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Pneumococcal carriage and serotype variation before and after introduction of pneumococcal conjugate vaccines in patients with acute otitis media in Switzerland

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ABSTRACT

Background: Acute otitis media (AOM) is an important cause for antibiotic prescription within the paediatric population and *Streptococcus pneumoniae* is a major pathogen associated with AOM episodes. This study aimed at analysing the influence of the heptavalent and 13-valent pneumococcal conjugate vaccines (PCV7 and PCV13) on pneumococcal carriage and serotype distribution in AOM.
Methods: Nasopharyngeal swabs (NPS) and middle ear fluid (MEF) were collected within a Swiss surveillance study of outpatients from all ages with AOM between 2004 and 2015, covering three vaccination eras (pre-PCV7, PCV7 and PCV13). Samples were cultured for pneumococcal identification, and the association of vaccine era with pneumococcal carriage was investigated by logistic regression analysis adjusting for sociodemographic factors.
Findings: In total, 3300 NPS and 620 MEF were included in this study. The number of samples from patients with AOM dropped over vaccination eras and *S. pneumoniae* was less frequently isolated in the PCV13 era as compared to the other two eras. The latest (PCV13) vaccination era was independently associated with a reduced pneumococcal carriage within NPS (adjusted odds ratio 0.65, 95%-CI 0.45–0.94). Investigating serotype epidemiology, vaccine serotypes decreased significantly after the conjugate vaccine introductions with the exception of serotype 3. Within the non-PCV13 serotypes, a particular increase of serogroups 11, 15 and 23 was observed in both NPS and MEF.
Conclusion: A substantial change in pneumococcal carriage and serotype epidemiology suggests an impact of the conjugate vaccines on pneumococcal AOM in Switzerland.

1. Introduction

Otitis media (OM) is one of the most frequent childhood infections, a main reason of paediatric doctors' visits, and has been described as one of the most common reasons for antibiotic prescription in children [1]. OM refers to an inflammation of the middle ear and comprises two main entities: acute OM (AOM) and OM with effusion (OME) [2]. AOM is characterized by acute onset of clinical symptoms of inflammation such as otalgia (ear pain), fever and clinical signs of inflammation which include middle ear effusion, redness of the tympanic membrane or perforation. OME on the other hand is defined as middle ear effusion without signs or symptoms of an ear infection [2]. The most frequently isolated bacterial pathogens in AOM are *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* [3], and nasopharyngeal carriage is generally regarded as the prerequisite leading to subsequent respiratory infections [4].

After the introduction of pneumococcal conjugate vaccines (PCVs) into routine vaccine schedules, the heptavalent and 13-valent pneumococcal conjugate vaccines (PCV7 and PCV13, respectively) induced significant changes in the epidemiology of *S. pneumoniae*, both in disease and in carriage in many different countries [5–8]. However, the influence of the polysaccharide vaccines on pneumococcal otitis media remains uncertain as

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results of previous studies have been controversial [5,9–11]. A systematic review on the impact of pneumococcal conjugate vaccine reported a 0–9% efficacy on all-cause AOM in randomized trials whereas the efficacy was higher in nonrandomized trials (17–23%) [12].

In Switzerland, PCV7 and PCV13 have been recommended and reimbursed by health insurance plans since late 2006 (for all children younger than two years) and 2011 (for all children younger than five years), respectively [13]. Between 2008 and 2010 the pneumococcal vaccine coverage had been estimated at approximately 50% for two, and 37% for three doses at the age of two years [13,14]. From 2011 to 2013 coverage increased to 79% for two and 75% for three doses, respectively [13,14]. Finally, for 2014 and 2015 the coverage for three doses was 80% and 81%, respectively.

In Switzerland AOM is monitored since 1998 within a prospective national surveillance system based on a network of sentinel paediatricians and general practitioners treating outpatients with AOM [15]. In addition, sentinel physicians were collecting nasopharyngeal swabs (NPS) and middle ear fluid (MEF) allowing the investigation of longitudinal changes in pneumococcal aetiology and serotype epidemiology in patients with AOM. However, these samples have not yet been comprehensively analysed, and the impact of the conjugate vaccines on the role of *S. pneumoniae* in AOM in Switzerland remained unknown.

Therefore, the overall aim of our study was to investigate changes in pneumococcal carriage in patients with AOM and serotype distribution before and after the introductions of the pneumococcal conjugate vaccines PCV7 and PCV13, and explore risk factors for nasopharyngeal pneumococcal carriage during AOM. In more detail, the objectives were (I) To describe changes in characteristics of patients with AOM within the Swiss sentinel network over the pre-PCV7, PCV7 and PCV13 eras. (II) To assess the association of pneumococcal conjugate vaccine eras and pneumococcal carriage within NPS, adjusted for confounding by demographic factors. (III) To describe temporal variations of the pneumococcal serotype distribution over pre-PCV7, PCV7 and PCV13 eras within NPS and MEF.

2. Material and methods

2.1. Study design, setting and participants

This retrospective analysis of surveillance data included outpatients of any age with AOM between 2004 and 2015. Patients were monitored within a Swiss nationwide prospective surveillance system for various different diseases, which is ongoing since 1998 and has been described in detail previously [15]. In brief, this network is composed of a convenience sample of general practitioners (GP) and paediatricians from all geographical regions of Switzerland. The diagnosis of AOM episodes was established according to Centers for Disease Control and Prevention (CDC) definitions, and patients were eligible to be enrolled repeatedly for each new episode of AOM [15].

Participating doctors seeing AOM patients were asked to sample the nasopharynx and/or provide a sample of the middle ear fluid in case of spontaneous rupture for the investigation of the presence of *Streptococcus pneumoniae*. Therefore, our study design was cross-sectional (i.e., a series of cross-sectional samples for each year). Patients of whom no NPS or MEF was available were excluded from this study. This surveillance program is part of the governmental public health surveillance and is therefore exempted from approval by Institutional Review Boards. There has been no ethical approval to perform tympanocentesis for routine surveillance.

2.2. Study outcomes

Pneumococcal carriage and the serotype epidemiology were specified as the primary study outcomes. Pneumococcal carriage was defined as the presence of *S. pneumoniae* detected by culture from NPS or MEF. Serotypes/serogroups were categorized according to their inclusion in the conjugated vaccines: PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F); PCV13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F); additional PCV13 serotypes not included in PCV7 (1, 3, 5, 6A, 7F, 19A; further referred to as "additional PCV13 serotypes") and non-PCV13 serotypes (all other serotypes detected in NPS or MEF).

2.3. Vaccination era and confounding demographic factors

As a measure of the Swiss population's exposure to the conjugate vaccines PCV7 and PCV13 the study era was categorized based on vaccine introduction: a pre-PCV7 era (2004–2006), PCV7 era (2007–2010) and PCV13 era (2011–2014). To take into account a possible change in demographic factors over time, which could potentially be confounding the association of any PCV era with pneumococcal carriage, we included age (categorized into $\leq$1 year, 2–4 years and 5–15 years) as the most important confounder, as it is established to be a well-known risk factor for varying pneumococcal carriage.

As further confounding factors we included gender, day care attendance, vaccination status (at least one dose of either PCV7 or PCV13), antibiotic therapy within the last 8 weeks (for any indication), AOM history (i.e., no previous AOM versus $\geq$1 episodes in the last 12 months) and geographical region. For the latter, Switzerland territory was categorized into two regions: the Eastern-Southern region including both the large German-speaking and the much smaller Italian- and Romansh-speaking regions, and the Western region (French speaking) region.

2.4. Data collection

NPS were collected using twisted wire rayon tipped applicators (Copan Ventury Transystem; Copan, Italy), and MEF were obtained using the same or cotton tip swabs. As for the latter, the time of collection of the samples after spontaneous perforation was not recorded. With each sample, practitioners submitted a standardized questionnaire containing patient information (age, gender, vaccination statement, preceding antibiotic therapy, day care attendance, AOM history (number of otitis media episodes within the last 12 months) and place of residence).

After collection, samples of NPS and MEF were sent to the Swiss pneumococcal reference laboratory for culture detection of *S. pneumoniae*. The presence of *S. pneumoniae* in MEF and NPS was detected and confirmed by culture, using alpha hemolysis morphology on blood agar plates and optochin sensitivity. Serotypes of all *Streptococcus pneumoniae* isolates were determined by Quellung reaction using antiserum from the Statens Serum Institute (Copenhagen, Denmark).

2.5. Statistical analysis

Variations in the number of NPS, pneumococcal carriage in NPS and MEF, and *S. pneumoniae* serotypes in both NPS and MEF over the pre-PCV7, PCV7 and PCV13 era were compared with Chi-square tests for trend.

The association of vaccine era with pneumococcal carriage was analysed using logistic regression, adjusting for potential confounding by age, gender, day care attendance, vaccination status, prior antibiotic therapy, AOM history and geographical region. Missing values for PCV13 vaccinations were assumed to be non-

vaccinated in the eras prior to the respective PCV7 or PCV13 introduction.

Statistical significance was claimed at an alpha level of 0.05 without adjustment for multiple hypothesis testing. All statistical analyses were performed using the software R (version 3.1.3, 2015, http://www.R-project.org) and Graph Pad Prism.

3. Results

3.1. Characteristics of NPS from AOM patients

In total, the general practitioners and paediatricians from the Swiss Sentinella Network collected 3300 NPS from patients with AOM between 2004 and 2015. Patients with AOM were mostly children and about a third of the patients from which NPS were received were less than 2 years old (Table 1). However, numbers of the young (≤1 year and 2–4 years of age) were higher in the pre-PCV7 as compared to the PCV13 era. In contrast, an increased proportion of patients with AOM of >15 years of age was identified for the PCV13 era (Table 1). Overall, the median age of patients with AOM increased from 4 to 5 and 12 years, in the pre PCV7, PCV7 and PCV13 era, respectively. There was a slightly higher number of NPS collected from patients from the Eastern-Southern region within the PCV13 as compared to the pre-PCV7 era. The distribution of gender, preceding antibiotic therapy, and AOM history was similar for all three eras (Table 1).

However, a considerable decrease of samples received from young patients with AOM in the more recent years was observed (Supplementary Table 1), despite that the numbers of general practitioners and paediatricians sending samples did not change substantially over the years (data not shown). This decrease was seen in the age groups (≤1 year, $p = 0.025$; 2–4 years, $p < 0.001$ and 5–15 years, $p < 0.001$; Supplementary Table 1). In contrast, an increase of samples was observed for patients >15 years of age ($p < 0.001$). Therefore, this indicates that AOM was less often diagnosed in the more recent years for the ages 0–15 years within our Network.

3.2. Variation of pneumococcal carriage within NPS over the vaccine eras

From a total of 3300 NPS, *S. pneumoniae* was detected in 1525 (46%) of NPS (Table 2). Overall nasopharyngeal pneumococcal carriage declined from 50% in the pre-PCV7 and 49% in the PCV7 era to 36% in the PCV13 era (Table 2).

Univariate and multivariate logistic regression was subsequently performed to identify demographic and individual risk factors associated with pneumococcal carriage (Table 2). The adjusted

Table 1
Characteristics of NPS from patients with acute otitis media, 2004–2015.

		Pre-PCV7 era: 2004–2006		PCV7 era: 2007–2010		PCV13 era: 2011–2015	
		N	%	N	%	N	%
Total number of NPS[a]		1588		961		751	
Age							
	≤1 years	522	32.9	343	35.7	203	27.0
	2–4 years	450	28.3	248	25.8	139	18.5
	5–15 years	433	27.3	204	21.2	156	20.8
	>15 years	176	11.1	165	17.2	250	33.3
	NA	7	0.4	1	0.1	3	0.4
Day care visits							
	Yes	383	24.1	283	29.4	196	26.1
	No	1105	69.6	603	62.7	488	65.0
	NA	100	6.3	75	7.8	67	9.0
Region							
	East	938	59.1	539	56.1	526	70.0
	West	649	40.9	422	43.9	222	29.6
	NA	1	0.1	0		3	0.4
Gender							
	Female	732	46.1	417	43.4	364	48.5
	Male	851	53.6	536	55.8	373	49.7
	NA	5	0.3	8	0.8	14	1.9
Vaccination status							
PCV7	Yes[b]	62	3.9	408	42.4	186	24.8
	No	1437	90.5	464	48.3	325	43.2
	NA	89	5.6	89	9.3	240	32.0
PCV13[c]	Yes[b]	0	0.0	0	0.0	202	26.9
	No	1588	100.0	961	100.0	268	35.7
	NA	0	0.0	0	0.0	281	37.4
Antibiotics during the last 8 weeks							
	No	1328	83.6	749	77.9	595	79.4
	Yes	235	14.8	158	16.4	108	14.4
	NA	25	1.6	54	5.6	47	6.3
AOM history during the last 12 months							
	No	929	58.5	524	54.5	440	58.6
	≥1 previous episode	529	33.3	319	33.2	218	29.0
	NA	130	8.2	118	12.3	93	12.4

NA; Not available (missing): PCV; pneumococcal conjugate vaccine: PCV7, 7-valent pneumococcal conjugate vaccine: PCV13, 13-valent pneumococcal conjugate vaccine.

[a] Indicated are all study participants who provided a nasopharyngeal swab (NPS); Samples from subsequent AOM episodes per patient were included. In total, this study included NPS from 2892 different patients.

[b] Indicates at least one dose of PCV7 or PCV13, respectively.

[c] In the eras prior PCV13 introduction (2004–2010), patients were assumed to be non-vaccinated with PCV13.

Table 2
Risk factors for pneumococcal carriage within nasopharyngeal swabs.

Demographic characteristics		Number of Swabs		Pneumococcal carriage					
		N	%	n	%	OR	95% CI	aOR	95% CI
Total swabs		3300		1525	46.2				
Time									
	Pre-PCV7 era: 2004–2006	1588	48.1	788	49.6	REF[a]		REF [a]	
	PCV7 era: 2007–2010	961	29.1	468	48.7	0.96	(0.82–1.13)	1.09	(0.87–1.37)
	PCV13 era: 2011–2015	751	22.8	269	35.8	**0.57**	**(0.47–0.68)**	**0.65**	**(0.45–0.94)**
Age (years)									
	≤1	1068	32.4	611	57.2	REF[a]		REF [a]	
	2–4	837	25.4	473	56.5	0.97	(0.81–1.17)	0.94	(0.76–1.18)
	5–15	793	24.0	331	41.7	**0.54**	**(0.44–0.64)**	**0.51**	**(0.40–0.65)**
	>15	591	17.9	108	18.3	**0.17**	**(0.13–0.22)**	**0.18**	**(0.12–0.25)**
Region									
	East	2003	60.7	833	41.6	REF[a]		REF [a]	
	West	1293	39.2	692	53.5	**1.62**	**(1.40–1.86)**	1.16	(0.96–1.39)
Gender									
	Female	1513	45.8	680	44.9	REF[a]		REF [a]	
	Male	1760	53.3	832	47.3	1.10	(0.96–1.26)	1.04	(0.87–1.23)
Day care attendance									
	No	2196	66.5	944	43.0	REF[a]		REF [a]	
	Yes	862	26.1	488	56.6	**1.73**	**(1.48–2.03)**	**1.33**	**(1.08–1.63)**
Vaccination type									
PCV7	No	2226	67.5	1006	45.2	REF[a]		REF [a]	
	Yes[b]	656	19.9	363	55.3	**1.50**	**(1.26–1.79)**	**0.76**	**(0.58–1.00)**
PCV13[c]	No	2817[c]	85.4	1325[c]	47.0	REF[a]		REF [a]	
	Yes[b]	202	6.1	99	49.0	1.08	(0.81–1.44)	1.14	(0.61–2.03)
Antibiotics during the last 8 weeks									
	No	2673	81.0	1233	46.1	REF[a]		REF [a]	
	Yes	501	15.2	229	45.7	0.98	(0.81–1.19)	**0.68**	**(0.53–0.88)**
AOM history during the last 12 months									
	No	1893	57.4	843	44.5	REF[a]		REF [a]	
	≥1 previous episode	1066	32.3	530	49.7	**1.23**	**(1.05–1.43)**	0.88	(0.72–1.08)

Abbreviations: aOR: adjusted Odds ratio; 95% CI: 95% confidence intervals; AOM: acute otitis media; PCV7 and PCV13: 7- and 13-valent pneumococcal conjugate vaccine. Confidence intervals that do not overlap the null value of OR = 1 are indicated in bold.
[a] REF, reference group for the regression analysis.
[b] Indicates at least one dose of PCV7 or PCV13, respectively.
[c] In the eras prior PCV13 introduction (2004–2010), we assumed patients were non-vaccinated with PCV13.

model provided strong evidence for a reduction in *S. pneumoniae* carriage during the PCV13 era (adjusted odds ratio (aOR) 0.65, 95% confidence interval (CI) 0.45–0.94; Table 2). Older children and adults were less likely to be pneumococcal carriers than younger patients (Table 2). Preceding antibiotic therapy was associated with a significant decrease of pneumococcal carriage in our adjusted model (OR 0.68, CI 0.53–0.88). An association of pneumococcal carriage with region and AOM history observed in univariate logistic regression could be explained by confounding factors included in our adjusted model. However, as for AOM history, if patients with one or more previous episodes during the last 12 months are excluded from the model, the pneumococcal carriage is still significantly reduced in the PCV13 era (data not shown).

3.3. Variation of the serotype epidemiology within NPS over the vaccine eras

While PCV7 serotypes within pneumococcal isolates from NPS dropped from 53.2% in the pre-PCV7 era to only 10.0% in the PCV13 era, the number of non-PCV13 serotypes increased substantially (Table 3 and Fig. 1). Compared to the pre-PCV7 era, additional PCV13 serotypes within pneumococcal isolates first increased in the PCV7 era, followed by a decrease in the PCV13 era. These findings were consistent over all age groups.

In total, twenty-two serotypes/serogroups with an overall proportion ≥1% were detected (Supplementary Table 2). Scrutinizing the individual serotypes/serogroups revealed that all PCV13 serotypes decreased, with the exception of serotype 3 for which proportions of total pneumococcal isolates increased from 8.5% in 2004 to 11.1% in 2015 (Chi square test for trend; $p = 0.013$; Fig. 1A). The highest proportion of serotype 3 was recorded in 2013 with 27.5%. However, in the PCV13 era, serotype 3 was less frequently isolated within the very young (≤1 year) as compared to older children (5–15 years) (Supplementary Table 3). As for serotype 19A, this serotype increased in the PCV7 but subsequently decreased significantly in the PCV13 era (Chi square test for trend; $p = 0.001$; Fig. 1A).

The proportions of non-PCV13 serotypes increased from 26.1% in 2004 to 85.2% in 2015 (Chi square test for trend; $p < 0.001$, Fig. 1B). Serogroups 11, 15 and 23 (excluding serotype 23F) were the most frequent emerging non-PCV13 serotypes (Fig. 1B). Similar findings were discovered if PCV7, additional PCV13, and (total) PCV13 serotypes were analysed stratified by age groups within the three different eras (Table 3).

3.4. Pneumococcal isolation of MEF from patients with AOM

Overall, 620 MEF were received of which 190, 180 and 250 MEF were collected in the Pre-PCV7, PCV7 and PCV13 era, respectively (Supplementary Table 4). As for the AOM history of these samples, 67 (35.3%), 63 (35.0%), and 95 (38.0%) were received from patients who had one or more previous otitis media episodes during the last 12 months.

Table 3
Pneumococcal culture results of nasopharyngeal swabs (NPS) from patients with acute otitis media.

	Pre-PCV7 era: 2004–2006		PCV7 era: 2007–2010		PCV13 era: 2011–2015		X^2 test for trend
	N	%	N	%	N	%	
Total isolates	788	100.0	468	100.0	269	100.0	
PCV7 isolates	419	53.2	130	27.8	27	10.0	<0.0001
PCV13-7 isolates	191	24.2	169	36.1	85	31.6	0.0008
Non-PCV13 isolates	178	22.6	169	36.1	157	58.4	<0.0001
Per age group							
≤1 year of age							
Total isolates	314	100.0	188	100.0	109	100.0	
PCV7 isolates	173	55.1	49	26.1	11	10.1	<0.0001
PCV13-7 isolates	67	21.3	65	34.6	24	22.0	0.3
Non-PCV13 isolates	74	23.6	74	39.4	74	67.9	<0.0001
2–4 years of age							
Total isolates	251	100.0	145	100.0	77	100.0	
PCV7 isolates	146	58.2	43	29.7	7	9.1	<0.0001
PCV13-7 isolates	48	19.1	51	35.2	22	28.6	0.009
Non-PCV13 isolates	57	22.7	51	35.2	48	62.3	<0.0001
5–15 years of age							
Total isolates	189	100.0	95	100.0	47	100.0	
PCV7 isolates	85	45.0	29	30.5	6	12.8	<0.0001
PCV13-7 isolates	63	33.3	38	40.0	25	53.2	0.01
Non-PCV13 isolates	41	21.7	28	29.5	16	34.0	0.05
>15 years of age							
Total isolates	33	100.0	40	100.0	35	100.0	
PCV7 isolates	15	45.5	9	22.5	3	8.6	0.004
PCV13-7 isolates	13	39.4	15	37.5	14	40.0	0.96
Non-PCV13 isolates	5	15.2	16	40.0	18	51.4	0.002

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine: PCV13, 13-valent pneumococcal conjugate vaccine.
PCV7 isolates include the isolates with the serotypes 4, 6B, 9V, 14, 18C, 19F and 23F.
PCV13-7 isolates include the isolates with the serotypes 1, 3, 5, 6A, 7F, 19A.
PCV13 isolates include the isolates with the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.

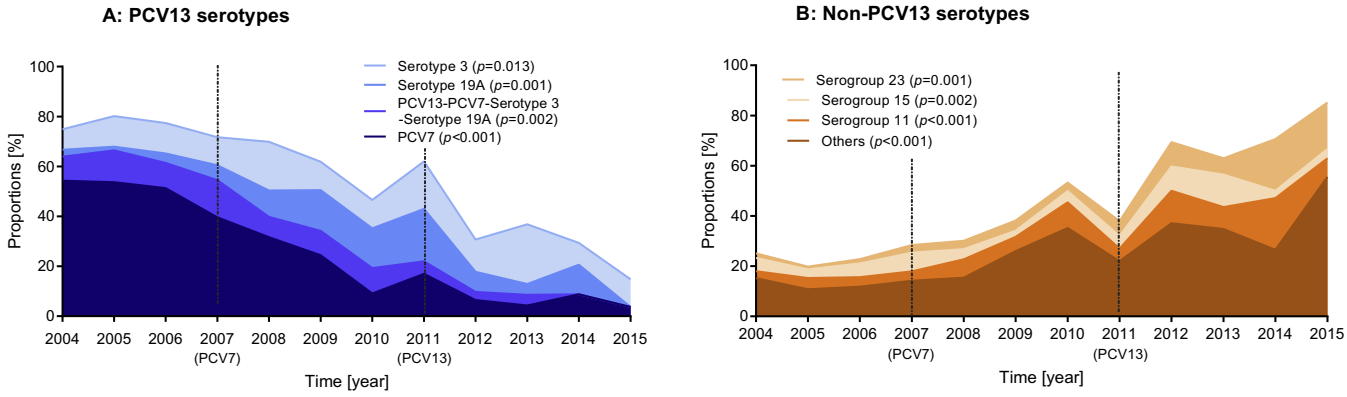

Fig. 1. Variation of pneumococcal serotypes isolated from nasopharyngeal swabs over vaccination eras. Indicated are in (A) the PCV7 (7-valent pneumococcal conjugate vaccine) and PCV13 (13-valent pneumococcal conjugate vaccine) serotypes. Furthermore, serotype 19A and serotype 3 and non-PCV7 but PCV13 serotypes excluding serotypes 3 and 19A (serotypes 1, 5, 6A, 7F) are illustrated. (B) Non-PCV13 serogroups/serogroups are also illustrated and the most frequent are shown individually. Serogroup 23 does not include serotype 23F. Data is plotted for each year and the introductions of the vaccines (PCV7 and PCV13) are indicated. Significant p-values (Chi square test for trend) are shown. PCV7 isolates include the isolates with the serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. PCV13-PCV7-serotype19A-serotype3 isolates include the serotypes 1, 5, 6A, 7F.

Within the collection of 620 MEF 146 (21.2%) were pneumococcal culture positive. A significantly decreased pneumococcal carriage was observed in the PCV13 era (Supplementary Table 4). However, no significant trend for pneumococcal carriage was detected if stratified according to every age category (data not shown). Similar as for the NPS, PCV7 and non-PCV13 serotypes were less and more often isolated in the PCV13 era as compared to the pre-PCV7 era, respectively (Table 4). Analysing individual serotypes, serotype 3 was detected most frequently within each of the three eras (Pre-PCV7, during the PCV7 and PCV13 era; Fig. 2A). However, investigating individual non-PCV13 serotypes, serogroups 11, 15 and 23 (excluding serotype 23F) were the most frequent within both MEF and NPS (Figs. 1B and 2B).

4. Discussion

This study reports the findings from a Swiss prospective surveillance system for *S. pneumoniae* including patients with AOM between 2004 and 2015, spanning over three major time periods defined by the introductions of PCV7 (2007) and PCV13 (2011). We found a decrease of NPS from patients with AOM in the more recent years despite the number of physicians sending samples

Table 4
Pneumococcal culture results of middle ear fluids (MEF) from patients with acute otitis media.

	Pre-PCV7 era: 2004–2006		PCV7 era: 2007–2010		PCV13 era 2011–2015		X^2 test for trend
	N	%	N	%	N	%	
Total isolates	46	100.0	57	100.0	43	100.0	
PCV7 isolates	21	45.7	12	21.1	7	16.3	0.002
PCV13-7 isolates	23	50.0	33	57.9	25	58.1	0.5
Non-PCV13 isolates	2	4.3	12	21.1	11	25.6	0.02
Per age group							
≤1 year of age							
Total isolates	17	100.0	20	100.0	16	100.0	
PCV7 isolates	14	82.4	6	30.0	5	31.3	0.003
PCV13-7 isolates	3	17.6	9	45.0	8	50.0	0.05
Non-PCV13 isolates	0	0.0	5	25.0	3	18.8	0.1
2–4 years of age							
Total isolates	8	100.0	16	100.0	8	100.0	
PCV7 isolates	3	37.5	5	31.3	2	25.0	0.6
PCV13-7 isolates	4	50.0	9	56.3	4	50.0	1
Non-PCV13 isolates	1	12.5	2	12.5	2	25.0	0.5
5–15 years of age							
Total isolates	18	100.0	13	100.0	15	100.0	
PCV7 isolates	4	22.2	1	7.7	0	0.0	0.04
PCV13-7 isolates	13	72.2	10	76.9	10	66.7	0.7
Non-PCV13 isolates	1	5.6	2	15.4	5	33.3	0.04
>15 years of age							
Total isolates	3	100.0	8	100.0	4	100.0	
PCV7 isolates	0	0.0	0	0.0	0	0.0	NA
PCV13-7 isolates	3	100.0	5	62.5	3	75.0	0.53
Non-PCV13 isolates	0	0.0	3	37.5	1	25.0	0.53

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine: PCV13, 13-valent pneumococcal conjugate vaccine: NA, not available.

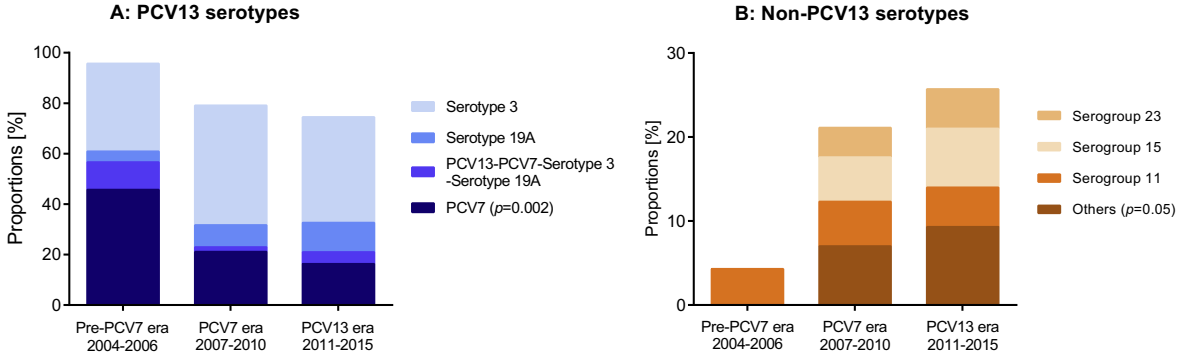

Fig. 2. Variation of pneumococcal serotypes over vaccination era within middle ear fluids (MEF). Indicated are in (A) the PCV7 (7-valent pneumococcal conjugate vaccine) and PCV13 (13-valent pneumococcal conjugate vaccine) serotypes. Furthermore, serotypes 19A and 3 as well as non-PCV7 but PCV13 serotypes excluding serotypes 3 and 19A (serotypes 1, 5, 6A, 7F) are illustrated. Data is plotted according to vaccine era i.e. pre-PCV7 (2004–2006), PCV7 (2007–2010) and PCV13 era (2011–2015). Non-PCV13, (B) serogroups/serogroups are also plotted and the most frequent are shown individually. Serogroup 23 does not include serotype 23F. Significant p-values (Chi square test of trend) are indicated. PCV7 isolates include the isolates with the serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. PCV13-PCV7-serotype19A-serotype3 isolates include the serotypes 1, 5, 6A, 7F.

remained more or less constant. Pneumococcal nasopharyngeal carriage decreased after vaccine introduction, and a decrease of *S. pneumoniae* within MEF of patients with AOM after PCV13 introduction was observed. Finally, a decrease of vaccine serotypes after PCV introduction and a corresponding increase of non-vaccine serotypes were noted.

Within this study we were not able to directly calculate incidence numbers from patients with AOM which is a major limitation but the identified decrease in the number of NPS from patients with AOM might be indeed due to a lower incidence of AOM in Switzerland in recent years. In addition, the median age of AOM patients increased during the more recent years, a characteristic often observed when an effective vaccine is introduced in the youngest of the population. Therefore, a drop of AOM incidence after vaccine introduction in young patients can be hypothesized [16,17]. However, we did not investigate important factors potentially influencing AOM incidents like changes in the patient or family healthcare seeking behavior though this may probably not have changed as health insurance is mandatory and health care services, including GP or paediatrician visits, are reimbursed. In addition, we did not analyze adherence of GPs towards the national AOM prescription guidelines.

A decline in AOM incidence would be consistent with reports from other countries: A recent study from England and Wales revealed an incidence of otitis media of 204.4 episodes per 1000 person-years (95% CI, 201.8–207.0) in <2 year-olds and 180.6 episodes/1000 (95% CI, 179.1–182.2) in 2–4 year-olds [18]. This data was derived from a primary care electronic healthcare database. In addition, Wau et al. discovered a decline in the number of AOM incidence after introductions of both, PCV7 and PCV13 [18].

Within our study, we specifically analysed pneumococcal carriage data of NPS, allowing the investigation of the associations between the pneumococcal conjugate vaccines PCV7 and PCV13 and pneumococcal carriage. In France, in a study using a design very similar to ours, PCV13 but not PCV7 had a strong impact on pneumococcal carriage during AOM [19]. Overall, pneumococcal carriage was reduced from 71.2% to 56.2% from 2001 to 2014 in the French study ($p < 0.001$). Similarly, no effect of PCV7 on pneumococcal carriage has been identified in Germany [20]. Within our study, the relative trends were found to be comparable. Interestingly, no clear effect of PCVs on pneumococcal carriage was identified within healthy individuals in England [21].

As for the changing serotype epidemiology within NPS, we detected a near elimination of PCV7 serotypes which is common in other settings, too [22–24]. Regarding the epidemiology of the additional five PCV13 serotypes, there was also a decrease, however less pronounced, which may be due to the fact that PCV13 has been introduced more recently as compared to PCV7. While serotype 3 (included in PCV13) persisted even in the PCV13 era, proportions of non-PCV13 serotypes increased during the PCV13 era. In comparison, a relatively moderate decline in pneumococcal carriage, featuring a substantial decrease of vaccine-serotypes and concomitant increase of non-vaccine serotypes was observed in the immediate period following PCV introduction in southern Israel [25].

Within this study, we didn't report any data on antimicrobial resistance patterns as this has been recently addressed within another study [14]. In brief, during 2004–2014, the proportion of non-susceptible isolates significantly decreased for penicillin, ceftriaxone, erythromycin and trimethoprim/sulfamethoxazole (TMP-SMX). This is partly due to the decrease of more resistant PCV13 serotypes (e.g. 6B, 9V, 14 and 19A) after the vaccine introduction [14].

We have additionally analysed pneumococcal culture data of MEF from patients with tympanic membrane perforation. Spontaneous perforation is a common complication reported among 15% of AOM episodes and caused by the same bacteria as uncomplicated AOM [26,27], and a positive pneumococcal culture of >30% has been described [27–29]. In our study we observed similar proportions for pneumococcal carriage in the pre PCV7 era, but this number decreased to 17% in the PCV13 era. In Israel, a near-elimination of PCV13 serotypes in MEF has been described [5]. In contrast, we observed a high proportion of serotype 3 (an additional PCV13 serotype) isolated from MEF even after the introduction of PCV13. This could explain why pneumococcal carriage within MEF did not decrease as within NPS in our setting. Serotype 3 has also been described as most frequently isolated from patients with OM in Germany [20]. It has been speculated that PCV13 triggers only weak immune reactions to their serotype 3 components [30–32]. Interestingly, pneumococcal culture results from MEF as compared to the NPS showed striking similarities including a decreasing trend on *S. pneumoniae* carriage after PCV13 introduction and similarities within the serotype epidemiology in our setting. More specifically, an increase of the non PCV13 serogroups/serotypes 11, 15 and 23 (excluding serotype 23F) was identified within both, MEF and NPS.

This study has several strengths. First, this is a very large surveillance-based study that included pneumococcal culture data for both NPS and MEF from patients with AOM. Although this surveillance network was initially composed of a convenience sample of GP and paediatricians, representativeness for the Swiss population has been approximated [15]. Second, the study duration was long and already starting three years before the introduction of PCV7 on a national level, making it one of only few studies to investigate AOM episodes using MEF and NPS cultures over such a long duration. Finally, the serotyping data is very detailed and available for both MEF and NPS, allowing concurrent analysis of the serotype epidemiology.

There are also limitations to this study. First, sampling of the NPS and/or MEF was not performed for all the patients with AOM visiting the doctors of the network due to patients or doctors not consenting to the sampling, which introduces a degree of uncertainty in generalizability to the total Swiss population. However, distribution of gender, preceding antibiotic therapy, and AOM history was evenly distributed for all the three eras. Second, our sample of MEF only included AOM cases with spontaneous perforation, which might introduce an overrepresentation of severe cases. Third, we did not collect any information re breastfeeding for the very young AOM patients, smoking exposures, number of siblings and/or presence/absence of infant influenza vaccination. Fourth, we did not assess a potential association of the number of AOM cases and regional variation in the vaccine coverage. This would have been a complex analysis necessitating coverage data from various regions during identical years, which was not available for this study. Finally, we did not assess for pneumococcal co-colonisation and/or other otopathogens (e.g. non typeable *Haemophilus influenzae* or *Moraxella catarrhalis*) but both issues have been addressed by us and within other studies [33–37]. More precisely, the presence of other otopathogens has been investigated by a non-culture approach, by 16S rRNA sequencing, allowing a whole microbiota characterization [37].

In conclusion, the combination of a decreasing number of samples from patients with AOM within the surveillance network, an increasing mean age of patients with AOM, and a substantial decrease of serotypes included in the conjugate vaccines suggest a considerable impact of the conjugate vaccines PCV7 and PCV13 on pneumococcal nasopharyngeal carriage and AOM incidence in Switzerland. However, it remains to be determined if serotype replacement poses a risk to undermine the effects of the vaccines in the future.

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Conflict of interest

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.02.010.

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