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PCV13 serotype decrease in Italian adolescents and adults in the post-PCV13 era: Herd protection from children or secular trend?

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ABSTRACT

Background and aim of the work: In 2010 PCV13 replaced PCV7 in the pediatric vaccination schedule for Italian children. While a strong herd effect was demonstrated for PCV7, a possible herd effect due to PCV13 is still under debate. Our aim was to evaluate differences in the distribution of pneumococcal serotypes between the pre and post-PCV13 eras in unvaccinated Italian adolescents and adults with laboratory-confirmed pneumococcal infection from 3 Italian Regions with a high rate of PCV13 vaccination of children.

Patients and methods: Adolescents and adults admitted with laboratory-confirmed pneumococcal infection in the hospitals of 3 Italian Regions (Friuli-Venezia Giulia, Emilia Romagna, and Tuscany) between April 2006 and June 2016 were included in the study. Diagnosis of pneumococcal infection and serotyping were performed with Real Time PCR directly on normally sterile fluids or on culture isolates.

Results: 523 patients with laboratory-confirmed pneumococcal infection were enrolled (Male/Female ratio was 300/223, 1.3; median age 67.1, IQR 53.4–74.9). None of the patients had been vaccinated with any pneumococcal vaccine; 96.4% were serotyped. Overall, the most frequent serotypes were 3 (67/504, 13.3%), 8 (43/504, 8.5%), and 19A (38/504, 7.5%). Serotype distribution differed among age classes and clinical presentations.

Overall, PCV13 serotypes accounted for 47.6% of cases: 62.3% in the pre-PCV13 era and 45.0% in the post-PCV13 era; ($p = 0.005$ OR = 2.03; CL 95%: 1.2–3.3). Serotype 7F accounted for 12/77 (15.6%) of all serotypes in the pre-PCV13 period and for 12/427 (2.8%) in the post-PCV13 period and was the only serotype significantly contributing to the difference in percentage between pre and post-PCV13 eras.

Conclusion: Our study demonstrated a difference in percentage in serotype distribution in adolescents and adults laboratory-confirmed pneumococcal infection between the pre and post-PCV13 eras. This difference is mainly due to the decrease of serotype 7F. Thus, in order to decrease disease burden, adults and in particular the elderly should be offered a specific vaccination program.

1. Introduction

Despite the availability of effective vaccines, *Streptococcus pneumoniae* continues to be the leading cause of pneumonia and invasive bacterial infections, in particular in elderly people and in children. Over 90 serotypes are known but only a subset causes the majority of pneumonia and invasive pneumococcal diseases (IPD). *Streptococcus pneumoniae* is also a common commensal inhabitant of the nasopharynx of healthy people, especially young children who, consequently, can transmit the pathogen to adults

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[1]. Nasopharyngeal colonization is considered an essential step to bacterial spreading and subsequent IPD.

Pneumococcal incidence, virulence, as well as circulating serotypes differ among countries and may vary in different periods in the same country [2]. These changes are caused both by selective pressure caused by vaccination and by secular trends [3]. Widespread vaccination with the 7 valent conjugate pneumococcal vaccine (PCV7) was highly effective in reducing IPD in vaccinated children and induced a significant decrease of IPD in unvaccinated adults as well [4–7]. Since 2010, 13-valent conjugate pneumococcal vaccine (PCV13) has replaced PCV7 in Italy and in over 120 other countries and has been included in pediatric vaccination schedules. In 2012, PCV13 was approved for use in adults over 50 years of age in Italy and in other countries. In 2013 PCV13 was approved for all ages. Since 2015, PCV13 has been recommended not only for children and high-risk groups but also for adults aged >65 in a limited number of Italian Regions.

Several studies demonstrated strong herd protection of adults after the introduction of PCV7 in pediatric vaccination programs, and other studies demonstrated a similar herd protection of adults after the introduction of PCV13 vaccination [4–6,8]. A number of studies have addressed the serotype distribution of adult IPD in different countries, and demonstrated serotype changes in the short term (1–3 years) between the pre and post-PCV13 eras. However, data regarding long-term serotype changes in adult and elderly IPD in the post-PCV13 era is still incomplete. That information is especially interesting and significant for Italy, one of the "super-aging" societies of the world, with a 21.2% of population >65 years in 2014, according to the Organization for Economic Co-operation and Development (OECD) [9].

Therefore, the aim of the present work was to evaluate the presence and the extent of PCV13 herd protection on laboratory-confirmed pneumococcal infection in unvaccinated Italian adolescents and adults 6 years after the introduction of PCV13 in Italy and to assess serotype changes between the pre and post-PCV13 eras in Italian adolescents and adults with laboratory-confirmed pneumococcal infection.

2. Materials and methods

2.1. Study design

This observational study was conducted from April 2005 through June 2016 in adolescents and adults (>14 years of age) admitted with a diagnosis of pneumococcal infection to hospitals in 3 Italian Regions. The 3 Regions (Friuli-Venezia Giulia, in the north-east, and Emilia Romagna and Tuscany, in the center of Italy) have very low and similar rates of polysaccharide pneumococcal adult vaccination (<10%). PCV7 had been progressively introduced since 2003 in Italy. The vaccine shift from PCV7 to PCV13 for children's vaccination was carried out in the 3 Regions in July 2010. The pneumococcal conjugated vaccine PCV13 is offered to all infants in those three Regions, and – according to the most recent survey performed in 2013 – the average vaccination rate is >70% (74.9% for Friuli, 94.1% for Emilia Romagna, and 93.5% for Tuscany) [10]. Clinical information was collected using a standardized questionnaire defining sex, age, clinical data, and pneumococcal vaccination. Stored samples (non-culturable lyophilized pneumococcal isolates), if available, were also accepted and associated clinical information was retrieved for clinical reports.

The study obtained the approval of the local Ethical Committee.

2.2. Laboratory methods

Clinical materials were obtained as part of standard care from patients included in the study; more than one specimen for patient could be available (blood, 356 samples, cerebrospinal fluid, 84 samples, pleural fluid, 22 samples, peritoneal fluid, 2 samples, articular fluid, 1 sample) or bronchoalveolar lavage (BAL, 76 samples).

Laboratory-confirmed pneumococcal infection was defined as the clinical suspicion of bacterial disease (pneumonia, meningitis, sepsis, other) and the laboratory confirmation of the presence of *S. pneumoniae* in a normally sterile fluid or bronchoalveolar lavage.

Laboratory confirmation was obtained by Realtime-PCR (RT-PCR) and/or culture methods as previously described [11,12]. Whole blood and/or other biological fluids, when appropriate, were collected from all patients as soon as possible after hospital admission for culture and/or molecular tests. For culture purposes, 4–6 mL of blood samples (up to 3 sets) were drawn and immediately sent to the local laboratory. Standardized procedures were used for collection and shipment of biological samples to local laboratories. Samples for molecular tests were sent by the participant centers on a voluntary basis to our Laboratory (Immunology Laboratory, Anna Meyer Children's University Hospital, Florence, Italy) at room temperature using an overnight freepost carrier and molecular tests were performed within 2 h of delivery. Stored isolates were also accepted.

All samples were tested with RT-PCR for the *lytA* gene as previously described [12]. Isolates already classified as *S. pneumoniae* by cultural methods were also sampled and analyzed for the *lytA* gene in order to confirm the diagnosis. A sample was considered negative if there was no increase in fluorescent signal before RT-PCR cycle 40. All samples were serotyped using RT-PCR.

For both diagnosis and serotyping by RT-PCR, 200 μL of available biological fluids or lyophilized isolates [13] were used.

For serotyping, 33 primer/probe sets targeting different regions of the *cpsA* gene were used, specific for 33 different serotypes. Twenty-nine primer-probe sets were previously published by our group [12,14,15]. The sequences of the 4 additional primer-probe sets are available upon request. Pneumococcal serotypes were classified as covered by PCV13 vaccines if they were included in the 13-valent (4, 6B, 9V, 14, 18C, 19F, 23F, 1, 3, 5, 6A, 7F, 19A) conjugate vaccine.

If no increase in fluorescent signal was observed after 40 cycles for any of the serotype-specific primer/probe sets, in spite of a positive result with both RT-PCR (*lytA* gene) and end-point PCR (*cpsA* gene) [12], the sample was considered non-typeable with the serotype-specific primers in RT-PCR.

2.3. Statistical analysis

Two tailed p values were used and p values <0.05 were considered statistically significant. Results were expressed as means and standard deviations (SD) or as median and interquartile range (IQR) as appropriate. The χ^2 test was used to assess group differences in categorical variables. Data were processed with the SPSSX statistical package (SPSS 11.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Diagnosis of pneumococcal infection

We identified a total of 523 patients, 89/523 pre-PCV13, 434 post-PCV13, 125/523 (6/125 pre-PCV13, 119/125 post-PCV13) from Friuli-Venezia Giulia, 84/523 (5/84 pre-PCV13, 79/84 post PCV13) from Emilia Romagna, and 314/523 (78/314 pre PCV-13 236/314 post-PCV13), from Tuscany in the period April 2006–June 2016. The 3 Regions are situated in the center or north of Italy and represent 16% of the Italian population >14 years.

The gender ratio M/F was 300/223, 1.3; age range was 14–94 years of age; median age and interquartile range (IQR) were

Table 1
Distribution of enrolled patients according to age.

Age groups	Pre-PCV13 n (%)	Post-PCV13 n (%)	Tot
14–18	8 (9.0%)	7 (1.6%)	15
19–30	4 (4.5%)	19 (4.4%)	23
31–45	13 (14.6%)	35 (8.1%)	48
46–60	16 (18.0%)	93 (21.4%)	109
61–75	30 (33.7%)	180 (41.5%)	210
76–100	18 (20.2%)	100 (23.0%)	118
Tot	89	434	523

67.1 and 53.4–74.9 respectively. Distribution of patients in age groups is shown in Table 1. None of the patients had a pneumococcal vaccination history (either conjugate or polysaccharide).

Pneumococcal serotype or serogroup was identified in 504/523 patients (96.4%). In particular, for serogroup 6, 11, 12, 15, 18, 22, 33, only serogroup and not serotype was detected.

3.2. PCV13 potential coverage and serotype distribution of laboratory-confirmed pneumococcal infection

Potential coverage (defined as the percentage of laboratory-confirmed pneumococcal infection due to serotypes included in PCV13) in the pre-PCV13 period was 62.3% (48/77), with a significant diminution to 45.0% (192/427) in the post-PCV13 period (p = 0.005 OR = 2.03; CL 95%: 1.2–3.3) (Fig. 1). The potential coverage for PCV7 serotypes and for 6 additional PCV13 serotypes was 19.0% (96/504) and 28.6% (144/504) respectively (Fig. 1). The potential serotype coverage of PCV13 trend year by year is shown in Fig. 2.

Overall, the most frequent serotypes were serotype 3 (67/504, 13.3%), 8 (43/504, 8.5%), 19A (38/504, 7.5%), 12 (33/504, 6.5%), and 22 (28/504, 5.6%). Serotypes that showed the greatest increase in percentage in the post-PCV13 era were 8 (+5.5%), 23A (+4.4%) and 33 (+3.5%). On the other hand, serotypes that showed the greatest reduction in percentage in the post-PCV13 era were serotype 7F (−12.8%; pre-PCV13: 12/77, 15.6%; post-PCV13: 12/427,2.8%), 15 (−4.0%; pre-PCV13: 6/77, 7.8%; post-PCV13: 16/427, 3.8%), and 12 (−3.0%; pre-PCV13: 7/77, 9.1%; post-PCV13: 26/427, 6.1%).

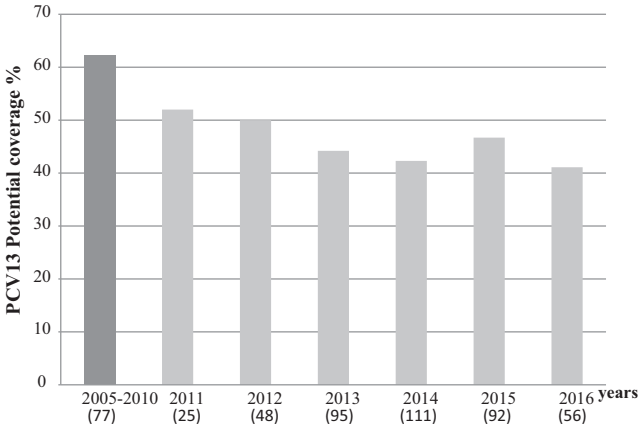


Fig. 2. Potential serotype coverage before (2005–2010) and after (2011–2016) the introduction of PCV13.

Potential coverage is different in different ages (Fig. 3A). Serotype 3 was the most frequent serotype in patients >45 years (18/104, 17.3% in 46–60 years; 27/203, 13.3% in 61–75 years and 13.7%, 16/117 in >76 years) while the most frequent serotypes in younger patients were 7F in 14–18 years patients (4/13, 30.7%), 23A in 19–30 years patients (3/21, 14.3%), and 12 in 31–45 years patients (9/46, 19.6%).

According to the Hospital Discharge Register (HDR), sepsis was the most frequent pneumococcal infection n = 247/523 (47.2%). Pneumococcal pneumonia was documented in n = 169/523 (32.3%) HDR (82/169, 48.5%, were bacteremic pneumococcal pneumonia). Meningitis was present in n = 94/523 HDR (18.0%) (in 43/94, 45.7% cases, meningitis was associated with sepsis and in 2/94, 2.1%, with pneumonia). In one HDR, sepsis was associated with both meningitis and pneumococcal pneumonia. In 12/523 (2.3%) patients other pneumococcal infection, including peritonitis,

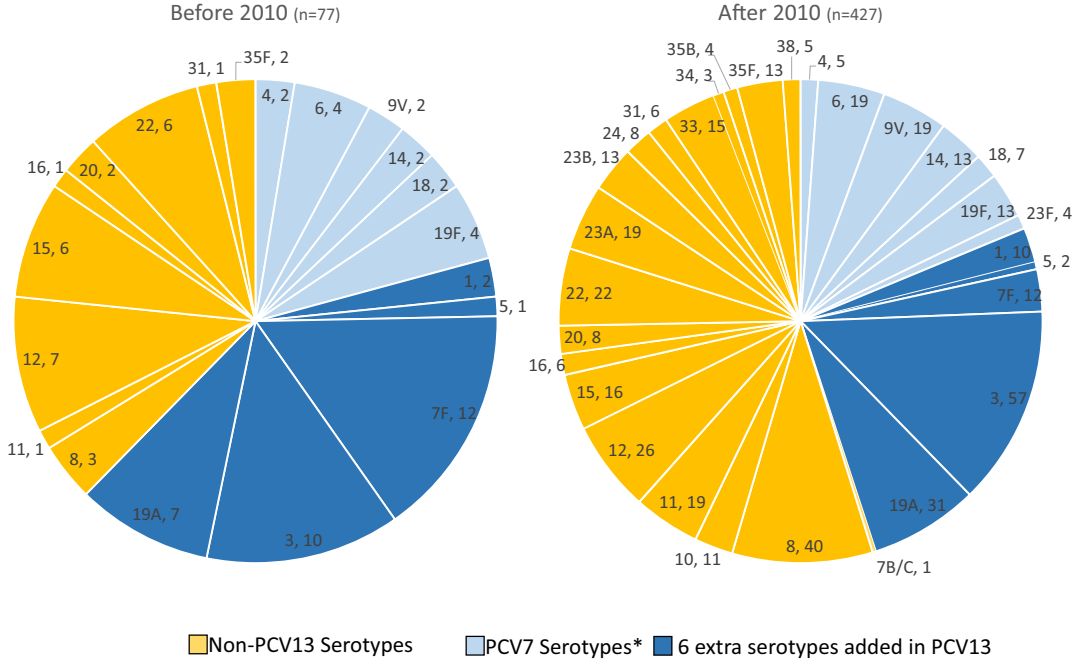


Fig. 1. Potential coverage in the pre-PCV13 period and post-PCV13 period.

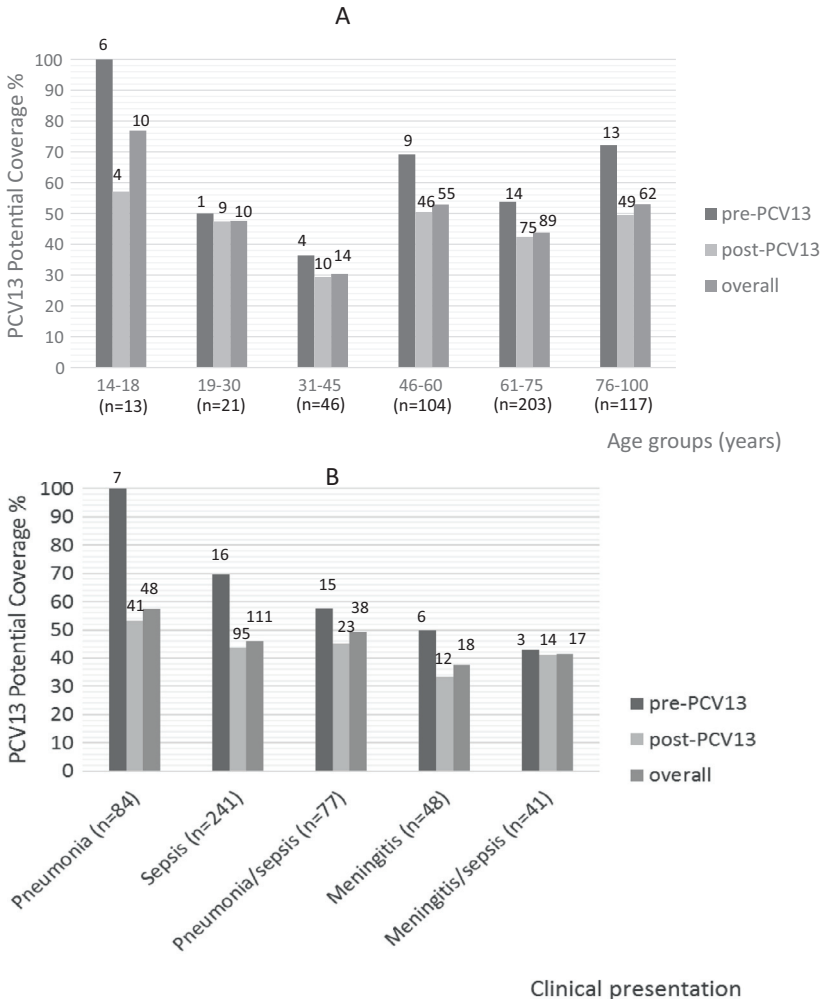


Fig. 3. Potential coverage of PCV13 according to age (A) or clinical presentation (B).

arthritis and otomastoiditis with sepsis, were found. Sepsis was the most frequent pneumococcal infection in any age group with the exception of the 14–18 years group where pneumonia was the most frequent pneumococcal infection. Meningitis was the least frequent pneumococcal infection in all age groups. Potential coverage for PCV13 according to clinical presentation is shown in Fig. 3B. Serotype 3 was the most frequent serotype in pneumonia (30/161; 18.6%) and meningitis (10/92, 10.9%), while serotype 8 was the most common in sepsis not associated with other clinical conditions (28/241, 11.6%).

3.3. Crucial role of serotype 7F

Serotype 7F accounted for 12/77 (15.6%) in the pre-PCV13 period and for 12/427 (2.8%) in the post-PCV13 period (Fig. 1). Serotype 7F represents the only statistically significant difference in the distribution of pneumococcal serotypes between the pre and the post-PCV13 eras. Differences in the distribution of other serotypes were not significant, independently of the discrepancy of sample size between the pre and the post-PCV13 eras (Fig. 4).

4. Discussion

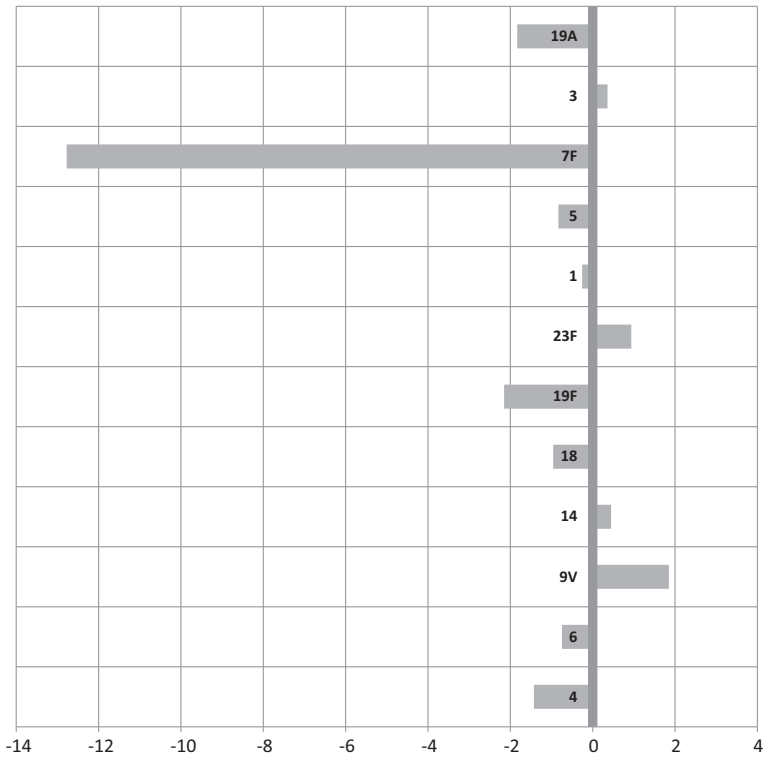
The study demonstrates that serotype percentage changes occurred after the introduction of PCV13 in the 3 Italian regions studied, but herd protection, if present, is mainly due to a significant reduction of serotype 7F. Similar results were found by other groups [16–22] but, up to now, the crucial role of 7F had not been clearly demonstrated. However, both in our study in Italy and in Kendall's study in Utah, USA, [20], the PCV13 "herd effect" was essentially due to serotype 7F.

It is unlikely that the decrease in 7F incidence is due to a stronger immunogenicity of that serotype in vaccinated children. Actually, Jackson et al. demonstrated that 7F OPA titers were similar to those induced by other PCV13 serotypes and even lower if compared to serotype 19A, 4, 6B, 18C and 23F [23].

It is also unlikely that the 7F decrease is due to the elimination of serotype 7F from the nasopharynx of vaccinated children. It is well known that the herd protection induced by PCV7 is due to the reduction of vaccine strains in the nose and in the pharynges of vaccinated children [24,25]. Actually, we have recently demonstrated [26,27] that in the pre-vaccination era, serotype 7F was very rarely found (less than 1%) in nasopharyngeal swabs of Italian children from the same Regions. So it seems quite unlikely that the elimination of a serotype that is very rare in children's pharynx would give a strong herd protection to adults.

Further studies will be necessary to evaluate whether the reduction of 7F-associated pneumococcal infection in adults may be due to the decrease of children IPD more than 7F carriage in children.

It should be considered that 7F reduction in percentage in unvaccinated adolescents and adults might be due to other factors, such as secular trends, more than being dependent on the herd effect associated with the PCV13 vaccination of children. Actually,



Pre-PCV13	Post-PCV13	*p*	OR	95% CI
Absolute numbers				
7	31	0.74	0.76	0.33-1.81
10	57	0.92	1.03	0.50-2.15
12	**12**	**$<10^{-5}$**	**0.13**	**0.06-0.31**
1	2	0.95	0.35	0.03-4.0
2	10	0.79	0.88	0.19-4.12
0	4	0.88	NC	NC
4	13	0.53	0.56	0.18-1.76
2	7	0.90	0.62	0.13-3.04
2	13	0.88	1.18	0.26-5.35
2	19	0.66	1.78	0.41-7.82
4	19	0.99	0.84	0.28-2.55
2	5	0.65	0.44	0.08-2.30

Fig. 4. Differences (in percentage) in the distribution of PCV13 serotypes between the pre and post PCV13 era.

Del Amo et al. demonstrated a strong reduction of 7F in adults in Catalonia, Spain, a region where the vaccine was only available in the private market, with a PCV13 estimate vaccination of 55% of children [28]. Moreover, a recent work, performed on a limited number of cases over a 30-year period in Italy, demonstrated the absence of that serotype in the previous decades, thus suggesting a spontaneous oscillation of 7F incidence [29].

Our data suggest that in those countries, such as Italy, where the only decreasing serotype is 7F, herd protection obtainable with PCV13 is very limited, if ever present.

As in other studies, the present work showed that the most frequent serotype was 3, followed by 8 and 19A. Those serotypes, taken together, account for about one third of all laboratory-confirmed pneumococcal infection. Similar findings have been reported across European countries: in Navarre, Spain, 3 and 19A were the most frequent serotypes in 2010–2013, followed by 7F [21]. Similar results were obtained in Norway [22]. Although several studies, both in United States and in Europe, [17,21,30,31] demonstrated an overall herd effect of PCV13 introduction in adults and elderly, in our study, as previously outlined, a limited change in percentage, with the exception of 7F, occurred among PCV13 serotypes between the pre-and post-PCV13 eras in adult pneumococcal infection.

As specified, in the present work serogroup 6 was not split for 6A/6B and serogroup18 was not split for its serotypes. This might lead to underestimation of the herd effects: for serotype 6 because herd effects for PCV7 serotypes may already have been maximized and for serotype 18 because non-PCV13 serotypes may be included.

Our unpublished data on pediatric surveillance, consistently with results obtained all over the world [30,32–37] on children vaccination effectiveness, indicated a rapid and substantial decline of pneumococcal infection due to all serotypes included in PCV13, with zero cases of 19A (that was >20% in the pre-PCV13 era) in 2015 and in the first six months of 2016 in vaccinated children. On the other hand, as far as carriage is concerned, recent studies performed in France suggest a permanence of vaccine serotypes in carriers after the PCV13 vaccination [38]. Those results are not dissimilar from what we demonstrated in 2014 [26,27]: actually, our data demonstrated that the disappearance of PCV7 serotypes from the nasopharynx after the vaccination is only partial and temporary. Moreover, the 6 additional serotypes included in PCV13 (1, 3, 5, 6A, 7F, 19A) are rarely found in carrier children. Consequently, the limited indirect protection of adults by vaccination of children could be expected and dedicated programs of vaccination of adults seem the only feasible strategy.

A new, larger formulation of conjugate pneumococcal vaccines is under preparation and will probably include 22F and 33, which accounted for about 10% of IPD cases in adults in the US in 2007 [39]. Both of these serotypes are not commonly found in Italian adults with IPD: 22F has decreased in the last 6 years and is now 5.1%, while serotype 33 was absent in the pre-PCV13 era and is now 3.5%.

Our study has some limitations. The main bias and limit of the study is related to absolute numbers of isolates before and after PCV13 (77 pre-PCV13 and 427 post-PCV13) due to a better monitoring after 2010. Therefore, absolute numbers of PCV13 serotypes are generally higher after than before vaccination. Anyway, we can suppose that poorer surveillance before vaccination did not influence serotype distribution. Another limitation was that our study population was obtained from three Italian regions only, which together correspond to about one sixth of the entire Italian population. However, these regions have a highly vaccinated child population ($\geqslant$75% of vaccinated children), compared to most

Italian regions and other countries. Consequently, the study is well generalizable to regions with high vaccination rates in children. Another limitation for generalizability is the low vaccination rate in adults.

Actually, since there was a difference in vaccine uptake between regions (minimum 74.9%, maximum 94.1%, difference 19.2%), it would be interesting to know if there were differences in serotypes distribution in pre- and post-PCV13 vaccine period in particular regions. However, given the limited number of isolates obtained in the pre-PCV13 era, only aggregate analysis of the 3 regions has been possible.

Another limitation was that samples were sent by the participant centers to our laboratory on a voluntary basis, so that the study cannot give data on IPD incidence or burden of disease on the adult population. However, the present study, performed on a large population of Italian adolescents and adults, represents, to our knowledge, the largest study on pneumococcal infection in Italian adults in the last decades and, for the first time, characterizes long-term serotype changes after the introduction of PCV13.

Pneumococcal pneumonia cases are probably underestimated since most pneumonia are usually cured by family practitioners and even in cases admitted to hospital, microbiological tests are not always performed. However, we believe that cases we described may be representative of pneumococcal serotype distribution in the studied Italian Regions, since our data set is the largest available in Italy, being even larger than datasets obtained on pneumonia by the National Institute of Health through pneumococcal surveillance [40].

Regarding clinical presentation, our study demonstrated that about 55% of pneumonia cases were caused by PCV13 serotypes (Fig. 3B). That is a very important finding, in light of the fact that pneumococcal pneumonia is one of the most frequent and serious clinical presentations of pneumococcal disease in older adults. For that reason, adult vaccination could be a very important tool in reducing the burden of pneumococcal infection in Italy.

5. Conclusions

Our study clearly demonstrated that the introduction of PCV13 is associated with a modification in percentage of serotypes causing pneumococcal infection in Italian adolescents and adults. That variation was essentially due to the strong decrease of serotype 7F while no similar effect was seen for any other vaccine serotype. Consequently, herd protection was probably weaker than expected, so that it may be speculated that other factors, such as secular trends, together with herd protection, might be the cause for that reduction. Therefore, in order to decrease disease burden, adults and, in particular, the elderly should be offered a specific vaccination program. Pneumonia is the leading cause of adult and elderly pneumococcal disease and is caused by PCV13 serotypes in about 60% of cases. Pneumonia prevention through vaccination is particularly important for Italy, which is one of the "super-ageing" societies of the world where elderly people represent a large percentage of the total population.

Conflict of interest statement

The authors have no conflict of interest.

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