

Indirect Effect of Pneumococcal Conjugate Vaccines on Pneumococcal Colonization: Persistence and Dynamics of Vaccine Serotypes in Sicily (Italy) 11 Years Postintroduction, 2009–2020

Fabio Tramuto,^{1,2} Giulia Randazzo,² Arianna Santino,² Giuseppe Sferlazza,² Adriana Previti,² Giorgio Graziano,² Claudio Costantino,^{1,2} Walter Mazzucco,^{1,2} Emanuele Amodio,¹ Francesco Vitale,^{1,2} and Carmelo Massimo Maida^{1,2}

¹Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties “G. D’Alessandro”—Hygiene Section, University of Palermo, Palermo, Italy; and ²Regional Reference Laboratory for Molecular Surveillance of Influenza, Clinical Epidemiology Unit, University Hospital “Paolo Giaccone”, Palermo, Italy

Background. In Italy, evidence on the long-term effects of pneumococcal conjugate vaccines on nasopharyngeal carriage is still limited. This study assessed pneumococcal carriage prevalence, serotype distribution, and temporal trends during the decade after 13-valent pneumococcal conjugate vaccine (PCV13) introduction and before the severe acute respiratory syndrome coronavirus 2 (COVID-19) pandemic.

Methods. Oropharyngeal samples were collected from 12 733 individuals of all ages presenting with influenza-like illness within the national respiratory pathogens surveillance network. *Streptococcus pneumoniae* detection and serotyping were performed using real-time PCR-based assays.

Results. Overall pneumococcal carriage was 27.1%. The highest prevalence occurred in children aged 2–4 years (51.6%), while colonization was about 10% among adults, including those ≥ 75 years. After vaccine introduction, PCV serotypes declined markedly, accompanied by increased nonvaccine serotypes. Following years of sustained pediatric vaccination, vaccine serotypes re-emerged, replacing previously expanding non-PCV types. Some vaccine serotypes associated with higher invasive disease risk persisted despite high vaccination coverage. Serotype distribution differed significantly by age, and viral coinfection—especially hRSV—appeared to increase pneumococcal colonization likelihood.

Conclusions. Pneumococcal carriage remained common across all ages despite long-standing pediatric vaccination, with continued circulation of both vaccine and nonvaccine serotypes. Viral coinfection may facilitate colonization, highlighting the need for ongoing surveillance and adaptive vaccination strategies.

Keywords. *Streptococcus pneumoniae*; colonization; serotype; vaccine; Italy.

Infections caused by *Streptococcus pneumoniae* (pneumococcus) continue to represent a major global cause of illness and death. This microorganism is responsible for a wide spectrum of clinical conditions, including severe manifestations collectively referred to as invasive pneumococcal disease (IPD) [1], as well as more frequent community-acquired infections, such as otitis media, sinusitis, conjunctivitis, and pneumonia. Invasive pneumococcal disease is characterized by the detection of pneumococcus in normally sterile body sites, such as

blood or cerebrospinal fluid, and is associated with a substantial burden of complications and high fatality rates.

The highest burden of IPD is among infants, the elderly, and immune-compromised patients, although younger adults are also at risk [2]. A key determinant of pneumococcal virulence is the polysaccharide capsule, which enables the pathogen to evade host immune responses. More than 100 immunologically distinct capsular serotypes have been identified, each differing in invasive potential and prevalence. Since currently licensed vaccines rely on the induction of serotype-specific antibodies targeting the capsular polysaccharides, they provide protection against the serotypes included in their formulation. Nevertheless, similarities in composition and structure of some polysaccharide antigens may result in cross-reactivity in immunized individuals against closely related serotypes [3]. Most commonly, vaccine-induced cross-reactive antibodies occur between those serotypes that are categorized in the same serogroup [4], as demonstrated within the serogroups 6, 9, 15, 19, 20, and 23 [5].

Extensive evidence demonstrates that the introduction of large-scale childhood vaccination programs has led to a marked

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Correspondence: Fabio Tramuto, PhD, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties “G. D’Alessandro”—Hygiene Section, University of Palermo, 133, via del Vespro, Palermo I-90127, Italy (fabio.tramuto@unipa.it).

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decline in pneumococcal disease in many countries. The benefits extend beyond vaccinated children, also providing indirect protection of nonvaccinated subjects linked to reduced pathogen transmission within a community [6]; nevertheless, some serotypes contained in pediatric pneumococcal conjugate vaccines (PCVs) have remained a persistent cause of disease among older adults [7]. In addition, following the resolution of pandemic-related restrictions associated with COVID-19, the burden of IPD increased again in several nations, including Italy. According to the most recent national surveillance data, the overall incidence of pneumococcal IPD in Italy rose from 0.84 cases per 100 000 inhabitants in 2021 to 3.02 per 100 000 in 2023, with the highest rates observed in children under 1 year of age (10.41 per 100 000) and adults over 65 years (7.45 per 100 000) [8]. Across Europe, estimates of IPD incidence range between 0.1 and 12.2 cases per 100 000 population [9], although the true incidence remains uncertain due to variability in reporting practices, diagnostic methods, and surveillance structures [10].

Sicily was the first Italian region to implement universal pneumococcal vaccination for infants within its regional immunization plan. In 2004, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced using a 2 + 1 schedule in infants aged 2 months or older and was replaced by PCV13 in 2010. Additionally, the 23-valent pneumococcal polysaccharide vaccine (PPSV23) was recommended for adults aged ≥ 65 years and for those aged ≥ 19 years with chronic conditions, to be administered sequentially after PCV13. Since 2023, this recommendation has shifted to a PCV20 + PPSV23 sequential schedule for individuals ≥ 60 years and for adults ≥ 19 years with clinically relevant comorbidities. In Sicily, vaccination coverage among newborns and toddlers has consistently exceeded 90% in birth cohorts from 2010 to 2020 [11], while uptake among adults and the elderly remains substantially lower [12].

Beyond clinical infections, *S. pneumoniae* frequently colonizes the nasopharynx of healthy individuals, particularly children, who serve as the main reservoir facilitating bacterial transmission. Colonization is recognized as a critical precursor to both disease development and community spread [13], although the mechanisms governing the transition from carriage to invasive disease are not fully understood.

One well-documented consequence of pediatric PCV vaccination is a marked reduction of vaccine serotypes (VTs) in carriage and disease, accompanied by an expansion of nonvaccine serotypes (NVTs), a process known as serotype replacement, observed both in vaccinated and unvaccinated populations [14–16]

In this context, surveillance of pneumococcal carriage represents an important tool to assess selective vaccine pressure and, therefore, to guide the adoption of higher-valent vaccines, such as PCV15, PCV20, and PCV21 [17]. However, most carriage studies have been limited to short- or medium-term

observation periods [18] or have focused at pediatric populations [19], making them inadequate for evaluating the long-term effects of vaccination on both carriage prevalence and serotype distribution, especially in older age groups.

The present observational study aims to retrospectively evaluate pneumococcal carriage and serotype-specific patterns across different age groups in Sicily, an Italian region characterized by high pediatric PCV coverage, over a decade following the introduction of universal childhood vaccination and preceding the onset of the COVID-19 pandemic.

METHODS

Study Design and Specimen Collection

A cross-sectional observational study was conducted to determine the prevalence, serotype composition, and temporal patterns of oropharyngeal colonization by *S. pneumoniae* among residents of Sicily, the fifth most populated region of Italy. To achieve this objective, oropharyngeal samples were obtained with the support of general practitioners and pediatricians participating in RespiVirNet, the Italian surveillance network monitoring influenza-like illnesses (ILIs) in Sicily [20]. Sampling took place during winter from 2009 until late January 2020, immediately preceding the onset of the COVID-19 public health emergency. Oropharyngeal specimens were collected using standardized flocked swabs and processed at the Sicilian Regional Reference Laboratory located at the “P. Giaccone” University Hospital in Palermo.

Laboratory Procedures

All respiratory samples were subjected to molecular screening to detect influenza viruses, respiratory syncytial virus (hRSV), and pneumococcus. Detection of influenza and hRSV was carried out using real-time PCR (RT-PCR) protocols previously published in the literature [21, 22], whereas pneumococcal carriage was identified through a specific RT-PCR assay targeting the pneumococcal autolysin gene (*lytA*). Samples testing positive for *S. pneumoniae* (*lytA*-positive) were subsequently serotyped using a panel of single-plex RT-PCR assays aimed at detecting serotypes included in the PPSV23. Because of the genetic similarity of capsular loci within defined serogroups, some assays were limited to serogroup-level identification, such as 6A/6B, 7A/7F, 9L/9N, 9A/9 V, 11A/11D/11E, 12F/44, 18B/18C, 19B/19F, 22A/22F, and 33A/33F/37. All tests were performed in duplicate, incorporating both negative and plasmid-positive controls, following the Centers for Disease Control and Prevention (CDC) recommendations [23] with minor laboratory modifications. An RT-PCR result was considered negative when no amplification occurred after 40 cycles.

Statistical Analysis

Pneumococcal serotypes were classified into 4 categories: “PCV7 serotypes,” “additional PCV13 serotypes,” “non-PCV13

PPSV23 serotypes,” and “NVTs.” Participants were grouped into 10 age classes: ≤ 12 months, 13–23 months, 2–4 years, 5–9 years, 10–14 years, 15–24 years, 25–44 years, 45–64 years, 65–74 years, and ≥ 75 years. Descriptive statistics (frequency, percentage, median, and interquartile range) were used to summarize demographic and clinical characteristics. Associations between pneumococcal carriage and participant factors were evaluated using logistic regression, with odds ratios (ORs/adjOR) and 95% CIs. Age and sex were included as a priori variables, and stratified analyses were conducted to assess effect modification. Statistical significance was defined as $P \leq .05$ (2-tailed). Data were analyzed using STATA MP v19.5 (StataCorp, College Station, TX, United States).

Ethical Considerations

The study followed Italian data protection laws and the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants or from parents/legal guardians of minors. All authors had full access to the data and took responsibility for the publication.

RESULTS

Study Population

A total of 12 733 individuals were included (Supplementary Figure 1) and analyzed according to their pneumococcal colonization status. Their demographic and clinical characteristics are summarized in Table 1. The overall male-to-female ratio

(M:F & 1.10) remained consistent throughout the surveillance period, ranging from 0.95 to 1.50 across individual seasons (data not shown). The median age of enrolled subjects was 13 years (interquartile range [IQR] & 43), and more than half of the participants (approximately 52%) were children or adolescents aged ≤ 14 years, although all age groups were represented. With the exception of the 2009–2010 surveillance season, heavily influenced by the influenza A(H1N1)pdm09 pandemic, most participants were recruited from the community (77.5%, n & 8240/10 625), rather than from hospital settings.

Oropharyngeal Carriage of *Streptococcus pneumoniae*

Overall, 27.1% of the population (n = 3452/12 733) tested positive for the pneumococcal *lytA* gene and were therefore classified as carriers. Carriage prevalence was similar between males and females (Table 1). However, the age distribution differed significantly between carriers and noncarriers: pneumococcal carriers had a median age of 5 years, compared with 24 years among noncolonized individuals. Colonization was markedly higher among children, particularly those ≤ 14 years, where prevalence ranged from 28.1% to 51.6%. The highest risk of carriage was observed in children aged 2–4 years (OR = 11.49; 95% CI: 8.67–15.23). In contrast, pneumococcal carriage was relatively uncommon in adults aged ≥ 45 years, where prevalence varied between 8.5% and 12.4% (Table 1; Supplementary Figure 2).

The majority of carriers were identified in the community, and individuals outside hospitals were nearly 3 times more

Table 1. Demographic Characteristics of the Study Population According to Pneumococcus Detection

		lytA-neg (%)	lytA-pos (%)	Logistic Regression		
				adjOR ^a	95% CI	P
Study population (n [%])	n = 12 733	9281 (72.9)	3452 (27.1)			
Sex (n [%])						
Male	6686 (52.5)	4865 (72.8)	1821 (27.2)	REF		
Female	6047 (47.5)	4416 (73.0)	1631 (27.0)	1.01	0.94–1.10	.738
Age (years; median [IQR])	13 (43)	24 (45)	5 (9)			
Age groups (years) (n [%])	n = 12 604					
≤ 12 m	448 (3.6)	279 (62.3)	169 (37.7)	6.52	4.70–9.05	<.001
13–23 m	595 (4.7)	331 (55.6)	264 (44.4)	8.58	6.28–11.73	<.001
2–4	1869 (14.8)	904 (48.4)	965 (51.6)	11.49	8.67–15.23	<.001
5–9	2359 (18.7)	1406 (59.6)	953 (40.4)	7.29	5.52–9.64	<.001
10–14	1290 (10.2)	927 (71.9)	363 (28.1)	4.21	3.14–5.65	<.001
15–24	874 (6.9)	763 (87.3)	111 (12.7)	1.57	1.12–2.18	.008
25–44	1749 (13.9)	1530 (87.5)	219 (12.5)	1.54	1.14–2.08	.005
45–64	2023 (16.1)	1772 (87.6)	251 (12.4)	1.52	1.13–2.05	.006
65–74	703 (5.6)	621 (88.3)	82 (11.7)	1.42	0.99–2.02	.051
≥ 75	694 (5.5)	635 (91.5)	59 (8.5)	REF		
Healthcare settings (n [%]) ^b	n = 10 625					
Community	8240 (77.5)	5174 (62.8)	3066 (37.2)	2.78	2.36–3.26	<.001
Hospital	2387 (22.5)	2166 (90.8)	219 (9.2)	REF		

^aadjOR: OR adjusted by age.

^bExcluding the surveillance season 2009–2010.

likely to carry pneumococcus than hospitalized subjects (adjOR = 2.78; 95% CI: 2.36–3.26). Colonization also demonstrated a strong seasonal pattern, peaking during winter months. Carriage rates increased by approximately 5–6-fold in December through February, compared to September, taken as the reference month (Supplementary Figure 3).

Because the study was conducted among individuals presenting with ILI, the role of coinfection with influenza viruses or respiratory syncytial virus was assessed (Table 2). Influenza virus alone was detected in 45.1% (n & 3590/7968) of tested individuals, predominantly type A (70.9%, n & 2547/3590). In contrast, hRSV alone was detected in 12.3% (n & 614/4992) of subjects, with subgroup A and B almost evenly distributed (46.4% vs 53.6%). Both influenza and hRSV were positively associated with pneumococcal carriage, but the strength of association was greater for hRSV (influenza adjOR & 1.19; 95% CI: 1.07–1.31; hRSV adjOR & 1.97; 95% CI: 1.64–2.36).

Fluctuations in pneumococcal carriage prevalence across surveillance seasons likely reflected year-to-year changes in population age structure. Figure 1 also overlays pneumococcal carriage trends with vaccine coverage in Sicily following the introduction of PCV13 in mid-2010. Notably, PCV7-/PCV13-type serotypes steadily decreased during the first 5 surveillance seasons, reaching a minimum in 2013–2014, followed by a gradual increase. A similar pattern was observed among additional PPSV23 serotypes. In contrast, the proportion of NVTs peaked in 2013–2014 before declining in subsequent years.

Serotype-Specific Carriage

Serotype distribution across the entire surveillance period is shown in Figure 2. The 5 most frequently detected serotypes/serogroups were 22A/22F, 4, 6A/6B, 19B/19F, and 9A/9V, each with a prevalence $\geq 5.5\%$. Four of these were included in

PCVs. All other detected serotypes/serogroups were less common, and 2.3% of carriers harbored serotypes absent from current vaccine formulations.

Age-stratified analysis revealed marked differences (Figure 3). In children ≤ 4 years, the serotype profile resembled that of the full population but showed a predominance of 6A/6B. Logistic regression confirmed strong associations between carriage in younger age groups and several vaccine serotypes: PCV types 4 (OR & 3.5; 95% CI: 2.1–5.9), 5 (OR & 2.1; 95% CI: 1.1–3.7), and 6A/6B (OR & 7.7; 95% CI: 4.2–14.1), as well as PPSV23 serotypes 10A, 11A/11D/11E, and 15B/15C, although some estimates presented wide CIs. Conversely, NVTs were significantly associated with individuals aged ≥ 65 years.

Importantly, analysis restricted to the most recent surveillance years (2017–2020) confirmed the persistence of vaccine serotypes and ongoing community transmission, despite high pediatric vaccination coverage (data not shown).

The distribution of colonizing serotypes was also compared based on viral coinfection. Influenza-positive subjects showed no substantial differences, except for serogroup 22A/22F, which was significantly associated with influenza infection, whereas 15B/15C showed a negative correlation (Supplementary Figure 4). Strong associations emerged between hRSV and serotypes 6A/6B (OR & 2.11; 95% CI: 1.67–2.67) and 23F (OR & 3.66; 95% CI: 2.09–6.43) and serogroup 15B/15C (OR & 1.86; 95% CI: 1.31–2.63) (Figure 4).

Long-term dynamics of circulating serotypes are illustrated in Figure 5 and Supplementary Figures 5–8. Among PCV7 types, serogroup 19B/19F predominated until 2016–2017 before declining sharply, while serotype 4 consistently ranked among the top 10 and showed a steady increase, as did 6A/6B and 9A/9V. In contrast, serotypes 18B/18C and 19A, initially frequent, dramatically decreased over time. Serotype 23F peaked in 2014–2015 and then declined.

Table 2. Pneumococcal Colonization and Codetection of Influenza Virus or hRSV

	n	lytA-neg (%)	lytA-pos (%)	Logistic Regression		
				adjOR ^a	95% CI	P
Influenza infection (n [%]) ^b	n = 7968					
No	4378 (54.9)	3139 (71.7)	1239 (28.3)	REF		
Yes	3590 (45.1)	2318 (64.6)	1272 (35.4)	1.19	1.07–1.31	.001
Influenza type A	2547 (70.9)	1647 (71.1)	900 (70.8)			
Influenza type B	1043 (29.1)	671 (28.9)	372 (29.2)			
hRSV infection (n [%]) ^c	n = 4992					
No	4378 (87.7)	3139 (71.7)	1239 (28.3)	REF		
Yes	614 (12.3)	292 (47.6)	322 (52.4)	1.97	1.64–2.36	<.001
hRSV-A	285 (46.4)	123 (42.1)	162 (50.3)			
hRSV-B	329 (53.6)	169 (57.9)	160 (49.7)			

Abbreviation: hRSV, respiratory syncytial virus.

^aadjOR: OR adjusted by age.

^bSubjects negative for hRSV.

^cSubjects negative for influenza virus.

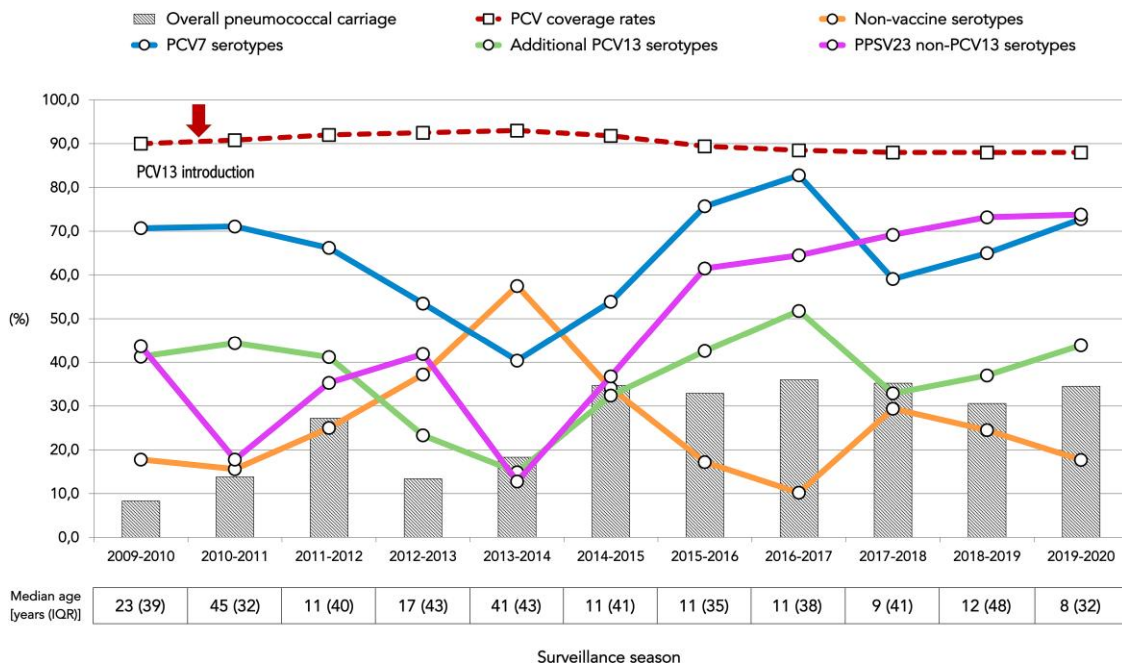


Figure 1. Prevalence of pneumococcal carriage, PCV coverage rates and frequency of pneumococcal serotypes according to different vaccine sets, by surveillance season. PCV, pneumococcal conjugate vaccine.

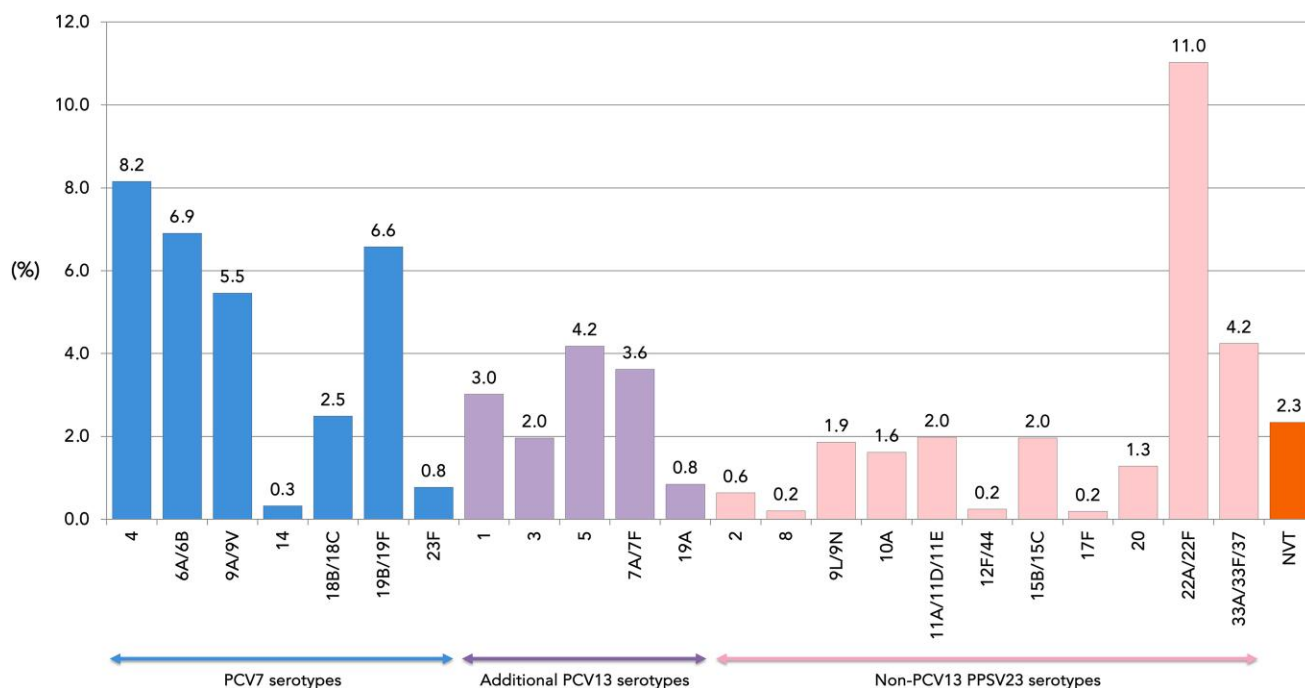


Figure 2. Prevalence of *Streptococcus pneumoniae* serotypes/serogroups included in PCV7, PCV13, or PPSV23 vaccines. Pooled surveillance seasons. NVTs, nonvaccine serotypes; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.

Finally, among those included in PPSV23, serogroups 22A/22F and 33A/33F/37 ranked within the highest position during the first 3 seasons monitored, although they circulated

at low prevalences in the population. These serotypes progressively increased their frequencies over time, becoming 2 of the predominant serogroups, whereas 8, 9L/9N,

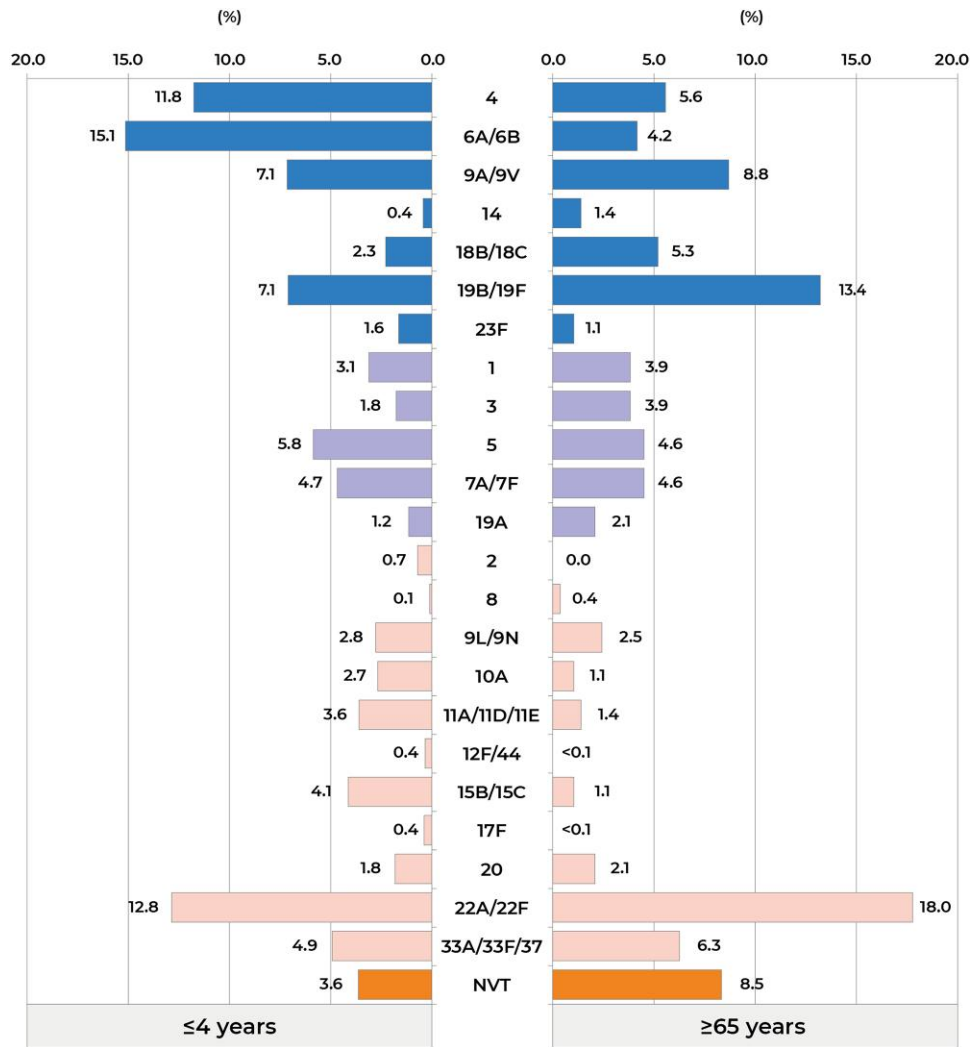


Figure 3. Relative frequencies of *Streptococcus pneumoniae* serotypes/serogroups among subjects aged ≤ 4 y and ≥ 65 y, according to PCV7, PCV13, or PPSV23. Pooled surveillance seasons. PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.

11A/11D/11E, 12F/44, and 20 showed the fastest increase in recent times.

DISCUSSION

The present study aimed to explore impact and serotype-specific patterns of pneumococcal oropharyngeal carriage in Sicily (Italy). It offers valuable insight into the burden of oropharyngeal carriage of *S. pneumoniae* and the long-term dynamics of circulating serotypes among individuals of all ages. The findings span a decade following the introduction of the 13-valent pneumococcal conjugate vaccine as a universal childhood immunization and extend up to the beginning of the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) pandemic. Several key outcomes emerged:

1. Pneumococcal colonization remained appreciable even among elderly individuals.
2. A marked decline in PCV serotypes (VTs) occurred following vaccine implementation, accompanied by a rise in NVTs.
3. Pneumococcal conjugate vaccine serotypes subsequently re-emerged several years later, despite long-term high vaccine coverage in children.
4. Serotype distribution showed strong age dependency.
5. Respiratory viruses, particularly hRSV, appeared to act as triggers for pneumococcal colonization.

Overall, pneumococcal carriage prevalence remained relatively stable throughout the study, except in periods when the median age of participants was higher, leading to a reduced

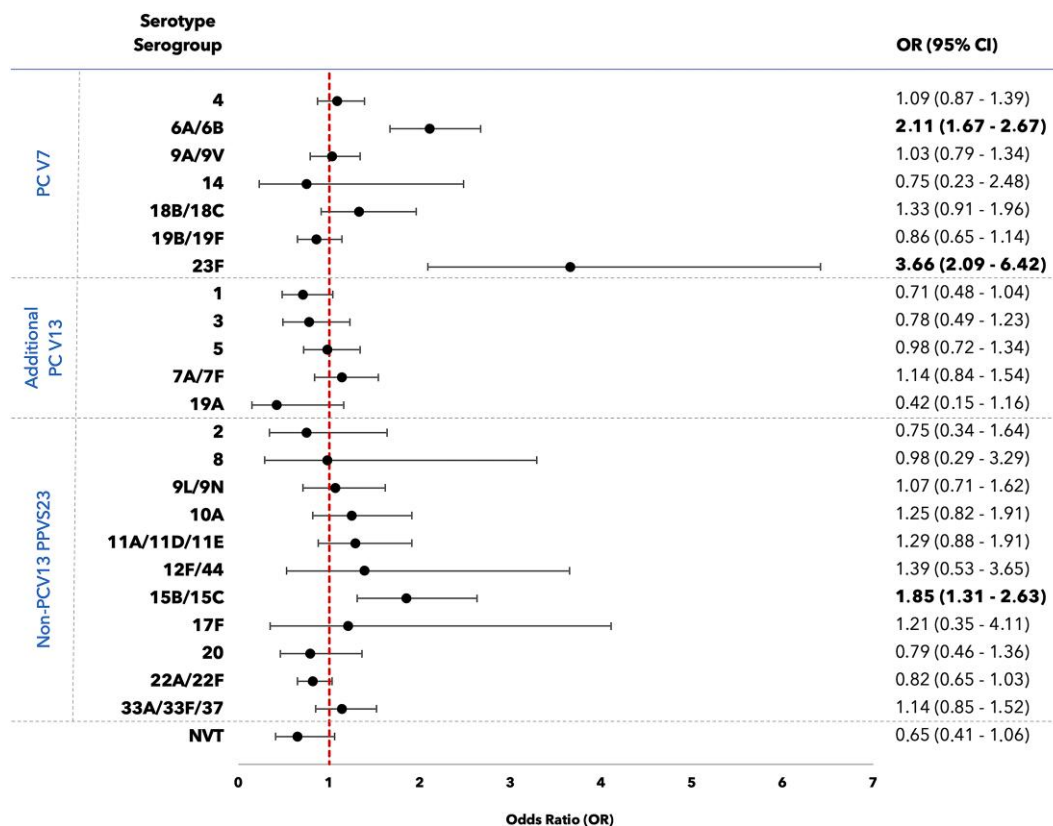


Figure 4. Forest plot showing the risk association between hRSV and pneumococcal serotype detection. hRSV-positive versus hRSV-negative (reference group: hRSV-negative). Pooled surveillance seasons. Statistically significant odds ratios are reported in bold (increased risk) and italic (decreased risk). NVTs, nonvaccine serotypes; hRSV, respiratory syncytial virus.

proportion of carriers. The odds of carriage were up to 12 times greater in children aged 2–4 years compared with elderly individuals, corroborating observations from other research reporting the highest carriage rates among young children [24], particularly within the first 2 years of life in high-income countries [25]. While the inverse association between age and pneumococcal colonization is well documented [26], our findings emphasize that an average of at least 10% of adults and elderly individuals were colonized. This highlights an often underestimated reservoir of pneumococcus among older populations.

Carriage in adults is comparatively understudied, and existing reports are insufficient to establish a comprehensive understanding of adult colonization dynamics. Moreover, low prevalence estimates in older individuals have largely come from culture-based methods [27], which, despite being highly specific, may lack sensitivity for pneumococcal detection [28]. For instance, studies from the United States and England have estimated carriage in older adults at only 1.8%–2.2% [29, 30], while similar levels were observed in Portugal [31]. In contrast, a meta-analysis from Latin America [32] reported a pooled prevalence of 26% among elderly individuals, suggesting that adults may serve as an important pneumococcal reservoir in specific contexts, particularly where vaccine coverage

differs [33]. These discrepancies underline the significance of local epidemiological and immunization conditions in shaping pneumococcal carriage trends.

Although young children remain the primary reservoir for pneumococcus and are the main source of transmission to older age groups, the presence of oropharyngeal colonization among elderly individuals warrants attention. Since colonization often precedes IPD, persistent carriage in older, vulnerable populations—who in many settings tend to be poorly vaccinated—raises considerable public health concerns [34].

As reported in multiple studies, our results demonstrate an initial post-PCV13 decline in colonization with VTs, accompanied by serotype replacement involving NVTs. This pattern has been observed globally following the uptake of PCVs, reflecting the impact of strong vaccine pressure on circulating serotypes. Portugal, for example, documented a drop in PCV13 serotype carriage from 47.6% prior to PCV13 introduction to just 10.7% by 2018–2020 [14]. Similar trends have been observed in England and Wales [35], Pakistan [15], the United States [36], and various European countries [37], where extensive childhood vaccination rapidly reshaped serotype ecology.

Despite the wealth of short-term data, fewer investigations have evaluated long-term serotype trends in regions with

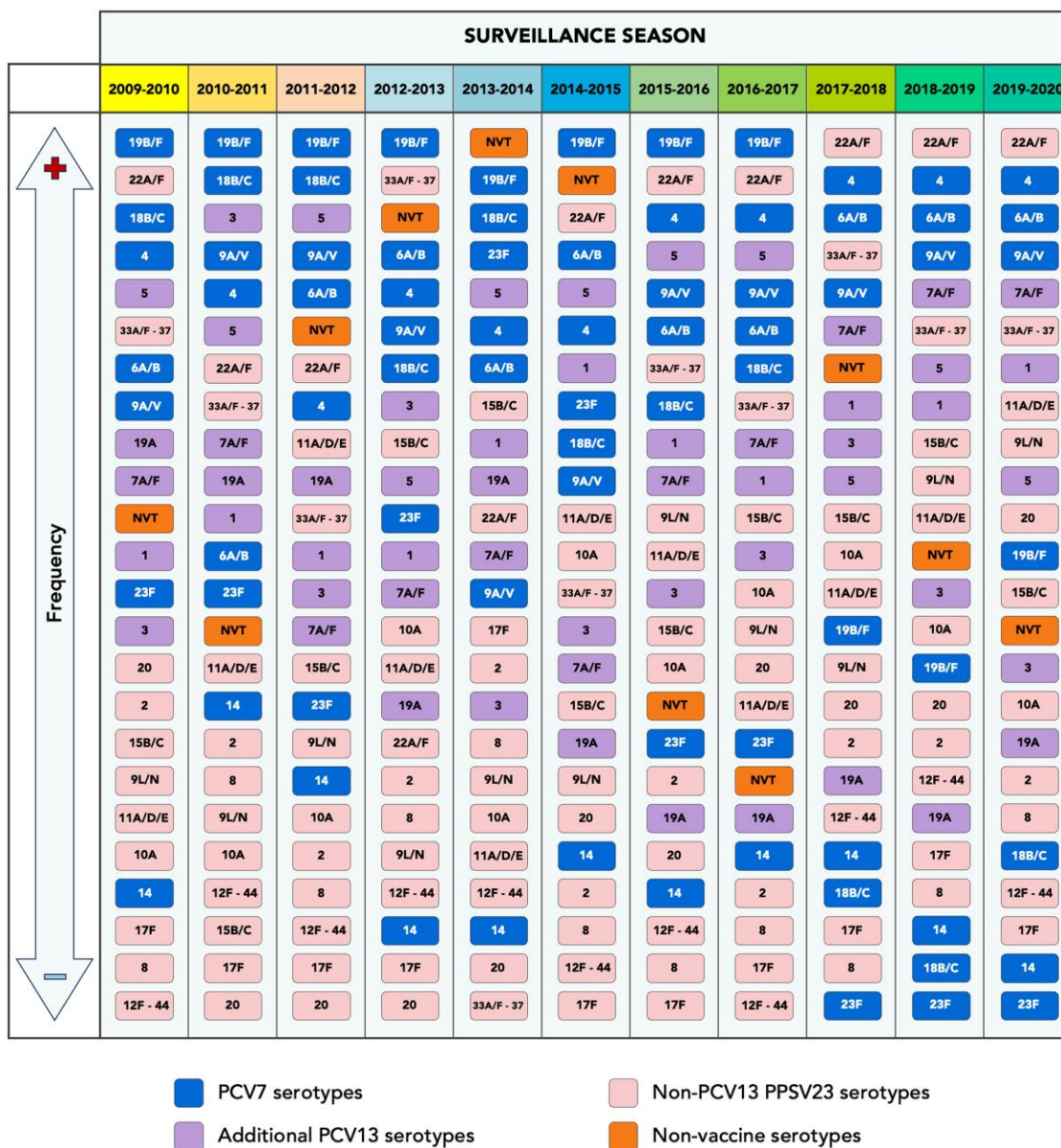


Figure 5. Seasonal fluctuation of pneumococcal serotypes included in PCV7, PCV13, or PPSV23 vaccines, according to each surveillance season. PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.

sustained high vaccine uptake [16, 33]. Our long-term analysis revealed, unexpectedly, a resurgence of some VTs in recent years. Although carriage levels varied by serotype and over time, certain PCV serotypes—such as 1, 4, 5, 6A/6B, 7A/7F, and 9A/9V—persisted in the population. While these serotypes currently do not represent a significant cause of IPD in infants in Sicily [8], their persistence in carriers is noteworthy. More importantly, serotypes 3, 14, 19A, 19B/19F, 8, and 10A demonstrated ongoing colonization and are associated with severe IPD risk [38]. Many of these serotypes have been implicated in vaccine breakthrough infections and continue to account

for a substantial proportion of IPD cases in Italy, especially among young children and the elderly [8].

Similar phenomena have been observed elsewhere. In France [39], serotypes 3, 19A, and 19F were linked to IPD vaccine failures 6 years post-PCV13 introduction, while microbiome analyses confirmed the persistence of vaccine serotypes in the respiratory tract [38]. In rural Gambia [40], PCV13 serotypes plateaued after a decade of vaccination, with serotypes 3, 6A, and 19F persisting across age groups. In the United Kingdom [24], after sequential PCV7 and PCV13 introduction, a long-term decline in vaccine serotypes was documented, except for

serotypes 3, 19A, and 19F, which continued to circulate into the 2020s, albeit at lower levels.

Age-related differences in serotype carriage were also prominent in our cohort. Children had higher odds of carrying serotypes 4, 6A/6B, and 5, as well as PPSV23-associated serotypes, such as 10A, 11A/11D/11E, and 15B/15C. Findings from other countries show similar trends but also underscore local variability. For example, in England [29], serotypes 10A, 11A, and 15B/15C predominated among children ≤ 4 years, whereas serotypes 4 and 6A/6B were more frequently detected in adults. In Gambia [16], serotype 19F persisted among children aged 0–4 years, while serotype 3 predominated in those aged 5–14 years. Israeli research further demonstrated different serotype patterns between children ≤ 2 years and those aged 2–5 years, with the latter group showing a distribution resembling that observed in adults with pneumococcal disease [41]. This may suggest waning postvaccination protection over time, especially among 5–9-year-olds, who in some studies showed higher odds of VT carriage than infants [16].

Because our surveillance enrolled individuals presenting with ILI, we also examined the effect of respiratory viruses as potential facilitators of pneumococcal colonization. Although carriage rates followed age patterns similar to those observed in healthy populations, viral codetection was strongly associated with pneumococcal carriage. This association was particularly marked for hRSV, which correlated with several clinically relevant serotypes, including PCV serotypes 6A/6B and 23F and PPSV23 serogroup 15B/15C.

Microbial interplay within the upper respiratory tract can be synergistic or competitive [42]. Several studies have shown that viral infections may enhance pneumococcal colonization density or facilitate acquisition either in older adults [43] or children [44]. Influenza virus can reshape the mucosal environment, suppress host immunity, and increase pneumococcal transmission among contacts [45]. These interactions have important implications for immunization strategies [46], particularly regarding live attenuated viral vaccines [47]. Respiratory syncytial virus has been demonstrated to directly increase pneumococcal adherence, proliferation, and virulence through altered immune responses [48]. Experimental studies suggest that hRSV infection increases susceptibility to secondary pneumococcal colonization and disease severity [49].

Some limitations of the study design should be taken into consideration. As the study was conducted within a single geographic region, the results may have limited generalizability and, not necessarily, represent the whole country. Nevertheless, because Sicily is one of the most populous regions of Italy and surveillance covered all provinces, we are confident in the representativeness of the results. A further limit concerns the lack of individual pneumococcal vaccination status, which did not allow for stratified analyses by immunization history.

Moreover, sampling was limited to oropharyngeal specimens, and this may have biased the real impact of pneumococcal carriage, particularly in the pediatric population. Additionally, all participants were symptomatic patients presenting ILI, and this health condition may have further influenced the estimate of carriage prevalence. However, available evidence suggests that symptom-based sampling does not substantially distort the distribution of pneumococcal serotypes [50].

Despite these limitations, our work has several significant strengths. This investigation represents, to our knowledge, the first long-term analysis in Italy, which allowed evaluating pneumococcal oropharyngeal carriage and serotype trends across all ages over more than a decade of PCV use. Moreover, the molecular approach also enabled assessment of multiple colonizing serotypes, offering a broader representation of pneumococcal ecology than culture-based methods.

Ultimately, we found that while pneumococcal colonization remains highest among children, a considerable proportion of elderly individuals also harbor the pathogen. Respiratory viral coinfection appears to promote carriage, and persistent circulation of vaccine serotypes raises important concerns.

Collectively, these findings highlight the persistent circulation of vaccine serotypes, despite 2 decades of high PCV coverage in the pediatric population, and underscore the importance of ongoing surveillance and re-evaluation of immunization strategies, especially as pneumococcal serotype ecology continues to evolve. Monitoring carriage through community-based respiratory surveillance systems may be key to guiding future vaccine policy, assessing indirect protection, and anticipating shifts in pneumococcal disease burden.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. Conceived and designed the study: F. T., C. M. M., E. A., and F. V. Performed the experiments: G. R., A. S., G. S., and A. P. Analyzed data: F. T., C. C., G. G., and W. M. Wrote the manuscript: F. T., C. M. M., and F. V. All authors have read and agreed to the final version of the manuscript

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