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# Pneumococcal carriage among adults aged 50 years and older with co-morbidities attending medical practices in Rome, Italy

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ABSTRACT

*Background:* Data on *Streptococcus pneumoniae* carriage in adults with co-morbidities are limited. In this study we estimated the pneumococcal carriage among adults with co-morbidities and evaluated socio-demographic and clinical risk factors. The potential coverage of the current pneumococcal vaccines recommended for adults (PCV13 and PPV23) was also investigated.

*Methods:* A cross-sectional study on *S. pneumoniae* carriage among unvaccinated adults ≥50 years with co-morbidities, presenting with or without acute respiratory symptoms at general practitioners in Rome, Italy, between October 2015 and July 2016 was conducted. Pneumococcal carriage was investigated by both cultural and molecular methods. Socio-demographic variables and co-morbidities were evaluated by logistic models as possible risk factors for pneumococcal carriage.

*Results:* Out of 248 patients (median age: 73 yrs; IQR: 65–79), 12 (4.8%) and 83 (33.5%) individuals were found colonized using cultural or molecular methods, respectively. Potential risk factors for pneumococcal colonization as ascertained by molecular methods were: low level of education (adjusted OR = 3.71, 95% CI: 1.62–9.40), winter months (December-March vs other months, adjusted OR = 2.56, 95% CI: 1.29–5.14), and presence of chronic lung diseases (adjusted OR = 2.18, 95% CI: 1.15–4.16). The combination of serotype-specific multiplex RT-PCR and conventional PCR allowed to identify 22 serotypes/group of serotypes, of which the most common were: 24F/24A/24B, 12F/12A/12B/44/46, 6A/6B, 14, 15B/15C, and 22F/22A. Prevalence of pneumococcal carriage due to PCV13 serotypes and non-PCV13 serotypes was 23.6% and 67.3%, respectively. Prevalence of colonization due to PPV23 serotypes was estimated to be 54.6%.

*Conclusions:* A high prevalence of *S. pneumoniae* carriage was observed among adults with co-morbidities, especially among individuals affected by chronic lung diseases. These results support vaccine strategies based on the sequential administration of PCV13 and PPV23 to control potentially invasive pneumococcal strains in adults, especially in subjects with co-morbidities.



## 1. Introduction

*Streptococcus pneumoniae* (the pneumococcus) is the most common bacterial respiratory pathogen and the most frequent cause of community-acquired pneumonia, particularly among the elderly. Pneumococcal infections include both invasive and non-invasive diseases and colonization is considered a pre-requisite for infection. The pneumococcus resides on the mucosal surface of the nasopharynx of healthy people, especially children, where it normally remains asymptomatic. Occasionally pneumococcus spreads locally to cause non-invasive infections such as otitis, sinusitis or pneumonia, or it may invade the bloodstream to cause invasive pneumococcal diseases (IPD), such as meningitis and/or bacteremia/sepsis [1]. The development of invasive disease depends on both the virulence of the pneumococcus and the immunological status of the host, hence the highest incidence of pneumococcal diseases occurs in the extremities of life, among young children and the elderly [2].

Substantial changes in the epidemiology of pneumococcal infections occurred after the introduction of conjugate vaccines

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for children immunization. The 7-valent pneumococcal conjugate vaccine (PCV7), introduced as from 2001 in several countries around the world and targeting the most common 7 serotypes in children's IPD, was replaced between 2008 and 2010 by high-valency vaccines that extended serotype coverage to 10 serotypes (PCV10) or 13 serotypes (PCV13). Overall, conjugate vaccines were effective in reducing IPD due to vaccine serotypes not only among vaccinated children but also among unvaccinated individuals across all age groups as a result of herd protection [3–6]. However, over the years, the positive impact of conjugate vaccines was jeopardized by the increase of non-vaccine serotype IPD [7,8]. To monitor vaccine impact, a large number of surveillance studies were carried out in countries where the vaccines were used [3–6]. Also carriage studies proved to be a valuable tool for evaluating indirect vaccine effects [9–11]. Since children are recognized to be the main reservoir for pneumococcal transmission in the community, the majority of carriage studies focused on this age group, while only recently a number of studies have addressed the carrier status in adults [12–15].

At present, two pneumococcal vaccines can be used for adult vaccination: the 23-valent polysaccharide vaccine (PPV23) and PCV13, the latter approved in 2011 for prevention of IPD and pneumonia in this age class. According to the CAPiTA study [16], PCV13 effectiveness against PCV13-type IPD and pneumococcal pneumonia among adults over 64 years of age was estimated to be 75% and 45%, respectively. In Italy, PCV13 entered the national immunization programme for adults over 64 years in 2017, with the recommendation to sequentially administer PCV13 followed by PPV23 according to the prime-boost strategy. This immunization schedule was recommended also for children over 5 years of age and younger adults with chronic health conditions, such as heart or respiratory diseases, impaired immune system, or presence of cochlear implants (http://www.salute.gov.it/imgs/C_17_pubblicazioni_2571_allegato.pdf).

In view of likely future changes in the circulation of pneumococcal serotypes due to the selective pressure of the vaccines in use, it is important to know the baseline pneumococcal carriage in the elderly, especially among individuals with predisposing conditions, that represent a high-risk population for pneumococcal diseases. Elderly patients attending general practitioners' offices can be a representative sample of this population.

The main objective of this study was to estimate the *S. pneumoniae* carriage rate among unvaccinated individuals aged ≥50 years with predisposing conditions attending medical practices in the city of Rome, Italy. Secondary objectives were to study the socio-demographic and clinical risk factors associated with colonization in this population, and to evaluate the potential coverage of the pneumococcal vaccines for adults.

## 2. Materials and methods

A cross-sectional study on *S. pneumoniae* carriage was conducted on a convenience sample of patients aged 50 years and older, consecutively attending medical practices in Rome between October 2015 and July 2016, and presenting with co-morbidities that are considered predisposing factors for invasive pneumococcal disease (IPD), such as chronic illnesses (including heart, liver, and kidney chronic diseases), lung diseases (chronic obstructive pulmonary disease, emphysema, and asthma), diabetes, presence of conditions that weaken the immune system (HIV/AIDS, cancer, absent or dysfunctional spleen), presence of cochlear implant or cerebrospinal fluid leak (http://www.salute.gov.it/imgs/C_17_pubblicazioni_2571_allegato.pdf).

Twenty-six general practitioners (GPs) were recruited in the city of Rome, 36% in urban and 64% in suburban areas. Each GP was asked to enrol 10 patients during routine visits. For each patient, the GP had to fill a structured questionnaire containing demographic and clinical data, and to obtain a nasopharyngeal swab. The questionnaire included information on: (1) socio-demographic data (age, sex, number and age of family members, smoking habits, educational level, occupational or retirement status); (2) presence of underlying conditions predisposing to IPD; (3) presence of acute respiratory symptoms at the time of recruitment.

The sample size was established on the basis of a published study estimating a carriage prevalence of 36% [17].

### 2.1. Ethics statement

The study protocol was approved by the Ethics Committee of the Regional Health System (Lazio Region, ASL RM E, Rome), and written informed consent was obtained from all recruited patients. All the procedures were applied in accordance with the European Statements for Good Clinician Practice and the Declaration of Helsinki of the World Medical Association.

### 2.2. Sample collection

A nasopharyngeal (NP) sample was collected from each patient using a nylon flocked swab (eSwab, Copan, Brescia, Italy) and transferred into a vial containing milk-tryptone-glucose-glycerol (STGG) medium, according to the WHO methods [18]. NP swabs were transferred to the laboratory within 6 h from collection.

### 2.3. S. pneumoniae culture and serotyping by the Quellung reaction

On arrival to the laboratory, an aliquot of STGG medium was streaked onto a 5% sheep blood Columbia agar plate supplemented with gentamicin 5 μg/mL, that was incubated overnight at 37 °C in 5% $CO_2$-enriched atmosphere. *S. pneumoniae* was identified according to standard procedures. Pneumococcal isolates were serotyped by the Quellung reaction using commercially available antisera (Statens Serum Institut, Copenhagen, Denmark). The vials containing NP swabs in STGG broth were stored at −80 °C for subsequent molecular analyses.

### 2.4. S. pneumoniae detection and serotyping by molecular methods

For molecular analysis, genomic DNA was extracted from culture-enriched STGG broths [19] by using QIAmp DNA Mini kit (Qiagen, Hilden, Germany). The *lytA, piaB*, and *cpsA* genes were used as targets for *S. pneumoniae* detection by Real time PCR (RT-PCR) [20–22]. Samples were classified as positive for *S. pneumoniae* when the cycle threshold (Ct) values for all targeted genes were ≤ 35. Molecular serotyping was obtained combining RT-PCR and conventional multiplex PCR according to published protocols (https://www.cdc.gov/streplab/pneumococcus/resources.html). In particular, the RT-PCR panel contained the following serotypes or groups of serotypes: 1, 2, 3, 4, 5, 6A/6B, 6C/6D, 7F/7A, 8, 9 V/9A, 10A/10B, 11A/11D, 12F/12A/12B/44/46, 14, 15A/15B/15C/15F, 15A/15F, 16F, 18C/18F/18B/18A, 19A, 19F, 20, 22F/22A, 23A, 23F, 33F/33A/37, 35B, and 38 [19,23,24]. Identification of serotypes/serogroups was considered adequate when the Ct value for the targeted capsular genes was ≤ 35. Conventional multiplex PCR included the following additional serotypes/ group of serotypes: 7C/7B/40, 9 N/9L, 10F/10C/33C, 13, 17F, 21, 23B, 24F/24A/24B, 31, 34, 35A, 35F, 38/25F/25A, and 39 (https://www.cdc.gov/streplab/pneumococcus/resources.html). Samples with no signal in RT-PCR (Ct value ≤ 35) and with no amplification in conventional

PCR for any serotype or group of serotypes tested, were defined as not determined (ND).

### 2.5. Statistical analysis

The prevalence of pneumococcal carriage was estimated separately according to the results of cultural or molecular methods. McNemar's test was applied for comparison of correlated proportions and chi-square for independent proportions. Univariate and multivariate logistic models were applied in order to investigate socio-demographic characteristics (sex, age, education, working or retirement status, smoking habits, presence of children in the same household) and clinical features (presence of co-morbidities, presence of acute respiratory symptoms) or other factors possibly associated with pneumococcal carriage. Profile-likelihood-based confidence intervals (PL-CI) were applied in order to limit sparse data bias, in other terms, to obtain more accurate estimates when case numbers were not adequate for some combination of variables and outcome levels [25,26]. Given the importance of age, this variable was entered in the analyses both as continuous (with increments of 10 yrs) or as dichotomous variable, i.e. less than or equal to the median age of individuals of the study sample (i.e., ≤73 yrs vs >73 yrs). Further, multivariate logistic analysis was also performed according to the group of participants with or without chronic lung diseases. Only variables with $p < 0.05$ were considered as risk-factors for *S. pneumoniae* carriage. All statistical analyses were performed using SAS version 9.4.

## 3. Results

### 3.1. Characteristics of the study population

A total of 260 patients were recruited by the GPs. Of these, 12 (5%) were excluded because they refused NP sampling or because their questionnaires were not completed; therefore, the final number of enrolled patients was 248. Patients were recruited from October 1, 2015 to July 31, 2016; 30.6% were recruited during winter months (from December 1, 2015 to March 31, 2016).

The characteristics of the population under study are summarized in Table 1: the median age was 73 years with the largest group aged between 71 and 80 years (35.9%); 50% were females and 20.2% had a university degree. The majority of the participants were retired (66.9%) and 18.9% were smokers. Almost 65% of participants lived in the same household with at least one child<5 year of age. According to the protocol, none of the patients had been vaccinated with either PCV13 or PPV23.

At the time of recruitment, half of the participants (n = 124) had acute respiratory symptoms, the most frequent being cough (80.6%), dyspnoea (38.7%), and purulent sputum (38.7); fever was present in 25.8% of the individuals. All participants had co-

**Table 1**
Characteristics of the study population and *S. pneumoniae* colonization by cultural and molecular methods.

| | **Patients (n = 248)** | | **Positivity by culture (n = 12; 4.8%)** | | **Positivity by RT-PCR (n = 83; 33.5%)** | |
|---|---|---|---|---|---|---|
| | n. | % | n. | % | n. | % |
| **Sex** | | | | | | |
| Females | 124 | 50.0 | 7 | 5.6 | 42 | 33.9 |
| Males | 124 | 50.0 | 5 | 4.0 | 41 | 33.1 |
| **Age groups** | | | | | | |
| 50–60 | 38 | 15.3 | 1 | 2.6 | 11 | 28.9 |
| 61–70 | 66 | 26.6 | 7 | 10.6 | 28 | 42.4 |
| 71–80 | 89 | 35.9 | 2 | 2.2 | 29 | 32.6 |
| 80–95 | 55 | 22.2 | 2 | 3.6 | 15 | 27.3 |
| **Median age** (range; IQR*) | 73 (50–95; 65–79) | | 71 (51–93; 65–78) | | 66 (55–84; 61.5–75.5) | |
| **Children cohabitants** | | | | | | |
| None | 87 | 35.1 | 4 | 4.6 | 28 | 32.2 |
| ≥1 | 161 | 64.9 | 8 | 5.0 | 55 | 34.2 |
| **Educational level** | | | | | | |
| Graduated | 50 | 20.2 | 1 | 2.0 | 10 | 20.0 |
| Non-graduated | 198 | 79.8 | 11 | 5.5 | 73 | 36.9 |
| **Occupation** | | | | | | |
| Retired | 166 | 66.9 | 7 | 4.2 | 51 | 30.7 |
| Non-retired | 82 | 33.1 | 5 | 6.1 | 32 | 39.1 |
| **Specimen collected** | | | | | | |
| Winter months** | 76 | 30.6 | 6 | 7.9 | 37 | 48.7 |
| Other months | 172 | 69.3 | 6 | 3.5 | 46 | 26.7 |
| **Smoker** | | | | | | |
| Yes | 47 | 18.9 | 2 | 4.2 | 18 | 38.3 |
| No | 201 | 81.0 | 10 | 5.0 | 65 | 32.4 |
| **Acute respiratory symptoms** | | | | | | |
| Presence | 124 | 50.0 | 10 | 8.1 | 48 | 38.7 |
| Absence | 124 | 50.0 | 2 | 1.6 | 35 | 28.2 |
| **Individuals with co-morbidities:** | | | | | | |
| Chronic cardiac diseases | 131 | 52.8 | 3 | 2.3 | 37 | 28.2 |
| Chronic lung diseases | 72 | 29.0 | 2 | 2.8 | 33 | 45.8 |
| Diabetes | 53 | 21.4 | 2 | 3.8 | 17 | 32.1 |
| Neoplasia | 25 | 10.1 | – | – | 6 | 24.0 |
| Asthma | 24 | 9.7 | 1 | 4.2 | 13 | 54.2 |

* IQR Interquartile range;
** Winter months (December-March) and other months (April-July and October-November).

morbidities, among which the most frequently reported were: chronic cardiac diseases (52.8%), chronic lung diseases (29%) and diabetes (21.4%).

### 3.2. S. pneumoniae carriage

The prevalence of *S. pneumoniae* carriage determined by cultural or by molecular methods was 4.8% (12/248) and 33.5% (83/248), respectively (Table 1). As expected, RT-PCR identified a higher carriage rate compared to culture. The colonization rate was highest in the age group 61–70 years, being 10.6% and 42.4% by cultural and by molecular methods, respectively. All the participants found colonized by culture were also positive by RT-PCR. The samples positive by culture were strongly positive in RT-PCR, yielding Ct values that were lower than those found in samples positive by the molecular method only, suggesting that positivity by the cultural method corresponds to a higher bacterial load in the nasopharynx.

To investigate socio-demographic and clinical features possibly associated with pneumococcal carriage, statistical analyses were separately applied to the results of the two identification methods used (Table 2). Data obtained by the cultural method (Table 2, Part A), were analysed only by univariate analysis since the low number of carriage events prevented a multivariate analysis. The univariate analyses indicated that the presence of at least one acute respiratory symptom was significantly associated with pneumococcal colonization (crude OR = 5.35, 95% CI: 1.37–35.30, $p = 0.014$). Data obtained by molecular methods (Table 2, Part B), were analysed by univariate and multivariate analyses showing that lower level of education (non-graduated vs. graduated, crude OR = 2.34, 95% CI: 1.14–5.19, $p = 0.019$; adjusted OR = 3.71, 95% CI: 1.62–9.40, $p = 0.001$), winter months (specimen collected in December-March vs. other months, crude OR = 2.17, 95% CI: 1.22–3.86, $p = 0.008$; adjusted OR = 2.56, 95% CI: 1.28–5.14, $p = 0.007$) and presence of chronic lung diseases (crude OR = 2.13, 95% CI: 1.21–3.77, $p = 0.009$; adjusted OR = 2.18, 95% CI: 1.15–4.16, $p = 0.017$) were significantly associated with carriage. Presence of asthma was a potential risk factor for *S. pneumoniae* carriage only at the univariate analysis (crude OR = 2.60, 95% CI: 1.09–6.20, $p = 0.028$), but was not confirmed by multivariate analysis.

Multivariate logistic models were also performed according to the group of participants with or without chronic lung diseases (Table 3). Pneumococcal colonization rates were 45.8% (33/72) and 28.4% (50/176) in individuals with or without chronic lung diseases, respectively. In participants affected by chronic lung diseases pneumococcal carriage had a very strong association with winter months (crude OR = 8.23, 95% CI: 2.57–32.29, p = <0.001; adjusted OR = 18.11, 95% CI: 3.95–112.55, p = <0.001).

### 3.3. Serotype distribution

The serotyping results obtained by the Quellung reaction on isolated strains and those obtained by molecular methods on NP samples are reported in Table 4. The 12 pneumococcal strains obtained belonged to the following serotypes: 6C and 8 (2 strains each), 7C, 11A, 15A, 15B, 31 and 35B (1 strain each); 2 strains were non-typeable (NT). Serotyping by RT-PCR or conventional PCR assigned a serotype/group of serotypes to 66.3% (55/83) of NP samples positive for *S. pneumoniae*. In 15 samples, more than one serotype or group of serotypes was detected. In particular, 2 different

**Table 2**
Analyses of risk-factors for *S. pneumoniae* carriage identified by culture (Part A, univariate analysis) or RT-PCR (Part B, univariate and multivariate analysis).

| | OR Crude | 95% PL-CI* | *p* | Adj** OR | 95% PL-CI* | *p* |
|---|---|---|---|---|---|---|
| **Part A** | | | | | | |
| Age × 10 yrs increments | 0.71 | (0.41–1.25) | 0.237 | – | – | – |
| Age ≤75 yrs vs >75 yrs | 2.09 | (0.61–9.61) | 0.253 | – | – | – |
| Females vs males | 1.42 | (0.44–4.93) | 0.553 | – | – | – |
| ≥1 child in the household vs. no | 1.08 | (0.33–4.16) | 0.896 | – | – | – |
| Non-graduated vs. graduated | 2.88 | (0.54–53.30) | 0.250 | – | – | – |
| Non-retired vs. retired | 1.47 | (0.42–4.77) | 0.523 | – | – | – |
| Winter months vs. other months | 2.75 | (0.83–9.08) | 0.095 | – | – | – |
| Smoking vs. non smoking | 0.85 | (0.13–3.36) | 0.833 | – | – | – |
| Acute respiratory symptoms | 5.35 | (1.37–35.30) | 0.014 | – | – | – |
| Co-morbidities: | | | | | | |
| Chronic cardiac diseases | 0.28 | (0.06–0.97) | 0.044 | – | – | – |
| Chronic lung diseases | 0.47 | (0.07–1.86) | 0.308 | – | – | – |
| Diabetes | 0.73 | (0.11–2.86) | 0.675 | – | – | – |
| Neoplasia | n.e.*** | – | – | – | – | – |
| Asthma | 0.84 | (0.04–4.63) | 0.869 | – | – | – |
| **Part B** | | | | | | |
| Age × 10 yrs increments | 0.89 | (0.69–1.15) | 0.377 | – | – | – |
| Age ≤75 yrs vs >75 yrs | 1.30 | (0.76–2.26) | 0.340 | 1.47 | (0.76–2.87) | 0.249 |
| Females vs males | 1.04 | (0.61–1.76) | 0.893 | 0.91 | (0.50–1.63) | 0.745 |
| ≥1 child in the household vs. no | 1.09 | (0.63–1.92) | 0.752 | 0.93 | (0.51–1.72) | 0.823 |
| Non-graduated vs. graduated | 2.34 | (1.14–5.19) | 0.019 | 3.71 | (1.62–9.40) | 0.001 |
| Non retired vs. retired | 1.44 | (0.83–2.51) | 0.195 | 1.63 | (0.85–3.17) | 0.143 |
| Winter months vs. other months | 2.17 | (1.22–3.86) | 0.008 | 2.56 | (1.28–5.14) | 0.007 |
| Smoking vs. non smoking | 1.30 | (0.66–2.49) | 0.439 | 1.02 | (0.48–2.13) | 0.949 |
| Acute respiratory symptoms | 1.61 | (0.94–2.75) | 0.080 | 1.09 | (0.56–2.12) | 0.789 |
| Co-morbidities: | | | | | | |
| Chronic cardiac diseases | 0.61 | (0.36–1.03) | 0.065 | 0.84 | (0.45–1.56) | 0.575 |
| Chronic lung diseases | 2.13 | (1.21–3.77) | 0.009 | 2.18 | (1.15–4.16) | 0.017 |
| Diabetes | 0.92 | (0.47–1.74) | 0.808 | 1.14 | (0.54–2.34) | 0.730 |
| Neoplasia | 0.60 | (0.21–1.48) | 0.278 | 0.57 | (0.19–1.51) | 0.265 |
| Asthma | 2.60 | (1.09–6.20) | 0.028 | 1.38 | (0.52–3.68) | 0.517 |

* Profile-Likelihood Confidence Intervals (95% PL-CI);
** Adjusted OR for age as dichotomous and all variables in the table;
*** n.e. = non estimable.

**Table 3**
Multivariate analyses of risk-factors for *S. pneumoniae* carriage identified by RT-PCR in subjects with (Part A) and without (Part B) chronic lung diseases.

| | OR Crude | 95% PL-CI* | *p* | Adj** OR | 95% PL-CI* | *p* |
|---|---|---|---|---|---|---|
| **Part A: with chronic lung diseases (n = 72)** | | | | | | |
| Age × 10 yrs increments | 1.05 | (0.65–1.71) | 0.838 | – | – | – |
| Age ≤75 yrs vs >75 yrs | 0.79 | (0.31–2.00) | 0.623 | 0.56 | (0.47–11.79) | 0.426 |
| Females vs males | 1.43 | (0.56–3.68) | 0.453 | 2.28 | (0.67–8.58) | 0.192 |
| ≥1 child in the household vs. no | 1.15 | (0.42–3.17) | 0.783 | 1.58 | (0.45–6.01) | 0.483 |
| Non-graduated vs. graduated | 1.68 | (0.51–6.04) | 0.394 | 2.17 | (0.48–11.51) | 0.318 |
| Non-retired vs. retired | 1.66 | (0.63–4.43) | 0.305 | 5.20 | (1.30–25.36) | 0.019 |
| Winter months vs. other months | 8.23 | (2.57–32.29) | <0.001 | 18.11 | (3.95–112.55) | <0.001 |
| Smoking vs. non smoking | 0.72 | (0.24–2.03) | 0.537 | 0.83 | (0.21–3.17) | 0.785 |
| Acute respiratory symptoms | 1.62 | (0.64–4.20) | 0.312 | 0.39 | (0.07–1.88) | 0.244 |
| Co-morbidities: | | | | | | |
| Chronic cardiac diseases | 0.88 | (0.34–2.22) | 0.782 | 0.99 | (0.27–3.68) | 0.987 |
| Diabetes | 0.98 | (0.26–3.60) | 0.978 | 1.72 | (0.36–8.28) | 0.493 |
| Neoplasia | 1.19 | (0.14–10.42) | 0.864 | 0.49 | (0.03–8.28) | 0.608 |
| Asthma | 2.18 | (0.65–7.96) | 0.209 | 1.94 | (0.41–10.00) | 0.402 |
| **Part B: without chronic lung diseases (n = 176)** | | | | | | |
| Age × 10 yrs increments | 2.15 | (1.05–4.66) | 0.036 | – | – | – |
| Age ≤75 yrs vs >75 yrs | 0.72 | (0.52–1.00) | 0.050 | 1.86 | (0.83–4.34) | 0.133 |
| Females vs males | 0.85 | (0.44–1.64) | 0.632 | 0.77 | (0.37–1.59) | 0.478 |
| ≥1 child in the household vs. no | 1.02 | (0.52–2.05) | 0.950 | 0.95 | (0.45–2.03) | 0.885 |
| Non-graduated vs. graduated | 2.94 | (1.15–9.04) | 0.022 | 4.36 | (1.51–15.34) | 0.005 |
| Non-retired vs. retired | 1.30 | (0.64–2.59) | 0.456 | 1.24 | (0.54–2.79) | 0.608 |
| Winter months vs. other months | 1.33 | (0.64–2.69) | 0.442 | 1.26 | (0.52–3.00) | 0.605 |
| Smoking vs. non smoking | 1.60 | (0.66–3.75) | 0.290 | 1.18 | (0.44–3.03) | 0.738 |
| Acute respiratory symptoms | 1.54 | (0.80–3.00) | 0.197 | 1.46 | (0.65–3.26) | 0.357 |
| Co-morbidities: | | | | | | |
| Chronic cardiac diseases | 0.53 | (0.27–1.03) | 0.062 | 0.63 | (0.28–1.37) | 0.240 |
| Diabetes | 1.01 | (0.46–2.14) | 0.979 | 0.93 | (0.37–2.25) | 0.882 |
| Neoplasia | 0.56 | (0.15–1.60) | 0.234 | 0.49 | (0.13–1.49) | 0.220 |
| Asthma | 2.22 | (0.61–7.73) | 0.214 | 0.97 | (0.23–3.89) | 0.961 |

* Profile-Likelihood Confidence Intervals (95% PL-CI);
** Adjusted OR for age as dichotomous and all variables in the table.

**Table 4**
Serotypes/group of serotypes detected by culture and the Quellung reaction or by molecular methods (RT-PCR or conventional PCR) in NP-samples.

| Serotype/group of serotypes | N° of NP-samples positive by culture | N° of NP-samples positive by molecular methods* |
|---|---|---|
| 2 | 0 | 1 |
| 3 | 0 | 1 |
| 5 | 0 | 1 |
| 6A/6B | 0 | 8 |
| 6C/6D | 2 (6C) | 3 |
| 7C/7B/40 | 1 (7C) | 3 |
| 8 | 2 | 2 |
| 10F/10C/33C | 0 | 1 |
| 11A/11D | 1 (11A) | 2 |
| 12F/12A/12B/44/46 | 0 | 9 |
| 14 | 0 | 6 |
| 15A/15F | 1 (15A) | 2 |
| 15B/15C | 1 (15B) | 5 |
| 19F | 0 | 2 |
| 20 | 0 | 1 |
| 22F/22A | 0 | 4 |
| 24F/24A/24B | 0 | 11 |
| 31 | 1 | 1 |
| 33F/33A/37 | 0 | 2 |
| 34 | 0 | 3 |
| 35B | 1 | 2 |
| 38 | 0 | 1 |
| NT** | 2 | – |
| ND*** | – | 28 |
| Tot | 12 | 99 |

* Fifteen samples contained combination of multiple serotypes/groups of serotype (see Table S1);
** NT, Non-typeable by the Quellung reaction;
*** ND, serotype not determined by molecular methods.

serotypes/group of serotypes were found in 14 samples and 3 different serotypes/group of serotypes in 1 sample (Supplementary Table 1). Concordance was found between the results obtained by the Quellung reaction and those obtained by RT-PCR/ conventional PCR, although in some cases the latter did not identify a univocal serotype but a group of serotypes including that obtained by the Quellung reaction (Table 4). The 2 NP samples from which NT pneumococcal strains were isolated, were both positive for pneumococcus by molecular methods, but serotype remained not determined.

A total of 22 serotypes/group of serotypes were detected by molecular methods, of which the most common were: 24F/24A/24B (identified in 11 samples), 12F/12A/12B/44/46 (in 9 samples), 6A/6B (in 8 samples), 14 (in 6 samples), 15B/15C (in 5 samples), and 22F/22A (in 4 samples) (Fig. 1).

According to these results, the prevalence of colonized individuals carrying at least one PCV13 serotype or one non-PCV13 serotype was 23.6% (13/55) and 67.3% (37/55), respectively. In 5 cases (5/55, 9.1%), both PCV13 and non-PCV13 serotypes were co-carried.

Since by molecular methods many of the serotypes included in PPV23 are grouped with non-PPV23 serotypes, it is difficult to evaluate the exact prevalence of PPV23 serotypes. However, if we consider that it is likely that in each group the most common serotype is that contained in PPV23, we can estimate that the prevalence of colonized individuals carrying at least one PPV23 serotype was 54.6% (30/55) while prevalence of individuals carrying non-PPV23 serotypes was 30.9% (17/55). Eight individuals (8/55, 14.5%) were colonized by both PPV23 and non-PPV23 serotypes.

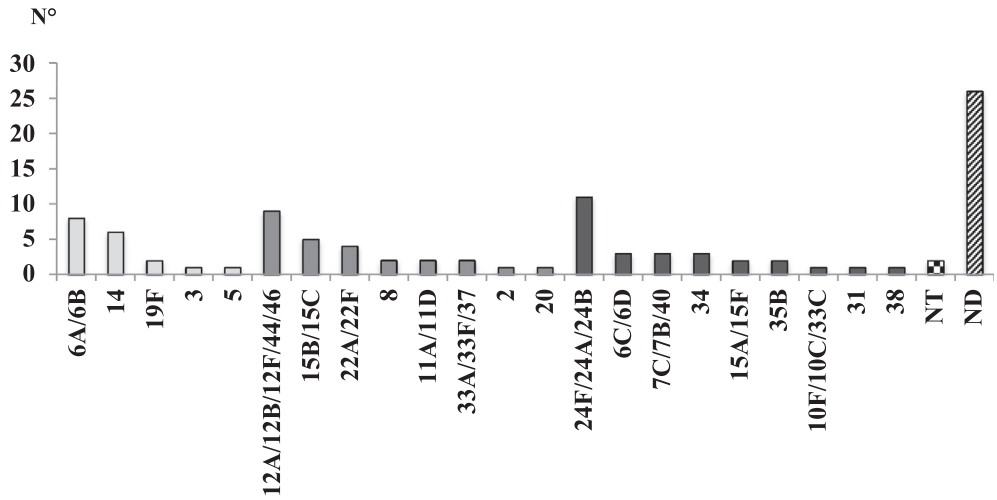


**Fig. 1. Frequency of *S. pneumoniae* serotype/group of serotypes in colonized adults aged ≥50 years. PCV13 serotypes are depicted in light grey; PPV23 serotypes in grey; Non-vaccine (non-PPV23) serotypes in dark grey; non typeable (NT) in dotted bar; not determined (ND) in striped bar.**

## 4. Discussion

*S. pneumoniae* is an important cause of morbidity and mortality among adults, especially in the elderly, to whom community –acquired pneumonia can represent a serious disease [27]. Our study provides a snapshot of the pneumococcal serotypes circulating in adults ≥50 years with underlying medical conditions in the city of Rome before the introduction of PCV13 for adult immunization. Pneumococcal carriage was assessed by both cultural and molecular methods, resulting in widely different pneumococcal carriage prevalence estimates. As expected, molecular methods revealed a higher carriage rate than conventional culture. The carriage rate obtained by culture (<5%) is in line with that reported in similar studies of adults, where rates ranged between 1% and 10% [28–30]. Molecular methods are more sensitive than cultural methods, being able to detect *S. pneumoniae* colonization also at low density. In molecular studies pneumococcal carriage rate in healthy adults reached proportions as high as 20% [12,13,31]. In our study, involving adults with co-morbidities and with or without acute respiratory diseases, the carriage rate was higher, reaching almost 33%, a percentage similar to that observed in the study by Krone et al. [17] on pneumococcal carriage in the elderly at the onset of influenza-like illness. In particular, very high carriage prevalence, almost 46%, was found among subjects suffering from chronic lung diseases. The two most recent Italian studies on pneumococcal colonization among adults performed by molecular methods reported lower carriage prevalence [12,15] (18.7% and 9.8%, respectively). Differences in the design of these studies, that included healthy adults, and different sampling methods, likely account for the different results.

When detecting pneumococcal colonization by molecular methods, an important issue to consider is the possibility of false positive results due to cross-reaction with other commensal species inhabiting the nasopharynx, such as streptococci of the *mitis*/*oralis* group [32]. Several molecular targets have been investigated for the specific detection of *S. pneumoniae*, such as the genes *lytA*, *ply*, *psaA*, *cpsA,* and *piaB* [20,22]. On the basis of recent carriage studies, we decided to use the combination of three of them as detection targets for the RT-PCR assay: genes *lytA,* coding for the autolysin and recommended by WHO [18], *piaB,* coding for the iron uptake ABC transporter lipoprotein [22], and *cpsA,* coding for the capsular biosynthesis gene A [21,22]. The strategy of using more than one target in culture-independent methods is widely applied in carriage studies to overcome the possibility of detecting false positive samples [15,17,21,33]. Therefore we consider our results to indicate actual colonization by pneumococcus.

The population investigated in this study is characterized by the presence of underlying co-morbidities; individuals suffering from chronic lung diseases were especially at risk for colonization. This finding correlates with studies demonstrating that this population is at increased risk for pneumococcal disease, both community-acquired pneumonia and invasive diseases [34–36]. In particular, in our study individuals suffering from chronic lung diseases are more prone to pneumococcal colonization during the winter months.

Conversely, the presence of acute respiratory tract infections was found to be associated (at univariate level) with pneumococcal carriage only when carriage was ascertained by conventional culture. This might be explained by the fact that during acute respiratory episodes, presumably of viral origin, the load of pneumococci in the nasopharynx increases to the level of detection by culture. Indeed it is known that viral respiratory tract infections are associated not only with higher rates of pneumococcal carriage but also with more abundant presence of pneumococci in the nasopharynx [17].

Lastly, from our study it appeared that having a lower level of education is also a potential risk factor for *S. pneumoniae* carriage; however, this poor education is likely to be a proxy for lower socio-economic level.

The pneumococci colonizing the adult patients in this study belong to a variety of serotypes. The two methods used for serotyping, the Quellung reaction on pneumococcal isolates and the molecular detection of serotypes on NP-samples, have both advantages and disadvantages. The Quellung reaction is based on a comprehensive panel of antisera and therefore allows the identification of almost all individual pneumococcal serotypes recognized to date but requires the isolated strain. On the other hand, molecular methods are able to identify serotypes in low-density pneumococcal carriage and colonization by multiple serotypes but are based on a more limited panel of selected serotypes or groups of serotypes and often do not allow discrimination of individual serotypes. The concurrent use of RT-PCR and conventional PCR for serotype detection has been recently proposed in carriage studies in order to combine the enhanced sensitivity of the former with the wider serotype coverage of the latter method [21,33]. This strategy allowed to assigning a serotype/group of serotypes in about 66% of NP samples positive for pneumococcus in our study. The failure of molecular serotyping in the remaining 34% of

samples might be due to the presence of serotypes not included in the molecular panel used, to the presence of NT pneumococci that lack capsular genes or to the lower sensitivity of serotype specific conventional PCR in samples with low bacterial load.

The most common carried serotype or group of serotypes was 24F/24A/24B, followed by 12F/12A/12B/44/46, 6A/6B, 14, 15B/15C, and 22F/22A. These serotypes partly resembled those found in colonized older adults in Milan in 2015 [15]. In particular, serogroup 24 (corresponding most probably to serotype 24F) was confirmed to be one of the most prevalent carried serogroup in children [11] and also in adults in Italy. Serogroups 12, 15, and 22 include frequent emergent non-PCV13 serotypes (e.g. serotypes 12F, 15B and 22F, respectively) recovered from IPD in the last few years in Italy (http://old.iss.it/mabi/) and in many other countries around the world [37]. On the contrary, other important serotypes recovered from IPD cases in adults in Italy at present, such as serotypes 8 and 3, were rarely found in carriage in our study.

This study was conducted prior to the introduction of PCV13 for adult immunization in Italy; participants were not vaccinated with PPV23 either, although they had co-morbidities, which should represent a strong recommendation for pneumococcal immunization. At present, the new national guidelines for elderly adults or at risk adults recommend sequential vaccination with PCV13 followed by PPV23 after 6–12 months. In our study, the prevalence of PCV13 serotypes colonization was about 24% of overall colonization events. The PCV13 use for children immunization, reducing the transmission of vaccine serotypes in the community, likely accounts for this low rate of PCV13 serotypes carriage in adults. Among PCV13 serotypes, 6A/6B, 14, and 19F were the most common serotypes, showing a persistent circulation in the adults, despite their decreased prevalence among children [37]. The majority of the serotypes found in this study are included in PPV23.

Our study has some limitations: first, this is a cross-sectional study, therefore we cannot exclude that we observed a transient presence of *S. pneumoniae* instead of a real colonization. The second limit is represented by the relatively small sample size, which could have affected the analysis for some of the variables considered. The last limit is the lack of oropharyngeal samples from the participants; recent recommendations suggest to combine naso- and oro-pharyngeal samples in order to achieve a more comprehensive carriage study. However, in our study, the carriage rate detected by RT-PCR was quite high and it is unlikely that testing also oropharyngeal samples could have further increased it.

In conclusion, our findings indicate that pneumococcal carriage among older adults with co-morbidities is high and confirm that the presence of co-morbidities involving the respiratory tract is a risk factor for pneumococcal colonization. Our study revealed that colonizing *S. pneumoniae* belong mainly to non-PCV13 serotypes, although a substantial proportion is contained in PPV23. The higher-valent conjugate vaccines currently under development could be useful in reducing pneumococcal carriage in this population. At present, the recommended sequential use of PCV13 and PPV23 for elderly immunization is expected to have an impact in curtailing IPD in this age class.

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## Declaration of Competing Interest

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## Authors' contributions

RC performed serotyping by Quellung and by conventional multiplex PCR, analysed results data and drafted the manuscript; CV and MC designed the study; MD performed statistical analysis; FF managed data collection; FM and AC performed *S. pneumoniae* detection and serotyping by RT-PCR; FPDA performed *S. pneumoniae* isolation by culture and DNA extraction; MC coordinated the General Practitioners' Group; AP finalized the manuscript; CV, MD, FF, FM, and AC contributed to draft the manuscript; GR critically revised the manuscript; the FIMMG group recruited patients, filled the questionnaires and performed the nasopharyngeal swabs. All authors have approved the final manuscript.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2019.06.052.

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