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Original Article

Impact of PCV7 vaccination on nasopharyngeal carriage and antimicrobial resistance among children in Turkey

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Abstract

Introduction: We aimed to evaluate the effects of 7-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae* and antibiotic resistance in children in a well-child clinic in a tertiary children's hospital in Turkey.

Methodology: We collected nasopharyngeal (NP) specimens from 557 two-month-old babies before vaccination. After the study population had received PCV7, NP samples were obtained from 135 babies. Antimicrobial susceptibility testing and serotyping were performed.

Results: *S. pneumoniae* colonized in 48 (8.6%) of the 557 two-month-old babies before vaccination. The follow-up cohort consisted of 135 subjects. The prevalence of PCV7 strain decreased from 33.3% to 19.3% after vaccination. However, non-PCV7 types increased from 66.6% to 80.6% ($p = 0.02$). Of PCV7 serotypes, 19F was the most frequent serotype before and after vaccination. There was an increase in 6A and 15 of non-PCV7 serotypes after vaccination. Penicillin non-susceptible increased from 56.3% to 80.6% after vaccination ($p = 0.03$). Serotypes 14, 18C, 9V and 6B, which were identified before vaccination, never colonized afterwards. Number of siblings and having sibling with older age of five were determined to be significant effective factors for SP colonization presence after vaccination and antibiotic use was negatively associated with pneumococcal carriage but associated with penicillin non-susceptibility.

Conclusions: Nasopharyngeal carriage rate of *S. pneumoniae* dropped after PCV7 vaccination, and replacement by NVT pneumococci were also observed. Risk factors for nasopharyngeal carriage included household crowding and having a sibling age five years or older. Penicillin non-susceptibility increased in both VT and NVT strains.

Key words: 7-valent pneumococcal conjugate vaccine; serotype replacement; nasopharyngeal carriers; penicillin susceptibility.

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Introduction

Streptococcus pneumoniae (SP) infections are known to be a prominent cause of mortality and morbidity in children under 2 years of age. Because asymptomatic nasopharyngeal carriage of S. pneumoniae is an essential element of the transmission of pneumococcal disease it is important to investigate carriage status for evaluating the effect of new pneumococcal vaccines. Pneumococcal conjugate vaccines (PCVs) are effective against invasive pneumococcal disease and nasopharyngeal carriage of vaccine serotypes (VT) [1]. Because heptavalent pneumococcal conjugate vaccine (PCV7) includes only 7 of the more than 92 pneumococcal serotypes, it has been shown that the prevalence of non-vaccine serotypes (NVTs) among asymptomatic carriers increased after vaccination [2]. Many of these NVT serotypes exhibit lower invasiveness and lower antibiotic resistance [3]. However, concerns have been raised about replacement of vaccine serotypes by nonvaccine serotypes that could be more virulent or more resistant to antimicrobials. In Turkey, the rate of high-level penicillin-resistant *Streptococcus pneumoniae* (SP) strains was quite low in healthy children [4]. Due to the widespread empirical use of antibiotics, there is a risk that the prevalence of penicillin non-susceptibility among SP might increase.

In 2008, PCV7 was introduced in Turkey with 3 doses + 1 boost dose schedule. Currently, vaccination coverage among children is over 95% [5]. Studies showed that 63-69% of invasive pneumococcal diseases in children under two years of age were caused by

PCV7 serotypes [6,7]. Ozdemir *et al.* found that the pneumococcal carriage rate was 22.5% and total coverage of vaccine and vaccine-related serotypes by PCV7, was 51.2% before vaccine introduction in healthy infants [8]. In Turkey however there is little published information regarding the effects of the implementation of PCV7 on nasopharyngeal carriage in healthy children. Many of the studies about post-PCV nasopharyngeal colonization were cross-sectional and were done in daycare centers. Prospective studies like ours on serotype changes before and after vaccination are rare [9,10]. The goals of this study are twofold: first is to determine the changes in SP serotypes and penicillin resistance in nasopharyngeal flora of healthy children before and after vaccination with PCV7, and the second is to evaluate the risk factors for carriage.

Methodology

The sample

Nasopharyngeal samples of 557 children who were followed up in the continuity clinics of Gazi University Medical Faculty were obtained at two months of age, before the first pneumococcal vaccination. Exclusion criteria were hypersensitivity to any component of the vaccine, including diphtheria toxoid. The sample was narrowed in order to cope with the difficulty of following the whole group of babies. This was achieved mainly by selecting the same babies who were colonized before vaccination so that we would be able to keep track of SP clearance and persistence in NP. In an attempt to keep track of SP acquisition, twice the numbers of these babies were randomly selected from among the all study group babies who were not colonized initially. For determining potential risk factors for nasopharyngeal carriage of *Streptococcus pneumonia* and penicillin-non-susceptible *Streptococcus pneumoniae* (PNSP) we developed a questionnaire. The questionnaire inquired the following data: age, sex, timing of nasopharyngeal samples, presence of siblings in the household, household size, daycare attendance, history of acute otitis media, and antimicrobial drug use within the month prior to nasopharyngeal samples.

Microbiological analysis

NP samples were obtained trans-nasally, by means of a flexible, sterile, dry cotton swabs. They were kept in Stuart medium at room temperature until they were processed in the microbiology laboratory within 3-4 hours. NP samples were plated onto trypticase soy agar with 5% sheep blood and 5µg/mL gentamycin. The plates were incubated at 37ºC in 5% CO_2 for 24-48 h. Pneumococci were distinguished from other alpha-hemolytic streptococci on the basis of colony morphology, optochin inhibition (ethylhydrocupreine; Difco, Detroit, USA), and bile solubility (10% sodium deoxycholate [Bactidrop; Difco, Detroit, USA]). Pneumococcal serogroups and serotypes were determined by latex agglutination test and the Quellung reaction method using polyclonal rabbit antisera and selected factor sera (Pneumotest-Latex kit; Statens Serum Institute, Copenhagen, Denmark) respectively. All pneumococci that did not react with the available antisera were confirmed as non-typeable (NT). To determine penicillin susceptibility, 1 µg oxacillin disks were used. When the inhibition zone measured ≥20 mm, the result was accepted as resistant according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method. MICs of penicillin were determined by using E test strips (Etest; AB Biodisk, Solna, Sweden).

Ethical approval

The local Ethics Committee of Gazi University approved the study (01/2010-63), and the study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each parent.

Statistical analysis

Data were analyzed with SPSS software (version 15.0 for Windows). All data are reported as median values (ranges) or as percentages (95% confidence interval (CI)). Depending on the normality of the data, variables were compared with an independent samples t-test or with a Mann-Whitney U test. All categorical variables were compared using the Pearson chi-square test, Yate's correction chi-square test, and Fisher's exact test. Multivariate logistic regression analyses were performed to evaluate the effects of the covariates (i.e., age, sex, timing of nasopharyngeal samples, presence of siblings in the household, daycare attendance, history of acute otitis media, and antimicrobial drug use within the month prior to nasopharyngeal samples) on the carriage of SP and penicillin-non-susceptible *Streptococcus pneumoniae*.

Results

Description of Sample

The study was conducted on 537 children who were two months old at the initial examination. The follow-up cohort consisted of 135 subjects (48 carriers and 87 babies randomized from the non-carriers). Forty-nine percent of these 135 children were males. The median

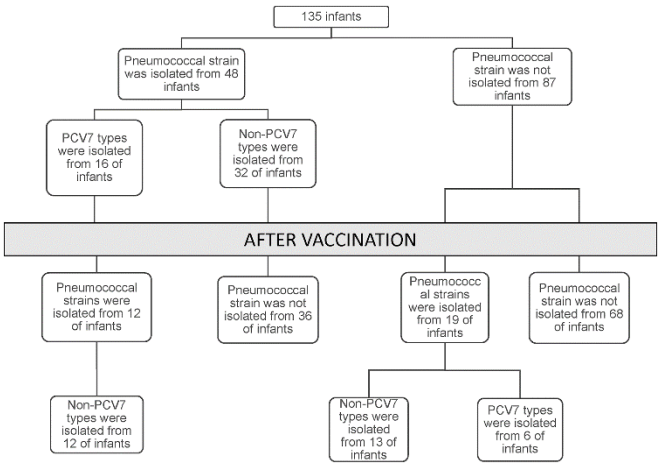
number of siblings was 1 (range 0-3) for the study sample. 34.8% (n = 47) of the infants had school-age siblings. The number of infants having at least one sibling was 71 (52.6%). Only one child was attending a daycare center. In cohort group (135 infants) a history of acute otitis media was reported for three children at the beginning of the study, whereas an additional twenty-two children were diagnosed with acute otitis media in the following months. The use of antibiotics within the month prior to first follow-up visit was reported in 10 children, while 54 children were treated with antibiotics in the month prior to the second follow-up visit.

SP colonization and serotyping before and after vaccination

S. pneumoniae colonization was present in 48 (8.6%) of the 537 two-month-old babies prior to vaccination. *S. pneumoniae* colonization was present in 48 (35.5%) of the 135 infants (cohort group). Among *S. pneumoniae* NP carrier group 16 (33.3%) of them carried VT, 32 (66.6%) of them carried NVT. After vaccination, SP colonization was present in 31 (23%) of the 135 babies. Among *S. pneumoniae* NP carrier group (n = 31) after vaccination 6 (19.4%) of them carried VT, 25 (80.6%) of them carried NVT. Pneumococcal strain colonization presence and distribution of VT/NVT before and after PCV7 vaccination in the cohort group is shown in Figure 1.

Before vaccination, no significant relationship was observed between colonization and seasons (p = 0.91). According to the developed regression model, having more than one sibling (OR**:** 1.56 [95% CI 1.01–1.87]) and having sibling with age five or older (OR: 1.97 [95% CI 1.35–2.13]) were determined to be significant effective factors for SP colonization presence after vaccination. Antibiotic use was negatively associated with pneumococcal carriage (OR**:** 0.12 [95% CI 0.05–0.43]). Factors associated with SP colonization after vaccination were illustrated in Table 1.

Figure 1. Descriptive illustration of the numbers regarding nasopharyngeal carriage of VT/NVT *Streptococcus pneumoniae* in cohort group before and after PCV7 vaccination.



The proportion of PCV7 serotypes declined from 33.3% (16 of 48) to 19.4% (6 of 31) after vaccination, whereas non–PCV7 serotypes increased from 66.7% (32 of 48) to 80.6% (25 of 31). Vaccine serotypes decreased significantly after the conjugate vaccine introduction, with the exception of serotype 23F. Prevalence of serotype 23F was increased from 2.1% to 6.5% after vaccination with PCV7. The six most frequent NVT serotypes after vaccination were 6A (16.1%), 11(9.7%), 33F (3.2%), 1 (3.2%), 10 (3.2%) and 34 (3.2%) respectively. Of the PCV7 serotypes, 19F was the most frequent serotype before (16.6%) and after vaccination (12.9%). Serotype 4 was not identified. Comparing prevalence before and after PCV7 vaccination, 1, 34, 6A and 10 were shown to have increased significantly ($p<0.05$). The changes in the prevalence percentages of the all types of serotypes (PCV7 and non-PCV7) before and after vaccination are shown in Figure 2.

Table 1. Factors associated with SP colonization after vaccination.

		Number of infant	Adjusted OR	Confidence Interval	p
Number of siblings	One or none	118	**Ref**		**0.03**
	More than one	17	**1.56**	1.01-1.87	
History of antibiotic use	Not present	81	**Ref**		**0.02**
	Present	54	**0.12**	0.05-0.43	
Presence of sibling	No	64	Ref		0.97
	Yes	71	0.99	0.69-1.41	
Having sibling with older than age of five	No	88	**Ref**		**0.03**
	Yes	47	**1.97**	1.35-2.13	
History of acute otitis media	No	113	Ref		0.48
	Yes	22	0.85	0.69-1.41	

CI, confidence interval; OR, odds ratio.

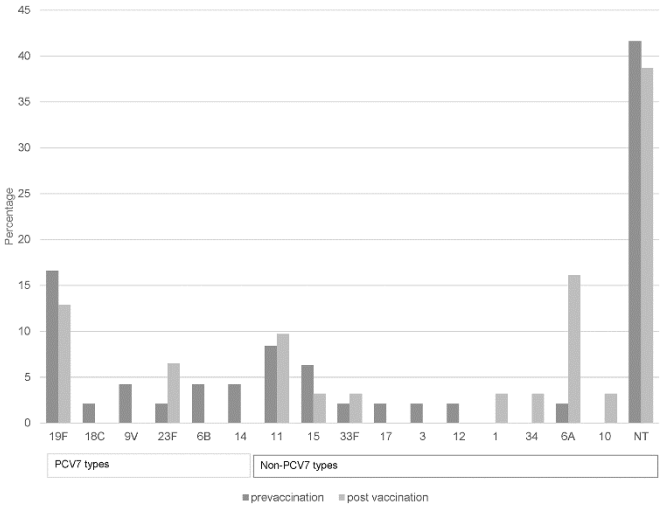
Penicillin resistance of SP serotypes before and after vaccination

Prior to vaccination, 56.3% (27 of 48) of the subjects with pneumococcal colonization were penicillin non-susceptible. It increased to 80.6% (25 of 31) post vaccination (p = 0.03). There was a significant relationship between penicillin susceptibility and history of acute otitis media (p = 0.02) and antibiotic use (p = 0.01). Penicillin non-susceptibility increased among both VT and NVT pneumococci. Before vaccination, 62.5% (10 of 16) of PCV7-included strains were penicillin non-susceptible, whereas penicillin non-susceptibility increased to 100% (6 of 6) after vaccination. Among non-vaccine serotypes, these rates were 53.1% (17 of 32) and 76% (19 of 25), respectively. Prior to PCV7 vaccination, 1.9% of the infants had used antibiotics. After vaccination, 40% of them had used antibiotics.

Acquisition, clearance, and persistence of SP colonization

Of the 87 babies with no SP colonization before vaccination, 19 (21.8%) acquired SP following the vaccination (new acquisition). Of the new acquisitions, 68.4% (13 of 19) were NVT, of which 64.2% were serotype 6A, 15, and non-typable serotypes. In 36 of 48 babies (75%) who had SP colonization before vaccination, no colonization was observed after vaccination (clearance) (p = 0.025). Pneumococcal strain colonization presence according to being VT or NVT before and after PCV7 vaccination in the cohort group is shown in Figure 1.

Figure 2. The changes in the prevalence percentages of the all types of serotypes (PCV7 and non-PCV7) before and after vaccination.



Serotypes 14, 18C, 9V and 6B, which were identified before vaccination, never colonized afterwards. This finding supports a vaccine efficacy of 100% for these serotypes. Serotype 19F and 23F persisted in colonization in 4 of 7 subjects after vaccination. Serotype 4 was not colonized before or after vaccination. Serotypes 11, 6A and nontypable serotypes were persistent non-PCV7 types.

Discussion

In this study, we analyzed the prevalence and distributions of nasopharyngeal carriage of *S. pneumoniae*, antibiotic resistance rates in *S. pneumoniae*, and new acquisition and clearance after PCV7 vaccination in Turkey. We found that the carriage of SP was 8.6% before vaccination with PCV7. In various studies conducted, carriage rate of SP in unvaccinated two-month-old children is found to be between 14 and 23% [11]. In our study the main factors associated with detection of pneumococcal carriage after vaccination were number of siblings and having sibling with age five or older. In this study, after vaccination we showed a significant decrease in PCV7 serotypes, and the clearance was found to be 75%. A similar reduction in vaccine serotypes was previously reported in other studies [12].

In our study, we demonstrated that over 80% of strains were NVT after vaccination, and new acquisitions of NVTs were greater than VTs. Among colonizing pneumococcal isolates, PCV7 serotypes decreased markedly (58%). Similar studies indicated that VT was replaced by NVT in the nasopharynx after vaccination with pneumococcal conjugate vaccine [13]. Likewise Keck *et al*. found that PCV7 serotype carriage declined by 97% after the introduction of PCV7 [14]. Serotype distribution also changes due to some other factors, i.e., antibiotic use, serotype distribution before PCV7 implementation in environment, vaccination coverage of region, etc. However, we regarded the vaccination of children as the most important factor, although we found that household crowding and having siblings over the age of five were independent risk factors for nasopharyngeal carriage. This association has been observed in other studies [15]. If the siblings had been vaccinated with PCV7, this might not have been a risk factor for nasopharyngeal carriage, but the history of PCV7 vaccination for the siblings of infants was not collected in the current study. Most likely, the siblings of the infants in our study had not been vaccinated because PCV7 was not included in national vaccination program prior to the study. Similar to our

study, Colins *et al*. showed that household crowding was associated with pneumococcal carriage [16].

Several studies demonstrated that the more frequent strain among NVT after PCV7 vaccination was 19A [17-19]. Because of its high penicillin resistance and its potential risk of invasive diseases, serotype 19A was placed in 13 valent pneumococcal vaccine. In contrast, among NVT both before and after vaccination, 19A did not colonize at all in our study. Serotype 6A turned out to be the most frequent serotype that we observed after vaccination. It has been shown that the NP carrier of this multidrug-resistant serotype is frequent and thus it was added to PCV13 as well. Serotype 1, which is included in PCV13, appeared in only one isolate in our study. None of the other serotypes in PCV13 colonized in this study. This result prompts us to ask if PCV13 vaccination is really necessary to replace PCV7. On the other hand, we can say serotype 6A is the most important reason for using PCV13. Previous studies have suggested that PCV7 provides lower levels of protection against serotype 19F [20,21]. We also found low efficacy against 19F.

Although serotype replacement has been a concern after the release of the conjugate pneumococcal vaccine, experts hoped that antibiotic resistance would decrease because resistance was highly concentrated in PCV7 strains [22]. In our study, penicillin resistance increased from 56.3% to 80.6% among both VT and NVT serotypes after vaccination. In addition, we found that among PCV7 serotypes, antimicrobial resistance was 100% after vaccination. In the same manner, Finkelstein found that non-susceptibility to penicillin was more common in PCV7-included strains than in non-PCV7 serotypes in a multi-community sample [23]. In our study, all of the resistant strains were intermediate except for one highly resistant strain. Increasing intermediate resistance was observed in Greece as well [24]. The colonization of intermediately penicillin-resistant VT and NVT serotypes continued to increase [25,26]. The increase in penicillin resistance most probably was related to inappropriate antibiotic use during childhood [27]. In our study, prior to PCV7 vaccination, 1.9% of the infants had used antibiotics; on the other hand, after vaccination, 40% of them had used antibiotics.

The main limitation of our study arises from narrowing down the sample size, which was used as a means of coping with social and economic constraints. Differences may appear more clearly in a larger sample.

The strength of this work was the study duration was long and there was a prospective cohort study before the introduction of PCV7.

Conclusion

We showed a significant decrease in PCV7 serotypes carriage rate after the PCV7 vaccination. Clearance was found to be 75%, and replacement by NVT pneumococci was observed in this study. We also found that household crowding and having a sibling age five or older were independent risk factors for nasopharyngeal carriage. Penicillin non-susceptibility increased in both VT and NVT strains, most probably due to increase in antibiotic usage. Future studies should continue to explore serotype changes and antibiotic susceptibility of new serotypes.

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References

1. Wang L, Fu J, Liang Z, Chen J (2017) Prevalence and serotype distribution of nasopharyngeal carriage of *Streptococcus pneumoniae* in China: a meta-analysis. BMC Infect Dis 17: 765.
2. Weinberger DM, Malley R, Lipsitch M (2011) Serotype replacement in disease after pneumococcal vaccination. Lancet 378: 1962-1973.
3. Flasche S, Van Hoek AJ, Sheasby E, Waight P, Andrews N, Sheppard C, George R, Miller E (2011) Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: a cross-sectional study. PLoS Med 8: e1001017.
4. Bayer M, Aslan G, Emekdaş G, Kuyucu N, Kanik A (2008) Nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children and multidrug resistance. Mikrobiyol Bul 42: 223-230. [Article in Turkish].
5. Mollahaliloğlu S, Başara Bora B, Eryılmaz Z (2010) The ministry of health of Turkey health statistics yearbook 2010. School of Public Health, Refik Saydam Hygiene Center Presidency, Ministry of Health of Turkey, Ankara. 2011: 37.
6. Ceyhan M, Ozsurekci Y, Gurler N, Oksuz L, Aydemir S, Ozkan S, Yuksekkaya S, Keser Emiroglu M, Gültekin M, Yaman A, Kiremitci A, Yanık K, Karli A, Ozcinar H, Aydin F, Bayramoglu G, Zer Y, Gulay Z, Gayyurhan ED, Gul M, Ozakin C, Guducuoglu H, Perçin D, Akpolat N, Ozturk C, Camcıoğlu Y, Karadağ Öncel E, Çelik M, Şanal L, Uslu H (2016) Serotype distribution of *Streptococcus pneumoniae* in children with invasive diseases in Turkey: 2008–2014. Hum Vaccin Immunother 12: 308-313.
7. Oksuz L, Gurler N (2017) Serotype distribution and antibiotic resistance of *Streptococcus pneumoniae* strains isolated from the adult patients in a Turkish University hospital. Mikrobiyol Bul 51: 195-208. [Article in Turkish].
8. Ozdemir B, Beyazova U, Camurdan AD, Sultan N, Ozkan S, Sahin F (2008) Nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy Turkish infants. J Infect 56: 332-339.
9. Ercan TE, Severge B, Topkaya A, Ercan RG, Altınkaya N (2011) Effect of the pneumococcal conjugate vaccine on pneumococcal carriage in Turkish children. Pediatr Int 53: 224-230.

10. Ozdemir H, Ciftçi E, Durmaz R, Güriz H, Aysev AD, Karbuz A, Gökdemir R, Acar B, Nar Ötgün S, Ertek M, Köse SK, Ince E. (2014) Nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy Turkish children after the addition of PCV7 to the national vaccine schedule. Eur J Pediatr 173: 313-320.
11. Hosseini SM, Poorolajal J, Karami M, Ameri P (2015) Prevalence of nasopharyngeal carriage of *Streptococcus* pneumonia in Iran: A meta-analysis. J Res Health Sci 15: 141-146.
12. Parra EL, De La Hoz F, Díaz PL, Sanabria O, Realpe ME, Moreno J (2013) Changes in *Streptococcus pneumoniae* serotype distribution in invasive disease and nasopharyngeal carriage after the heptavalent pneumococcal conjugate vaccine introduction in Bogotá, Colombia. Vaccine 31: 4033-4038.
13. Vissers M, Wijmenga-Monsuur AJ, Knol MJ, Badoux P, van Houten MA, van der Ende A, Sanders EAM, Rots NY (2018) Increased carriage of non-vaccine serotypes with low invasive disease potential four years after switching to the 10-valent pneumococcal conjugate vaccine in The Netherlands. PloS one 13: e0194823.
14. Keck JW, Wenger JD, Bruden DL, Rudolph KM, Hurlburt DA, Hennessy TW, Bruce MG (2014) PCV7-induced changes in pneumococcal carriage and invasive disease burden in Alaskan children. Vaccine 32: 6478-6484.
15. Usuf E, Bojang A, Camara B, Jagne I, Oluwalana C, Bottomley C, D'Alessandro U, Roca A (2018) Maternal pneumococcal nasopharyngeal carriage and risk factors for neonatal carriage after the introduction of pneumococcal conjugate vaccines in the Gambia. Clin Microbiol Infect 24: 389-395.
16. Collins DA, Hoskins A, Snelling T, Senasinghe K, Bowman J, Stemberger NA, Leach AJ, Lehmann D (2017) Predictors of pneumococcal carriage and the effect of the 13-valent pneumococcal conjugate vaccination in the Western Australian Aboriginal population. Pneumonia 9: 14.
17. Tocheva AS, Jefferies JM, Rubery H, Bennett J, Afimeke G, Garland J, Christodoulides M, Faust SN, Clarke SC (2011) Declining serotype coverage of new pneumococcal conjugate vaccines relating to the carriage of *Streptococcus pneumoniae* in young children. Vaccine 29: 4400-4404.
18. Park SY, Moore MR, Bruden DL, Hyde TB, Reasonover AL, Harker-Jones M, Rudolph KM, Hurlburt DA, Parks DJ, Parkinson AJ, Schuchat A, Hennessy TW (2008) Impact of conjugate vaccine on transmission of antimicrobial-resistant *Streptococcus pneumoniae* among Alaskan children. Pediatr Infect Dis J 27: 335-340.
19. Wroe PC, Lee GM, Finkelstein JA, Pelton SI, Hanage WP, Lipsitch M, Stevenson AE, Rifas-Shiman SL, Kleinman K, Dutta-Linn MM, Hinrichsen VL, Lakoma M, Huang SS (2012) Pneumococcal carriage and antibiotic resistance in young children before 13-valent conjugate vaccine. Pediatr Infect Dis J 31: 249.
20. Rodrigues F, Foster D, Caramelo F, Serranho P, Gonçalves G, Januário L, Finn A (2012) Progressive changes in pneumococcal carriage in children attending daycare in Portugal after 6 years of gradual conjugate vaccine introduction show falls in most residual vaccine serotypes but no net replacement or trends in diversity. Vaccine 30: 3951-3956.
21. Rinta-Kokko H, Dagan R, Givon-Lavi N, Auranen K (2009) Estimation of vaccine efficacy against acquisition of pneumococcal carriage. Vaccine 27: 3831-3837.
22. Vestrheim DF, Høiby EA, Aaberge IS, Caugant DA (2010) Impact of a pneumococcal conjugate vaccination program on carriage among children in Norway. Clin Vaccine Immunol 17: 325-334.
23. Finkelstein JA, Huang SS, Daniel J, Rifas-Shiman SL, Kleinman K, Goldmann D, Pelton SI, DeMaria A, Platt R (2003) Antibiotic-resistant *Streptococcus pneumoniae* in the heptavalent pneumococcal conjugate vaccine era: predictors of carriage in a multicommunity sample. Pediatrics 112: 862-869.
24. Grivea IN, Panagiotou M, Tsantouli AG, Syrogiannopoulos GA (2008) Impact of heptavalent pneumococcal conjugate vaccine on nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* among day-care center attendees in central Greece. Pediatr Infect Dis J 27: 519-525.
25. Huang SS, Platt R, Rifas-Shiman SL, Pelton SI, Goldmann D, Finkelstein JA (2005) Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. Pediatrics 116: e408-e413.
26. Dagan R, Klugman KP (2008) Impact of conjugate pneumococcal vaccines on antibiotic resistance. Lancet Infect Dis 8: 785-795.
27. Koliou MG, Andreou K, Lamnisos D, Lavranos G, Iakovides P, Economou C, Soteriades ES (2018) Risk factors for carriage of *Streptococcus pneumoniae* in children. BMC Pediatr 18: 144.

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