906. doi:10.1097/INF.0000000000001206.

Emerging *Streptococcus pneumoniae* Strains Colonizing the Nasopharynx in Children after 13-Valent (PCV13) Pneumococcal Conjugate Vaccination in Comparison to the 7-Valent (PCV7) Era, 2006-2015

Ravinder Kaur, PhD^a, Janet R. Casey, MD^b, and Michael E. Pichichero, MD^a

^a Center for Infectious Diseases and Immunology, Rochester General Hospital Research Institute, Rochester, NY

^bLegacy Pediatrics, Rochester, NY

Abstract

Background—After introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in the US in 2000, emergence of replacement serotypes occurred leading to the introduction of a 13-valent pneumococcal conjugate vaccine (PCV13) in 2010 that contained all PCV7 serotypes plus six additional serotypes (1, 3, 5, 6A, 7F & 19A). Here, we describe a 9 year prospective, longitudinal study characterizing *S. pneumoniae* (*Spn*) strains colonizing the nasopharynx (NP) of young children based on serotype, sequence type and antibiotic susceptibility during the PCV7 and PCV13 eras.

Methods—NP samples were obtained for pneumococcal identification from prospectively followed children at 6, 9, 12, 15, 18, 24 & 30 months of age. A total of 1072 visits during the PCV7 era (Jun 2006-Sep 2010) and 2044 visits during the PCV13 era (Oct 2010-Sep 2015) were included from 665 children. Serotyping and MLST (multi-locus sequence typing) types of *Spn* isolates were evaluated along with their antibiotic resistance pattern.

Results—A total of 1045 *Spn* were isolated; 350 during the PCV7 era and 685 during the PCV13 era. The most common serotypes identified during the PCV7 era were 19A and 23B compared with 35B, 23B and 21 in PCV13 era. Serotypes 15A/B/C emerged in equal proportion during the PCV13 era. Serotypes 16 and 20 were only observed in the PCV13 era. NP carriage of 19A persisted 5 years after PCV13 introduction (5% of all isolates). MLST sequence type (ST) 199 remained a dominant ST during both the PCV7 and PCV13 eras and ST558 and ST62 emerged following PCV13. Antibiotic resistance to penicillin, ceftriaxone, cefotaxime, erythromycin, tetracycline, and trimethoprim/sulfamethoxazole significantly decreased from the PCV7 to the PCV13 era.

Conclusion—Serotypes 35B, 23B, 21, and 15A/B/C rapidly emerged as NP colonizers in the early PCV13 era. Genetically divergent strains with ST558 and ST62 emerged. Resistance to common antibiotics declined following the introduction of PCV13.

Keywords

Streptococcus pneumoniae; PCV13; PCV; Serotypes; Sequence types

Introduction

Streptococcus pneumoniae (*Spn*) Nasopharyngeal (NP) colonization occurs commonly in the first two years of life and the prevalence of carriage in children under 2 years of age varies from 30% to 60% (1, 2). There are 94 *Spn* serotypes and their frequency, in both NP colonization and invasive disease, differs by age, geographic region, and socioeconomic conditions (3-5). NP colonization is the first step in disease pathogenesis of *Spn*, leading to non-invasive (e.g. acute otitis media (AOM), sinusitis and non-bacteremic pneumonia) and invasive infections (e.g. bacteremic pneumonia, bacteremia and meningitis) (6). The introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) represented an important step towards the prevention and control of invasive pneumococcal diseases (IPD) and non-invasive diseases associated with vaccine serotypes (7, 8).

Following the introduction of PCV7, there was an initial decrease in the incidence of NP colonization, IPD and AOM by vaccine serotypes; however, within 2 years there was an increase in NP colonization, IPD and AOM rates by non-PCV7 serotypes (9-12). Despite the change in the serotype distribution, the proportion of *Spn* NP colonization remained unchanged from the PCV7 into the PCV13 eras (13). Serotype 19A became the predominate *Spn* isolate in both NP colonization and disease, was frequently resistant to penicillin, and was associated mainly with sequence types (ST) 320 and ST199 indicate clonal expansion of serotype 19A (14-16).

In 2010, the PCV13 vaccine, including serotype 19A and five additional serotypes (1, 3, 5, 6A, 7F), was introduced in the US and several other countries. The present study reports on the changes in NP colonization in US children ages 6-30 months old following the introduction of PCV13. We describe the overall and serotype-specific trends in *Spn* colonization and compare them with the late PCV7 era in Rochester, NY, US. The antibiotic susceptibility pattern and molecular sequence typing patterns of the *Spn* serotypes to observe if particular clones are emerging, among *Spn* strains are compared.

Methods

Study population and sample processing

Subjects participated in our 9-year, prospective, longitudinal study of NP colonization and AOM in young children funded by the National Institutes of Deafness and Communication Disorders (R0108671) from Jun 2006 to Sep 2015. Children were enrolled from a middle-class, suburban socio-demographic population in Rochester, New York at 6 months of age and prospectively followed to 30 months of age. The details of the study design have been previously described (17).

Children received PCV7 vaccine from the introduction of study in 2006 until April 2010 and PCV13 vaccine after April 2010. Children received 3 doses of PCV vaccine according to the

U.S. schedule at 2, 4, and 6 months of age; either PCV7 or PCV13 depending on the date of enrollment period, a booster dose was given at 15 months of age. Written informed consent was obtained from parents prior to enrollment in the study and the IRBs of the University of Rochester and the Rochester Regional Health System approved this study. In this study data obtained from children is compared between the PCV13 (Oct2010-Sep 2015) and the late PCV7 (2006-sept 2010).

Demographic data collected included daycare attendance, breast-feeding, sibling information, family history of AOM, and tobacco smoke exposure. NP wash and NP swab samples were collected at the well child visits at 6, 9, 12, 15, 18, 24, and 30 month of age and processed as previously described (16). Occasionally a child presented with AOM at the time of the well child visit; these visits were excluded in this analysis. Standard microbiology processing and identification techniques were used to process and identify the organism nasal wash cultures. The NP samples were inoculated on chocolate and blood agar plates, and *Spn* was identified first by colony morphology, α -hemolysis of blood agar plate, and optochin disc differentiation. Rough strains that demonstrate α -hemolysis and positive optochin zone were selected for further characterization. Serotypes were determined by Quellung reaction using Latex pool and serotype-specific pneumococcal antisera (Serum Staten Institute, Denmark) on pure cultures of *Spn* isolates.

Antibiotic Susceptibility

Oxacillin susceptibility of each isolate was determined using Sensi-Disc from Becton and Dickinson. The antibiotic susceptibility of *Spn* isolates to 16 different antibiotics including penicillin, ceftriaxone, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, and erythromycin were determined with the VITEK 2 Gram Positive Susceptibility Card-AST-GP68 (BioMerieux, Inc.) in the clinical laboratories of Rochester General Hospital. *Spn* strain ATCC49619 was used as a control for each batch of antibiotic susceptibility testing. The numerical values for each antibiotic were expressed in $\mu\text{g/ml}$ and *Spn* isolates were classified as susceptible, intermediate, or resistant based on current Clinical and Laboratory Standard Institute (CLSI) breakpoints for non-meningeal isolates (18).

Multilocus Sequence Typing (MLST)

Sequence types (STs) of some randomly picked *Spn* isolates from our population were determined using the MLST technique as described previously (19, 20). An eBURST analysis was performed to compare ST of *Spn* in the PCV7 vs. PCV13 eras as described previously (21, 22).

Statistics

The statistical analysis was performed on GraphPad Prism version 6. The statistical significance was assessed using Fisher's exact test with a significance level of p value 0.05.

Results

Study Population and Microbiology

Six hundred sixty-five subjects were enrolled and had study visits from June 2006 to September 2015. 52% of the children were male. Table 1 shows the demographic data of our study population.

There were a total of 1072 well child visits during PCV7 period (Jul 2006-Sept 2010) and 2044 well child visits during the PCV13 period (Oct 2010-Sept 2015). Data during the transition period of vaccines from April-September 2010 were included in the PCV7 era. Table 2 shows the percentage of the three otopathogens isolated from NP samples of healthy children during PCV7 and PCV13 eras. Overall *Spn* colonization rates remained stable during the two time periods. There was a significant decrease in nontypeable *H. influenzae* (NT*Hi*) NP colonization while NP colonization by *Moraxella catarrhalis* (*Mcat*) remained stable following the introduction of PCV13.

S. pneumoniae Serotypes

Figure 1 shows the percentage distribution of the most commonly isolated *Spn* serotypes during the PCV7 and PCV13 eras. There was a significant decrease in vaccine serotypes 19A and 6A in the PCV13 era, $p < 0.0001$ and $p < 0.001$ respectively. Colonization of *Spn* serotype 3 did not differ between the PCV7 and PCV13 eras. Non-vaccine serotypes 35B, 35 and 16 increased significantly, $p < 0.0001$, $p < 0.001$ and $p < 0.05$ respectively. Within serogroup 15 there were equal proportions of serotypes 15A, 15B and 15C. Serotypes 16 and 20 (>6 isolates each) were only observed in the post-PCV13 era.

Sequence types

A total of 289 *Spn* isolates from the PCV7 era and 503 isolates from the PCV13 era were characterized using MLST which represented typing of 59% of the isolates and 73% of the isolates from the PCV7 and the PCV13 eras, respectively. Table 3 shows the most common STs, and the associated serotypes observed in the PCV7 and PCV13 eras. ST 199, 558 and 62 were seen most often following PCV13 introduction. There was a significant increase ($p < 0.0001$) in ST 558 associated with emergence of serotype 35B. Despite the significant decrease in serotype 19A, ST 199 remained unchanged due to the increase in serotypes 15A, B, and C. ST 320, associated with serotype 19A, significantly decreased following the introduction of PCV13 ($p < 0.0001$). ST 36, 235, 448, 1840, 3280 and 3811 were only observed following introduction of PCV13.

Figure 2 shows an eBURST analysis comparison of STs observed during the PCV7 and PCV13 eras. Each ST is represented by a point and its size is determined by the number of isolates. Groups of related STs are referred to as clonal complexes (CC). STs differing at a single allele are linked by straight line. Those STs, which cannot be linked to any other in the sample, are termed singletons. STs shown in pink were found in both the PCV7 and PCV13 eras, in black when found from the PCV7 era only and in green when found only during the PCV13 era.

Antibiotic susceptibility

There was a higher percentage of *Spn* isolates that were oxacillin resistant in PCV7 era and the percentage of β -lactamase producing *NTHi* and *Mcat* strains remained stable (Table 2). Table 4 shows the antibiotic susceptibility to 16 different antibiotics among 210 *Spn* isolates during the PCV7 and 438 *Spn* isolates during the PCV13 era. Among *Spn* isolates the prevalence of penicillin-nonsusceptible strains decreased (33% vs. 26%, $p<0.05$) and the prevalence of penicillin-resistant strains decreased (13% vs. 4%, $p<0.001$) in the PCV13 era. Conversely, 56% of *Spn* isolates were penicillin susceptible (MIC value ≤ 0.06 $\mu\text{g/ml}$) in the PCV7 era compared to 69% in the PCV13 era ($p<0.001$). Antibiotic resistance to Cefotaxime, Ceftriaxone, Erythromycin, Tetracycline and Trimethoprim/Sulfamethoxazole significantly decreased in the PCV13 era. *Spn* strains carrying multiple drug resistance significantly decreased ($p<0.0001$) in the PCV13 era; 10.5% *Spn* isolates exhibited resistance to 3 antibiotics among strains isolated in the PCV7 era compared to 2.8% in the PCV13 era ($p<0.0001$).

Discussion

Pneumococcal conjugate vaccines containing 7, 10 and 13 serotypes are in use in multiple countries resulting in a decline in colonization and both invasive and non-invasive pneumococcal infections with vaccine serotypes. However, the emergence of replacement serotypes occurred (15, 23, 24) and continues to occur as shown from the results presented here. Our group has been prospectively monitoring the serotype distribution, molecular type diversity and antibiotic susceptibility of *Spn* strains since 1995 (14, 16, 25). Here we provided analysis of 9 years of results, 2006 – 2015, and showed the adaptability of the pneumococcus to respond to vaccine pressure.

A consistent observation among most studies [19-22], including ours, but not all studies [23, 24] is that PCVs do not result in an overall reduction in the frequency of *Spn* NP colonization. PCVs effectively eliminate all or nearly all of the strains expressing capsules corresponding to the polysaccharide-protein antigens in the vaccines. However, new serotypes quickly emerge to take the place of the strains eliminated.

We found a reduction in the NP colonization of vaccine serotype 19A after introduction of PCV13. Consistent with our results, Kaplan *et al* (26) and Bruce *et al* (9) have previously reported a decline in the prevalence of serotype 19A among children hospitalized with IPD after introduction of PCV13. However, we note that there is still a substantial frequency of *Spn* 19A colonization (5% of total *Spn* strains) in our population 5 years after PCV13 introduction. Olarte *et al* (27) found that 19A was still the most common serotype causing *Spn* meningitis in children during 2011-2013.

From an international perspective, another important serotype is 3. We did not observe a change in the frequency of serotype 3 NP colonization since introduction of PCV13. However, isolation of serotype 3 is relatively uncommon in our population so it is not possible to draw a conclusion of vaccine effectiveness on the reduction in NP colonization. Similarly, a study in France by Cohen *et al* (28) found no change in NP colonization by serotype 3.

We observed only 1 isolate of 7F and have not observed serotypes 1 and 5 in our population. Low detection rates of the serotypes 1, 3, 5 and 7F are expected irrespective of vaccination because they are rarely found in the NP, probably due to low density and short time of colonization (29).

Non-PCV13 serotypes accounted for 91% of the isolates in the PCV13 era, with 35B, 21 and 23B being the most commonly isolated (9-15% of total *Spn* isolates each). Other emergent serotypes of potential importance were non-vaccine serotypes 15A, 15B, 15C, 23A and 11A. Martin *et al* (30) recently reported the emergence of serogroup 15 and 35 but they did not subtype the strains. This is highly relevant as the serotypes 15A, 15B and 15C do not cross protect (31) and are observed in equal proportion in our population. The clinical relevance of serotypes 15B, 15C, 23A and 11 has been noted recently in invasive diseases (26). Among serogroup 35, 13% were 35B and 7% were either serotypes 35A/C/or F. Serotype 6C, which is not included in PCV13, accounted for ~2% of the total *Spn* isolates in our study during both the PCV7 and PCV13 eras. Some authorities hoped that inclusion of 6A in PCV13 might induce cross functional anti-6C opsonophagocytic responses and reduce 6C colonization (32) but this is not what we observed.

We found that the frequency of isolation of other potential respiratory bacterial pathogens, *NTHi* and *Mcat* remained stable across the PCV7 and PCV13 eras indicating that strains expressing non-vaccine *Spn* serotypes occupy the niche emptied by vaccine serotypes. The impact of age on *Spn* carriage and multicarriage analysis is not provided here and has been described previously (33).

Antibiotic resistance in the pneumococcal isolates decreased in the PCV13 era. Because serotype 19A accounted for the majority of antibiotic resistant strains during the PCV 7 era (15, 34) the decline in prevalence of serotype 19A was the major contributor to the significant decrease in nonsusceptible *Spn* strains during the PCV13 era. Our results differ from those of Lee *et al* (35), among children from Boston where they did not find a substantial change in the pattern of antibiotic nonsusceptibility in 2011, despite a modest decline in the rate of *Spn* serotype 19A carriage. Dagan *et al* (36) had similar findings among children in Israel.

Previous reports from our lab and other groups showed a predominance of ST320 and ST199 during the PCV7 era (14, 37). ST320 emerged as a predominant 19A clone following the introduction of PCV7 but was nearly eliminated following the change to PCV13. The *Spn* 19A serotypes we isolated during the PCV13 era were mainly ST199 and ST695. It is unclear why vaccination pressure by PCV13 appeared to eliminate clone ST320 but not ST199 or ST695. A newly predominant ST558 emerged in the PCV13 era associated with serotype 35B. ST558 was the main clone observed (13% of all isolates) in our population followed by ST199 (8%) and ST62 (9%) in the PCV13 era. This is the first report of the emergence of ST558 in the PCV13 era. Special note is taken of the emergence of serogroup 15 strains that share a ST199 origin. ST199 strains expressing a 19A capsule emerged in the PCV7 era to predominate and acquire antibiotic resistance. Additional studies are needed to see if strains expressing capsules of serogroup 15 become the next serotype 19A to cause invasive diseases.

Our study population is derived from a predominantly middle class, suburban population of children in upstate NY and may not be representative of other types of populations in the US or those in other countries. We showed that four years after the introduction of PCV13, *Spn* NP colonization rates have remained stable, there has been a reduction in vaccine serotypes 19A and 6A and an emergence in non-vaccine serotypes, 35B, 23B, 21, 15A, 15B and 15C. As occurred after introduction of PCV7, there was a decrease in antibiotic non-susceptible *Spn* strains with the elimination of 19A and 6A. The elimination of NP colonization of vaccine serotypes following PCV13 vaccination led to herd immunity and herd immunity extends the impact of PCV13 beyond vaccinated children to unvaccinated children and adults (38).

Acknowledgments

Financial Disclosure: This study was funded by the Pfizer and NIH NIDCD RO1 08671.

References

1. Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *The Journal of antimicrobial chemotherapy*. 2002; 50(Suppl S2): 59–73. [PubMed: 12556435]
2. Ghaffar F, Friedland IR, McCracken GH Jr. Dynamics of nasopharyngeal colonization by *Streptococcus pneumoniae*. *The Pediatric infectious disease journal*. 1999; 18:638–646. [PubMed: 10440444]
3. Sniadack DH, Schwartz B, Lipman H, et al. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children--implications for vaccine strategies. *The Pediatric infectious disease journal*. 1995; 14:503–510. [PubMed: 7667055]
4. Scott JA, Hall AJ, Dagan R, et al. Serogroup-specific epidemiology of *Streptococcus pneumoniae*: associations with age, sex, and geography in 7,000 episodes of invasive disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1996; 22:973–981. [PubMed: 8783696]
5. Musher DM. Infections caused by *Streptococcus pneumoniae*: clinical spectrum, pathogenesis, immunity, and treatment. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1992; 14:801–807. [PubMed: 1576274]
6. Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *The Lancet Infectious diseases*. 2004; 4:144–154. [PubMed: 14998500]
7. Weil-Olivier C, van der Linden M, de Schutter I, Dagan R, Mantovani L. Prevention of pneumococcal diseases in the post-seven valent vaccine era: a European perspective. *BMC infectious diseases*. 2012; 12:207. [PubMed: 22954038]
8. Kaplan SL, Mason EO Jr, Wald ER, et al. Decrease of invasive pneumococcal infections in children among 8 children's hospitals in the United States after the introduction of the 7-valent pneumococcal conjugate vaccine. *Pediatrics*. 2004; 113:443–449. [PubMed: 14993532]
9. Bruce MG, Singleton R, Bulkow L, et al. Impact of the 13-valent pneumococcal conjugate vaccine (pcv13) on invasive pneumococcal disease and carriage in Alaska. *Vaccine*. 2015; 33:4813–4819. [PubMed: 26247901]
10. Cohen R, Varon E, Doit C, et al. A 13-year survey of pneumococcal nasopharyngeal carriage in children with acute otitis media following PCV7 and PCV13 implementation. *Vaccine*. 2015; 33:5118–5126. [PubMed: 26271823]
11. Mendes RE, Hollingsworth RC, Costello A, et al. Noninvasive *Streptococcus pneumoniae* serotypes recovered from hospitalized adult patients in the United States in 2009 to 2012. *Antimicrobial agents and chemotherapy*. 2015; 59:5595–5601. [PubMed: 26124173]

12. Griffin MR, Zhu Y, Moore MR, Whitney CG, Grijalva CG. U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. *The New England journal of medicine*. 2013; 369:155–163. [PubMed: 23841730]
13. Gounder PP, Bruce MG, Bruden DJ, et al. Effect of the 13-valent Pneumococcal Conjugate Vaccine on Nasopharyngeal Colonization by *Streptococcus pneumoniae* -- Alaska, 2008–2012. *The Journal of infectious diseases*. 2013
14. Casey JR, Kaur R, Friedel VC, Pichichero ME. Acute otitis media otopathogens during 2008 to 2010 in Rochester, New York. *The Pediatric infectious disease journal*. 2013; 32:805–809. [PubMed: 23860479]
15. Pichichero ME, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *Jama*. 2007; 298:1772–1778. [PubMed: 17940232]
16. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *The Pediatric infectious disease journal*. 2010; 29:304–309. [PubMed: 19935445]
17. Friedel V, Zilora S, Bogaard D, Casey JR, Pichichero ME. Five-year prospective study of paediatric acute otitis media in Rochester, NY: modelling analysis of the risk of pneumococcal colonization in the nasopharynx and infection. *Epidemiology and infection*. 2014; 142:2186–2194. [PubMed: 24480055]
18. CaLS, I. Seventeenth Informational Supplement. CLSI; Wayne, PA: 2007. Performance Standards for Antimicrobial Susceptibility Testing.; p. 126–128.
19. Kaur R, Chang A, Xu Q, Casey JR, Pichichero ME. Phylogenetic relatedness and diversity of non-typable *Haemophilus influenzae* in the nasopharynx and middle ear fluid of children with acute otitis media. *Journal of medical microbiology*. 2011; 60:1841–1848. [PubMed: 21799196]
20. Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiology*. 1998; 144(Pt 11): 3049–3060. [PubMed: 9846740]
21. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *Journal of bacteriology*. 2004; 186:1518–1530. [PubMed: 14973027]
22. Spratt BG, Hanage WP, Li B, Aanensen DM, Feil EJ. Displaying the relatedness among isolates of bacterial species -- the eBURST approach. *FEMS microbiology letters*. 2004; 241:129–134. [PubMed: 15598523]
23. Pelton SI, Loughlin AM, Marchant CD. Seven valent pneumococcal conjugate vaccine immunization in two Boston communities: changes in serotypes and antimicrobial susceptibility among *Streptococcus pneumoniae* isolates. *The Pediatric infectious disease journal*. 2004; 23:1015–1022. [PubMed: 15545856]
24. Farrell DJ, Klugman KP, Pichichero M. Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *The Pediatric infectious disease journal*. 2007; 26:123–128. [PubMed: 17259873]
25. Casey JR, Pichichero ME. Changes in frequency and pathogens causing acute otitis media in 1995–2003. *The Pediatric infectious disease journal*. 2004; 23:824–828. [PubMed: 15361720]
26. Kaplan SL, Barson WJ, Lin PL, et al. Early trends for invasive pneumococcal infections in children after the introduction of the 13-valent pneumococcal conjugate vaccine. *The Pediatric infectious disease journal*. 2013; 32:203–207. [PubMed: 23558320]
27. Olarte L, Barson WJ, Barson RM, et al. Impact of the 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Meningitis in US Children. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2015; 61:767–775. [PubMed: 25972022]
28. Cohen R, Levy C, Bingen E, Koskas M, Nave I, Varon E. Impact of 13-valent pneumococcal conjugate vaccine on pneumococcal nasopharyngeal carriage in children with acute otitis media. *The Pediatric infectious disease journal*. 2012; 31:297–301. [PubMed: 22330166]
29. Simell B, Auranen K, Kayhty H, et al. The fundamental link between pneumococcal carriage and disease. *Expert review of vaccines*. 2012; 11:841–855. [PubMed: 22913260]

30. Martin JM, Hoberman A, Paradise JL, et al. Emergence of *Streptococcus pneumoniae* Serogroups 15 and 35 in Nasopharyngeal Cultures From Young Children With Acute Otitis Media. *The Pediatric infectious disease journal*. 2014; 33:e286–290. [PubMed: 24911895]
31. Rajam G, Carlone GM, Romero-Steiner S. Functional antibodies to the O-acetylated pneumococcal serotype 15B capsular polysaccharide have low cross-reactivities with serotype 15C. *Clinical and vaccine immunology : CVI*. 2007; 14:1223–1227. [PubMed: 17609392]
32. Cooper D, Yu X, Sidhu M, Nahm MH, Fernsten P, Jansen KU. The 13-valent pneumococcal conjugate vaccine (PCV13) elicits cross-functional opsonophagocytic killing responses in humans to *Streptococcus pneumoniae* serotypes 6C and 7A. *Vaccine*. 2011; 29:7207–7211. [PubMed: 21689707]
33. Kaur R, Czug K, Casey JR, Pichichero ME. Correlation of nasopharyngeal cultures prior to and at onset of acute otitis media with middle ear fluid cultures. *BMC infectious diseases*. 2014; 14:640. [PubMed: 25475135]
34. Cohen R, Levy C, Bonnet E, et al. Risk factors for serotype 19A carriage after introduction of 7-valent pneumococcal vaccination. *BMC infectious diseases*. 2011; 11:95. [PubMed: 21501471]
35. Lee GM, Kleinman K, Pelton SI, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccination on Carriage in Young Children in Massachusetts. *Journal of the Pediatric Infectious Diseases Society*. 2014; 3:23–32. [PubMed: 24567842]
36. Dagan R, Juergens C, Trammel J, et al. Efficacy of 13-Valent Versus 7-Valent Pneumococcal Conjugate Vaccine Against Nasopharyngeal Colonization of Antibiotic-Nonsusceptible *Streptococcus pneumoniae*. *The Journal of infectious diseases*. 2014
37. Hanage WP, Bishop CJ, Huang SS, et al. Carried pneumococci in Massachusetts children: the contribution of clonal expansion and serotype switching. *The Pediatric infectious disease journal*. 2011; 30:302–308. [PubMed: 21085049]
38. van Hoek AJ, Sheppard CL, Andrews NJ, et al. Pneumococcal carriage in children and adults two years after introduction of the thirteen valent pneumococcal conjugate vaccine in England. *Vaccine*. 2014; 32:4349–4355. [PubMed: 24657717]

Figure 1.

Comparison of most common serotypes of *S. pneumoniae* isolated from nasopharynx of healthy children in the PCV7 versus PCV13 eras.

**** $p < 0.0001$, *** $p < 0.001$ and * $p < 0.05$.

Notes: Dotted line represent to separate vaccine types from non-vaccine types.

Y-axis is split at 15 to zoom in to show proportion of below 15% serotypes on the same graph.

Figure 2.

A Comparative eBURST diagram showing the combined NP colonized *S. pneumoniae* isolated from children during PCV7 and PCV13 period. The Sequence Type numbers are colored according to whether they are found only during PCV13 (green), during PCV7 (black) or in both (pink). Size of the point that represents each sequence type is determined by the number of isolates it contains. Sequence types differing at a single allele are linked by straight line. Those sequence types, which cannot be linked to any other in the population, are termed singletons.

Table 1

Demographic information of Children at Enrollment

Number of Children	665
Sex	
Male	52%
Female	48%
Race/Ethnicity	
Caucasian	70.9%
African American	11.8%
Hispanic	3.9%
Asian	1.4%
Other	12%
Family history of AOM	49.6%
Exposed to tobacco smoke	12.7%
Day care attendance	43.9%
Breast-feeding >6 mo	27.2%

Table 2

Comparison of otopathogens causing Nasopharyngeal Colonization in Healthy children during PCV7 vs. PCV13 eras

Bacterial Respiratory	PCV7 (n=1072)	PCV13 (n=2044)
<i>S. pneumoniae</i>	350 (32.7%)	685 (33.5%)
Oxacillin resistance	115 (32.8%)	177 (25.8%) *
Nontypeable <i>H. influenzae</i>	181(16.9%)	252(12.3%) ***
β -lactamase positive	60 (33.2%)	87 (34.5%)
<i>M. catarrhalis</i>	403 (37.6%)	737 (36.1%)
β -lactamase positive	403 (100%)	737 (100%)

* Significant decrease ($p < 0.05$) in oxacillin susceptibility among strains between PCV7 vs. PCV13.

*** Significant decrease ($p < 0.001$) in proportion difference among NTHi strains between PCV7 vs. PCV13.

Table 3

Comparison of the most commonly observed sequence types (STs) and associated serotypes of *Streptococcus pneumoniae* in the PCV7 vs. PCV13 eras

Common Sequence types	# (%) of <i>Spn</i> MLST typed in PCV7 period (n=289)	# (%) of <i>Spn</i> MLST typed in PCV13 period (n=503)	Main Serotypes associated with ST
199	29 (10.0%)	42 (8.3%)	19A, 15B, 15A, 15C
558 [†]	12 (4.2%)	66 (13.1%) [†]	35B, 35 (not B)
62	19 (6.6%)	44 (8.8%)	11A, 11B, 11C, 6A, 35, 9
432	7 (2.4%)	32 (6.4%)	21
439	6 (2.1%)	23 (4.6%)	23A, 23B
320 [†]	21 (7.3%)	5 (1.0%)	19A
338	8 (2.8%)	20 (4.0%)	23A, 23B, 21
433	13 (4.5%)	16 (3.2%)	2211C
1262	6 (2.1%)	13 (2.6%)	15A, 15B, 15C
816	5 (1.7%)	7 (1.4%)	10, 39
695	7 (2.4%)	6 (1.2%)	19A
1847	10 (3.5%)	2 (0.4%)	23B, 23A
63	5 (1.7%)	6 (1.2%)	15A
2733	3 (1.0%)	8 (1.6%)	19A
473	3 (1.0%)	6 (1.2%)	6A, 6C, 21
1292	5 (1.7%)	3 (0.6%)	6A, 6C, 11C, 21
7927	4 (1.4%)	4 (0.8%)	21, 12
448 [*]	0	7 (1.4%)	NT
498	2 (0.7%)	9 (1.8%)	35 (not B)
36 [*]	0	5 (1.0%)	23B
1379	5 (1.7%)	1 (0.2%)	6A, 6C
3280 [*]	0	5 (1.0%)	35B, 15B, 15C
1840 [*]	0	6(1.2%)	23B, 15B
235 [*]	0	5(1.0%)	20
3811 [*]	0	13(2.5%)	15A

Note

^{*} represent STs only observed in PCV13 period.

[†] represent significant difference (p<0.0001) between PCV7 and PCV13.

Table 4

Comparison of antibiotic susceptibilities of the *Streptococcus pneumoniae* nasopharyngeal isolates during PCV7 versus PCV13 eras.

Antibiotics	Antibiotics sensitivity range in µg/ml	PCV7			PCV13			p-value of change in Resistance
		Percentage of Spn isolates (n=210)			Prcentage Spn isolates (n=438)			
		(S; I; R)	S	I	R	S	I	
Benzylpenicillin	<=.06;12-1;>=2	55.7	31.4	12.9	68.5	27.2	4.3	<0.001
Amoxicillin /Clavulanic Acid	<=2;14;R>=8	100.0	0.0	0.0	98.4	1.1	0.5	ns
Cefotaxime	<=.5;1;>=2	84.3	10.0	5.7	93.4	0.5	6.1	ns/0.01 *
Ceftriaxone	<=1;2;>=4	84.7	8.6	6.7	96.8	2.3	0.9	<0.0001
Ertapenem	<=1;2;>=4	98.6	1.4	0.0	98.1	0.9	0.0	ns
Meropenem	<=.25;.5;>=1	84.8	12.9	2.4	87.8	10.1	2.1	ns
Levofloxacin	<=2;4;>=8	100.0	0.0	0.0	100.0	0.0	0.0	ns
Moxifloxacin	<=1;2;>=4	100.0	0.0	0.0	100.0	0.0	0.0	ns
Ofloxacin	<=2;4;>=8	100.0	0.0	0.0	99.6	0.2	0.2	ns
Erythromycin	<=.25;.5;>=1	70.0	0.0	30.0	84.2	0.0	15.8	<0.0001
Telithromycin	<=1;2;>=4	100.0	0.0	0.0	100.0	0.0	0.0	ns
Linezolid	<=2	100.0	-	0.0	100.0	-	0.0	ns
Vancomycin	<=1	100.0	-	0.0	98.6	-	1.4	ns
Tetracycline	<=2;4;>=8	80.5	0.5	19.0	94.1	0.9	5.0	<0.0001
Chloramphenicol	<=4S;>=8R	98.6	-	1.4	100.0	-	0.0	0.01
Trimethoprim/Sulfamethoxazole	<=9.5;19-38;>=76	73.3	4.3	22.4	87.2	6.1	6.6	<0.0001

S: sensitive; I: intermediate; R: resistant; ns: not significant

* significant decrease in Cefotaxime Intermediate resistance level among strains between PCV7 vs. PCV13