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Original Article

Streptococcus pneumoniae oropharyngeal colonization in children and adolescents with cystic fibrosis

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Abstract

Background: This study was designed to evaluate *Streptococcus pneumoniae* (*S. pneumoniae*) carriage rates in patients with cystic fibrosis (CF).
Methods: An oropharyngeal swab was obtained from 212 CF children and adolescents enrolled during routine clinical visits. DNA from swabs was analyzed by real-time polymerase chain reaction.
Results: A total of 42 (19.8%) CF patients (mean age ± standard deviation [SD], 12.0 ± 3.3 years) were colonized by *S. pneumoniae*. Carriage was more common in younger patients and tended to decline with age. Administration of systemic and/or inhaled antibiotics in the last 3 months significantly correlated with a reduced carrier state [odds ratio (OR) 0.23, 95% confidence interval (CI) 0.07–0.69, and OR 0.26, 95% CI 0.08–0.77, respectively]. Vitamin D serum levels ≥30 ng/mL were less common in carriers than that in non-carriers (OR 0.35; 95% CI 0.08–1.49). In both the vaccinated and unvaccinated subjects, serotypes 19F, 5, 4, and 9V were the most commonly carried serotypes.
Conclusions: S. pneumoniae carrier state of school-age children and adolescents with CF is more prevalent than previously thought, and pneumococcal conjugate vaccination administered in the first year of life does not reduce the risk of re-colonization in later childhood and adolescence.

Keywords: Cystic fibrosis; Pneumococcal carrier; Pneumococcal colonization; Pneumococcal conjugate vaccine; Pneumococcal vaccination; *Streptococcus pneumoniae*

1. Introduction

Cystic fibrosis (CF) is considered a clinical condition at increased risk of pneumococcal invasive disease (IPD) for which pneumococcal vaccination is recommended [1]. However, the clinical relevance of *Streptococcus pneumoniae* (*S. pneumoniae*) in CF patients as well as the pathological importance of the different pneumococcal serotypes and the preventive efficacy offered by the presently available vaccines are poorly understood. This could explain why the vaccination coverage of CF patients is frequently suboptimal [2].

Pharyngeal colonization by *S. pneumoniae* is considered the basis of pneumococcal transmission among humans and a

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prerequisite for both invasive (IPD) and non-invasive pneumococcal diseases [3]. Pneumococcal conjugate vaccines significantly influenced pneumococcal colonization by the reduction of acquisition rates and density of serotypes included in each preparation [4]. The evaluation of pneumococcal carriage before and after vaccine administration has been considered an effective measure to estimate potential coverage and real efficacy of these preventive measures [5,6]. This study was designed to evaluate *S. pneumoniae* carriage rates in a group of school-age children and adolescents with CF for two reasons: 1) to gain information about the potential risk of *S. pneumoniae* infections in these patients, and 2) to evaluate the potential coverage offered by the pneumococcal conjugate vaccine including the greatest number of serotypes, the 13-valent preparation (PCV13). Moreover, because in Italy the first conjugate pneumococcal vaccines [those containing seven serotypes (PCV7)], was administered until 2008 to no more than 50% of infant population [7], our study could permit a comparison of *S. pneumoniae* carriage in vaccinated and unvaccinated subjects and evaluation of the long-term impact of PCV7 on colonization.

2. Material and methods

2.1. Enrolment of patients and swab collection

The study was conducted at four Regional Fibrotic Cystic Centers situated in Italy: Milan, (Lombardia), Verona (Veneto), Rome (Lazio), and Naples (Campania) from January 1, 2014, to June 30, 2014. The study was approved by the Ethics Committee of all the hospitals in which each center is located. Patients aged 6–17 years with documented diagnosis of CF regularly followed in each center were screened and enrolled during a routine clinic visit. Subjects with an active respiratory infection at the time of sampling, and those with a further chronic underlying disease different from CF were excluded from the study.

The children were enrolled after parental consent and subject assent had been obtained. After enrollment, clinical and laboratory data for each child collected during the previous 3 months were retrieved from the clinical records of the hospital and recorded in an electronic file specifically prepared for the study. Pneumococcal vaccination status was established by consulting the official vaccination chart issued by the Vaccination Services of each region.

The pneumococcal immunization schedule recommended by the Italian Ministry of Health for children born before 2008 included three options: 1) three doses of PCV7 in the first year of life, 2) two doses in the second year, or 3) a single dose after the second year until the fifth [8]. Children were considered fully vaccinated if one of these recommendations had been met by the time of enrolment, and not fully vaccinated if they had started but not completed the vaccination schedule. The latter group comprised only 1% of the enrolled subjects and was not compared with the groups of fully vaccinated or unvaccinated children.

The oropharyngeal samples were obtained using an ESwab kit containing a polypropylene screw-cap tube filled with 1 mL of liquid Amies medium (Brescia, Copan, Italy). The sampling was conducted by pressing the tongue downward to the floor of the mouth with a spatula and swabbing both tonsillar arches and the posterior nasopharynx, without touching the sides of the mouth. All of the swabs were immediately refrigerated at −20 °C, transported to the central laboratory within a week, and processed within 2 h from arrival.

2.2. Identification of S. pneumoniae

Bacterial genomic DNA was extracted from the samples using a NucliSENS easyMAG automated extraction system (BioMeriéux, Bagno a Ripoli, Florence, Italy), a 250 μL sample input, and a generic protocol. The DNA was analyzed for the autolysin-A-encoding gene (*lytA*) and the *wzg* (*cpsA*) gene of *S. pneumoniae* by real-time polymerase chain reaction (PCR) as previously described [9]. Each sample was tested in triplicates and was considered positive if at least two of the three tests revealed the presence of both genes. The levels of detection of the test were 16 genome copies. In order to maximize sensitivity, no internal amplification control was used in the reaction, but there was an external control. The real-time PCR negative specimens were also tested for the presence of an RNase P-encoding gene to exclude PCR inhibition and DNA extraction failure. All of the positive cases were serotyped using primers and probes designed on the basis of the GenBank database sequences (www.ncbi.nlm.nih.gov) of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (i.e., those in the 13-valent pneumococcal conjugate vaccine, PCV13), and synthesized by TIB Molbiol (Genoa, Italy) as previously described [9]. Analytical specificity was pre-evaluated by means of computer-aided analyses using Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast) and BLAST (www.blast.ncbi.nlm.nih.gov/Blast.cgi) software to compare the sequences with all of the sequences listed under bacteria and *Homo sapiens*.

2.3. Statistical analysis

The groups were compared using the X^2 or Fisher's exact test, when appropriate. The ordered categorical data were compared using a Cochran–Armitage trend test. Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional multiple logistic regression models to measure the association between the following: i) pneumococcal vaccination and pneumococcal carrier status and ii) selected demographic and clinical characteristics and pneumococcal carrier status. Adjustment was made for a priori defined covariates such as age, gender, number of siblings, and parental smoking habits. Stratified analyses for the two major age subgroups (<10 and 10–14) were also performed. All of the analyses were two tailed, and p-values <0.05 were considered statistically significant. All analyses were conducted using the SAS version 9.2 (Cary, NC, USA).

3. Results

A total of 212 CF children and adolescents (mean age ± SD, 12.0 ± 3.3 years) were enrolled. Their demographic and

Table 1
The main characteristics of 212 children and adolescents with cystic fibrosis (CF) by pneumococcal carriage.

	All children (n = 212)	Carriers (n = 42)	Non-carriers (n = 170)	p-Value
Age, years				
Mean ± SD	12.0 ± 3.3	10.7 ± 3.1	12.3 ± 3.3	0.005
<10	63 (29.7)	18 (42.9)	45 (26.5)	
10–14	102 (48.1)	18 (42.9)	84 (49.4)	
≥15	47 (22.2)	6 (14.3)	41 (24.1)	0.09
Sex				
Male	102 (48.1)	22 (52.4)	80 (47.1)	
Female	110 (51.9)	20 (47.6)	90 (52.9)	0.54
Ethnicity[a]				
Caucasian	210 (99.5)	41 (100.0)	169 (99.4)	
Non-Caucasian	1 (0.5)	0 (0.0)	1 (0.6)	0.99
No. of siblings[a]				
0	57 (28.1)	11 (29.0)	46 (27.9)	
1	115 (56.6)	23 (60.5)	92 (55.8)	
2	26 (12.8)	4 (10.5)	22 (13.3)	
≥3	5 (2.5)	0 (0.0)	5 (3.0)	0.43
Parental smoking habit[a]				
Both non-smokers	133 (64.2)	25 (65.8)	108 (63.9)	
At least one smoker	74 (35.7)	13 (34.2)	61 (36.1)	0.83
Gestational age (weeks)[a]				
<37	20 (10.0)	7 (18.9)	13 (8.0)	
≥37	179 (90.0)	30 (81.1)	149 (92.0)	0.07
Birth weight (g)[a]				
<2500	20 (10.3)	6 (15.8)	14 (8.9)	
≥2500	175 (89.7)	32 (84.2)	143 (91.1)	0.23
Exclusive breastfeeding[a]				
No	68 (33.0)	12 (30.8)	56 (33.5)	
Yes	138 (67.0)	27 (69.2)	111 (66.5)	0.74
Allergy				
No	159 (77.2)	28 (71.8)	131 (78.4)	
Yes	47 (22.8)	11 (28.2)	36 (21.6)	0.37
Allergic sensitization				
No	162 (78.6)	29 (74.4)	133 (79.6)	
Yes	44 (21.4)	10 (25.6)	34 (20.4)	0.47
Meningococcal vaccination				
No	102 (51.3)	21 (55.3)	81 (50.3)	
Yes	97 (48.7)	17 (44.7)	80 (49.7)	0.58
Influenza vaccination during the current season[a]				
No	65 (31.5)	9 (23.1)	56 (33.5)	
Yes	141 (68.5)	30 (76.9)	111 (66.5)	0.21

[a] Some missing values.

clinical characteristics according to pneumococcal carriage are reported in Table 1. Among the study patients, 42 (19.8%) and 170 (80.2%) were colonized or non-colonized by *S. pneumoniae*, respectively. Carriage was more common in younger patients and tended to decline with age. It was found in 28.6% of children <10 years, in 17.6% of those aged 10–14 years, and in 12.7% of those ≥15 years. Mean age of those who were pneumococcal carriers was significantly lower than that of those who were non-carriers (p = 0.005). No significant difference was found between carriers and non-carriers for any of the studied demographic and clinical variables, including sex, ethnicity, number of sibling, birth characteristics, allergies, and meningococcal and influenza vaccinations.

Table 2 shows the association between demographic, clinical characteristics, and pneumococcal carriage in the study population. Multivariate analysis showed that carriage was not influenced by sex (OR 0.93; 95% CI 0.43–1.98), presence of siblings (OR 1.27; 95% CI 0.54–2.90), parental smoking habit (OR 0.92; 95% CI 0.42–2.04), hospitalization (OR 0.79; 95% CI 0.36–1.77), or administration of steroids in

Table 2
Association between demographic and clinical characteristics and pneumococcal carriage in children with cystic fibrosis (CF).

	Carriers (n = 42)	Non-carriers (n = 170)	OR (95% CI)[a]
Age			
<10	18 (42.9)	45 (26.5)	1 (reference)
10–14	18 (42.9)	84 (49.4)	0.42 (0.18–0.95)
≥15	6 (14.3)	41 (24.1)	0.16 (0.04–0.60)
Sex			
Male	22 (52.4)	80 (47.1)	1 (reference)
Female	20 (47.6)	90 (52.9)	0.93 (0.43–1.98)
Siblings[b]			
No	11 (29.0)	46 (27.9)	1 (reference)
Yes	27 (71.0)	119 (72.1)	1.27 (0.54–2.98)
Parental smoking habit[b]			
Both non-smokers	25 (65.8)	108 (63.9)	1 (reference)
At least one smoker	13 (34.2)	61 (36.1)	0.92 (0.42–2.04)
Hospitalization (last 3 months)[b]			
No	15 (35.7)	48 (28.4)	1 (reference)
Yes	27 (64.3)	121 (71.6)	0.79 (0.36–1.77)
Systemic antibiotic therapy (last 3 months)[b]			
No	38 (90.5)	104 (61.9)	1 (reference)
Yes	4 (9.5)	64 (38.1)	0.23 (0.07–0.69)
Antibiotic inhalation therapy (last 3 months)[b]			
No	37 (88.1)	109 (64.9)	1 (reference)
Yes	5 (11.9)	59 (35.1)	0.26 (0.08–0.77)
Steroid therapy (last 3 months)[b]			
No	37 (88.1)	145 (85.8)	1 (reference)
Yes	5 (11.9)	24 (14.2)	0.96 (0.30–3.09)
The main bacteria at the last expectoration control[b]			
None	4 (9.8)	12 (7.1)	1 (reference)
Staphylococcus aureus	26 (63.4)	96 (56.8)	1.22 (0.24–6.17)
Haemophilus influenzae/ parainfluenzae	7 (17.1)	20 (11.8)	2.10 (0.35–12.6)
Pseudomonas aeruginosa	0 (0.0)	27 (16.0)	NE
Others	4 (9.8)	14 (8.3)	1.20 (0.17–8.50)
Vitamin D level[b]			
<30 ng/mL	18 (81.8)	42 (64.6)	1 (reference)
≥30 ng/mL	4 (18.2)	23 (35.4)	0.35 (0.08–1.49)

NE: not estimable.
[a] Multivariate models included terms for age, gender, number of siblings, and parental smoking plus, in turn, each clinical characteristic analyzed.
[b] Some missing values.

the last 3 months (OR 0.96; 95% CI 0.30–3.09). Moreover, no correlation was found between the carriage of *S. pneumoniae* and evidence of *Staphylococcus aureus* (OR 1.22; 95% CI 0.24–6.17), *Haemophilus influenzae/parainfluenzae* (OR 2.10; 95% CI 0.35–12.6), or other bacteria different from *Pseudomonas aeruginosa* (OR 1.20; 95% CI 0.35–12.6) at the last expectoration collection. In contrast, an inverse association emerged between age and pneumococcal carrier status: the OR of pneumococcal carriage for subjects aged ≥15 years compared to those <10 years was 0.16 (95% CI, 0.04–0.60). None of the *S. pneumoniae* carriers were concurrently colonized by *P. aeruginosa*. Furthermore, the administration of systemic and/or inhaled antibiotics in the last 3 months was significantly associated with a reduced carrier state (OR 0.23, 95% CI 0.07–0.69 and OR 0.26, 95% CI 0.08–0.77, respectively). Finally, vitamin D serum level ≥30 ng/mL was less common in carriers than that in non-carriers, although the difference between the groups did not reach statistical significance (OR 0.35; 95% CI 0.08–1.49).

Table 3
Relationship between pneumococcal vaccination status and pneumococcal carriage in children with cystic fibrosis (CF).[a]

	Vaccinated with PCV7 (n = 35)	Not vaccinated against pneumococcus (n = 177)	OR (95% CI)
Pneumococcal carrier status			
Any serotype			
Non-carriers	25 (71.4)	145 (81.9)	1 (reference)
Carriers	10 (28.6)	32 (18.1)	1.20 (0.44–3.29)
Serotypes in PCV7			
Non-carriers	25 (71.4)	148 (83.6)	1 (reference)
Carriers	10 (28.6)	29 (16.4)	1.33 (0.48–3.66)
Six additional serotypes in PCV13			
Non-carriers	29 (82.9)	163 (92.1)	1 (reference)
Carriers	6 (17.1)	14 (7.9)	1.17 (0.29–4.63)
Subgroup aged <10 years	(n = 16)	(n = 47)	
Any serotype			
Non-carriers	13 (81.2)	32 (68.1)	1 (reference)
Carriers	3 (18.8)	15 (31.9)	0.47 (0.08–2.85)
Serotypes in PCV7			
Non-carriers	13 (81.2)	33 (70.2)	1 (reference)
Carriers	3 (18.8)	14 (29.8)	0.48 (0.08–2.95)
Six additional serotypes in PCV13			
Non-carriers	15 (93.7)	41 (87.2)	1 (reference)
Carriers	1 (6.2)	6 (12.8)	NE
Subgroup aged 10–14 years	(n = 17)	(n = 85)	
Any serotype			
Non-carriers	11 (64.7)	73 (85.9)	1 (reference)
Carriers	6 (35.3)	12 (14.1)	3.22 (0.83–12.40)
Serotypes in PCV7			
Non-carriers	11 (64.7)	75 (88.2)	1 (reference)
Carriers	6 (35.3)	10 (11.8)	3.52 (0.90–13.83)
Six additional serotypes in PCV13			
Non-carriers	13 (76.5)	79 (92.9)	1 (reference)
Carriers	4 (23.5)	6 (7.1)	3.31 (0.61–17.86)

PCV7: 7-valent pneumococcal conjugate vaccine; PCV13: 13-valent pneumococcal conjugate vaccine.

[a] ORs adjusted for age (using a continuous term), gender, number of siblings, and parental smoking.

The relationship between pneumococcal vaccination status and pneumococcal carriage is detailed in Table 3. Carriage of at least one pneumococcal serotype was slightly more common in PCV7 immunized than in PCV7 non-immunized children (28.6% vs 18.1%, OR 1.20; 95% CI 0.44–3.29). The same results were observed when the only serotypes included in PCV7 and PCV13 were considered. Serotypes in PCV7 were detected in 10/35 (28.6%) and in 29/177 (16.4%) vaccinated and unvaccinated children, respectively (OR 1.33; 95% CI 0.48–3.66). Moreover, at least one of the six additional serotypes contained in PCV13 was detected in 6/35 (17.1%) and in 14/177 (7.9%) vaccinated and unvaccinated subjects, respectively (OR 1.17; 95% CI 0.29–4.63). However, none of these differences reached statistical significance. No significant difference between the vaccinated and unvaccinated subjects was observed also when carriage was evaluated according to the age of the patients. However, while the carriage of any pneumococcal serotype and of PCV7 and PCV13 serotypes in children aged <10 years was lower in vaccinated patients (OR 0.47, 95% CI 0.08–2.85; OR 0.48, 95% CI 0.08–2.95; OR not estimable, respectively), the opposite was found in children 10–14 years old (OR 3.22, 95% CI 0.83–12.40; OR 3.52, 95% CI 0.90–13.83; OR 3.31, 95% CI 0.61–17.86, respectively). Data regarding children ≥15 years were not compared due to the very low number of vaccinated subjects carrying any serotype in this age group.

Table 4 lists the single serotypes identified in children with CF according to the vaccination status. Only two children (4.8% of carriers), both in the group of unvaccinated patients,

Table 4
Carriage of specific pneumococcal subtypes in children with cystic fibrosis (CF) by pneumococcal vaccination status.

	Vaccinated with PCV7, n. (%)	Not vaccinated with PCV7, n. (%)	OR (95% CI)[a]
Total carriers	10	32	
Carriers of non-typeable serotypes	0 (0.0)	2 (6.2)	0.97 (0.25–3.77)
Carriers of PCV13 serotypes			
1	3 (30.0)	13 (40.6)	1.36 (0.33–5.59)
≥2	7 (70.0)	17 (53.2)	1.22 (0.35–4.31)
Carriers of different PCV13 serotypes			
Serotype 1	1 (2.9)	4 (2.3)	NE
Serotype 3	0 (0.0)	1 (0.6)	NE
Serotype 4	2 (5.7)	6 (3.4)	0.71 (0.07–7.18)
Serotype 5	4 (11.4)	6 (3.4)	4.44 (0.96–20.46)
Serotype 6A	1 (2.9)	1 (0.6)	NE
Serotype 6B	0 (0.0)	0 (0.0)	NE
Serotype 7F	0 (0.0)	2 (1.1)	NE
Serotype 9V	2 (5.7)	5 (2.8)	0.98 (0.10–9.52)
Serotype 14	0 (0.0)	1 (0.6)	NE
Serotype 18C	0 (0.0)	0 (0.0)	NE
Serotype 19A	1 (2.9)	2 (1.1)	NE
Serotype 19F	9 (25.7)	26 (14.7)	1.30 (0.45–3.73)
Serotype 23F	0 (0.0)	0 (0.0)	NE

ORs adjusted for age (using a continuous term), gender, ethnicity, number of sibling, and parental smoking. Reference category is non-carrier of corresponding serotypes.

NE: not estimable; PCV7: 7-valent pneumococcal conjugate vaccine; PCV13: 13-valent pneumococcal conjugate vaccine.

were exclusively colonized by serotypes not included in PCV13. Of the 10 vaccinated children who were colonized by *S. pneumoniae*, three and seven were colonized by 1 or ≥2 serotypes included in PCV13, respectively. Among the unvaccinated patients, those carrying 1 or ≥2 PCV13 serotypes were 13 (40.6%) and 17 (53.1%), respectively. The use of PCV7 did not influence carriage of one or two PCV13 serotypes. In both the vaccinated and unvaccinated subjects, serotypes 19F, 5, 4, and 9V were the most commonly carried serotypes. None of the serotypes included in PCV7 was associated with vaccination status and similar conclusions could be drawn for the PCV13 serotypes.

4. Discussion

This study shows that about 20% of school-age children and adolescents with CF are carriers of *S. pneumoniae*. This colonization rate was higher than that previously reported [10,11]. The advanced methods used in this study to identify *S. pneumoniae* could explain the differences and support the hypothesis that this is the real pharyngeal pneumococcal colonization rate of CF children. In previous studies, traditional microbiological methods were used. In contrast, this study was conducted by means of molecular methods which, albeit with some exceptions, have been found to be significantly more reliable than traditional non-enriched cultures in routine practice [9,12–14]. Oropharyngeal sampling was used to collect the pharyngeal secretions; it has recently been shown that it is a significantly better means of detecting the colonization by *S. pneumoniae* in adolescents, and young adults than the nasopharyngeal sampling used in the previous studies of younger children [13,15,16]. Finally, a flocked nylon fiber tip was used for sampling because previous studies have shown that this ensures the highest rate of detection of *S. pneumoniae*, particularly when compared with the more widely used Dacron and rayon swabs [17].

In this study, a control group of healthy subjects of the same age, living in the same geographic area, and with the same PCV7 coverage was not included. Consequently, it is not possible to state whether school-age children and adolescents with CF are colonized differently by *S. pneumoniae* than non-CF patients. Moreover, data regarding density of *S. pneumoniae*, sensitivity to antibiotics and biofilm production by colonizing strains were not obtained. Finally, immune characteristics of enrolled children were not studied and non-responders to vaccine stimulation were not identified. Despite these limits, the significantly higher than expected pneumococcal colonization rate of CF patients deserves attention. Further studies are needed to evaluate the real risks deriving from *S. pneumoniae* carriage in CF subjects.

The prevalence of pneumococcal colonization in CF patients was strictly age-related and significantly lower in children who have recently received antibiotic therapy. Both these findings were partially expected. A progressive reduction in pneumococcal colonization with an increase in age has been repeatedly demonstrated in healthy children [18,19]. This is explained by the maturation of the immune system and by the reduced role of factors such as day-care attendance that can significantly enhance the horizontal spread of pneumococcal strains among attendees.

Antimicrobial use reduces carriage, independently from the route of the administration, when drugs active against *S. pneumoniae* are used. In this study, both routes of antibiotic administrations correlated with a significant reduction of pneumococcal carriage. This finding is surprising considering the inhaled antibiotics. Tobramycin and sodium colistimethate are poorly active in vitro against this pathogen [20]. On the other hand, several other factors, such as season, viral respiratory infections, and other environmental variables can influence colonization [21,22] leading to *S. pneumoniae* carriage completely independent from therapy. Similar conclusions could be drawn for the finding that children carrying *S. pneumoniae* were not positive for *P. aeruginosa* colonization, a result that deserves attention for the possible protective role exerted by *S. pneumoniae* against this dangerous pathogen.

In contrast to what was expected, corticosteroid therapy was not significantly associated with an increased risk of *S. pneumoniae* carriage. Corticosteroids exert an immunosuppressive activity and their administration was thought able to favor pharyngeal bacterial colonization in children with asthma treated with inhaled drugs [23].

Children with vitamin D deficiency (those with serum vitamin D concentration <30 ng/mL [24]), were more frequently colonized than children with normal vitamin D levels. Despite the fact that the differences between the groups did not reach statistical significance, this finding is in line with the potential role played by vitamin D in immune system regulation and suggests that periodical monitoring of vitamin D levels prevents deficiency.

Pneumococcal carriage was not influenced by the previous PCV administration. Vaccinated children were frequently carriers of serotypes included in PCV7 with colonization rates quite similar or even higher than those of unvaccinated subjects. This finding seems to indicate that the well-known protective effect of conjugate vaccines (including PCV7) against carriage recently observed in healthy children tends to wane with time [4,19], and to suggest the need for systematic booster doses of pneumococcal vaccines to maintain protection during school-age and adolescence. All the *S. pneumoniae* carriers (except two), independent of vaccinations, were colonized by at least one serotype included in PCV13. Because in the study only PCV13 serotypes were detected, it cannot be excluded than in some patients, carriage could include PCV13 and non-PCV13 serotypes. However, the data collected with this study seem to suggest that PCV13 use offers good protection against *S. pneumoniae* colonization and infection in CF children.

In conclusion, although further researches on this topic are needed, this study seems to indicate that *S. pneumoniae* carrier state of school-age children and adolescents with CF is more prevalent than previously thought. Several factors might influence the carriage rates and require adequate evaluation to limit their influence. PCVs administered in the first years of life cannot prevent the risk of re-colonization in later childhood and adolescence. A persistent reduction in carriage and related risks

can only be obtained by a systemic booster at the beginning of the school age.

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References

[1] Centers for Disease Control and Prevention (CDC). Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among children aged 6–18 years with immunocompromising conditions: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 2013;62:521–4.

[2] Giannattasio A, Squeglia V, Lo Vecchio A, Russo MT, Barbarino A, Carlomagno R, et al. Pneumococcal and influenza vaccination rates and their determinants in children with chronic medical conditions. Ital J Pediatr 2010;36:28.

[3] Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL. The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines 2012;11:841–55.

[4] O'Brien KL, Millar E, Zell E, Bronsdom M, Weatherholz R, Reid R, et al. Effect of pneumococcal conjugate vaccine on nasopharyngeal colonization among immunized and unimmunized children. J Infect Dis 2007;196:1211–20.

[5] Marchisio P, Esposito S, Schito GC, Marchese A, Cavagna R, Principi N. Nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children: implications for the use of heptavalent pneumococcal conjugate vaccine. Emerg Infect Dis 2002;8:479–84.

[6] Auranen K, Rinta-Kokko H, Goldblatt D, Nohynek H, O'Brien KL, Satzke C, et al. Colonisation endpoints in *Streptococcus pneumoniae* vaccine trials. Vaccine 2014;32:153–8.

[7] Istituto Superiore di Sanità. Centro Nazionale di Epidemiologia, Sorveglianza e Promozione della Salute. Dati e evidenze disponibili per l'utilizzo dei vaccini anti-pneumococcici nei soggetti a rischio di qualsiasi età e per l'eventuale ampliamento dell'offerta ai soggetti anziani. Available at: http://www.epicentro.iss.it/temi/vaccinazioni/pdf/Dati%20e%20evidenze%20vaccini%20antipneumococcici.pdf. [Accessed on February 19, 2015].

[8] Ministero della Salute. Piano Nazionale Vaccini 2005–2007. Available at: http://www.salute.g., ov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=543. [Accessed on February 6, 2015].

[9] Marchese A, Esposito S, Coppo E, Rossi GA, Tozzi A, Romano M, et al. Detection of *Streptococcus pneumoniae* and identification of pneumococcal serotypes by real-time polymerase chain reaction using blood samples from Italian children ≤ 5 years of age with community-acquired pneumonia. Microb Drug Resist 2011;17:419–24.

[10] Pimentel de Araujo F, D'Ambrosio F, Camilli R, Fiscarelli E, Di Bonaventura G, Baldassarri L, et al. Characterization of *Streptococcus pneumoniae* clones from paediatric patients with cystic fibrosis. J Med Microbiol 2014;63:1704–15.

[11] Burns J, Emersaon J, Strapp JR, Yim DL, Krzewinski J, Louden L, et al. Microbiology of sputum from patients at cystic fibrosis centers in the United States. Clin Infect Dis 1998;27:158–63.

[12] Carvalho MG, Pimenta FC, Moura I, Roundtree A, Gertz Jr RE, Li Z, et al. Non-pneumococcal mitis-group streptococci confound detection of pneumococcal capsular serotype-specific loci in upper respiratory tract. Peer J 2013;1:e97.

[13] Trzciński K, Bogaert D, Wyllie A, Chu ML, van der Ende A, Bruin JP, et al. Superiority of trans-oral over trans-nasal sampling in detecting *Streptococcus pneumoniae* colonization in adults. PLoS ONE 2013;8:e60520.

[14] Cvitkovic Spik V, Beovic B, Pokorn M, Drole Torkar A, Vidmar D, Papst L, et al. Improvement of pneumococcal pneumonia diagnostics by the use of rt-PCR on plasma and respiratory samples. Scand J Infect Dis 2013;45:731–7.

[15] O'Brien KL, World Nohynek H, Health Organization Pneumococcal Vaccine Trials Carriage WorkingGroup. Report from a WHO working group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. Pediatr Infect Dis J 2003;22:133–40.

[16] Principi N, Terranova L, Zampiero A, Manzoni F, Senatore L, Rios WP, et al. Oropharyngeal and nasopharyngeal sampling for the detection of adolescent *Streptococcus pneumoniae* carriers. J Med Microbiol 2014;63:393–8.

[17] Dube FS, Kaba M, Whittaker E, Zar HJ, Nicol MP. Detection of *Streptococcus pneumoniae* from different types of nasopharyngeal swabs in children. PLoS ONE 2013;8:e68097.

[18] Le Polain de Waroux O, Flasche S, Prieto-Merino D, Edmunds WJ. Age-dependent prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae* before conjugate vaccine introduction: a prediction model based on a meta-analysis. PLoS ONE 2014;9:e86136.

[19] Principi N, Terranova L, Zampiero A, Montinaro V, Ierardi V, Peves Rios E, et al. Pharyngeal colonisation by *Streptococcus pneumoniae* in older children and adolescents in a geographical area characterised by relatively limited pneumococcal vaccination coverage. Pediatr Infect Dis J 2015;34:426–32.

[20] Ryan G, Singh M, Dwan K. Inhaled antibiotics for long-term therapy in cystic fibrosis. Cochrane Database Syst Rev 2011;3:CD001021.

[21] Principi N, Marchisio P, Schito GC, Mannelli S. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Ascanius Project Collaborative Group. Pediatr Infect Dis J 1999;18:517–23.

[22] Hendley JO, Hayden FG, Winther B. Weekly point prevalence of *Streptococcus pneumoniae*, *Hemophilus influenzae* and *Moraxella catarrhalis* in the upper airways of normal young children: effect of respiratory illness and season. APMIS 2005;113:213–20.

[23] Zhang L, Prietsch SO, Mendes AP, Von Groll A, Rocha GP, Carrion L, et al. Inhaled corticosteroids increase the risk of oropharyngeal colonization by *Streptococcus pneumoniae* in children with asthma. Respirology 2013;18:272–7.

[24] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.