

BMJ Open Colonisation of Irish patients with chronic obstructive pulmonary disease by *Streptococcus pneumoniae* and analysis of the pneumococcal vaccine coverage: a non-interventional, observational, prospective cohort study

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ABSTRACT

Objectives To characterise the pattern of colonisation and serotypes of *Streptococcus pneumoniae* among patients with chronic obstructive pulmonary disease (COPD) who currently receive the 23-valent pneumococcal polysaccharide vaccine (PPV-23) according to vaccination status, use of antibiotics and steroids. To investigate the prevalence of PPV-23 and 13-valent pneumococcal conjugate vaccine (PCV-13) serotypes within the study cohort.

Design A non-interventional, observational, prospective cohort study with a 12-month follow-up period inclusive of quarterly study visits.

Setting Beaumont Hospital and The Royal College of Surgeons in Ireland Clinical Research Centre, Dublin, Ireland.

Participants Patients with an established diagnosis of COPD attending a tertiary medical centre.

Primary outcome measure Colonisation rate of *S. pneumoniae* in patients with COPD and characterisation of serotypes of *S. pneumoniae* with correlation to currently available pneumococcal vaccines. Sputum and oropharyngeal swab samples were collected for the isolation of *S. pneumoniae*.

Secondary outcome measure Seasonality of colonisation of *S. pneumoniae* and its relationship with the incidence of exacerbations of COPD.

Results *S. pneumoniae* was detected in 16 of 417 samples, a colonisation incident rate of 3.8% and in 11 of 133 (8%) patients at least once during the study. The majority of *S. pneumoniae* isolates were identified in spring and were non-vaccine serotypes for either the PPV-23 or PCV-13 (63%). The colonisation incident rate of *S. pneumoniae* fluctuated over the four seasons with a peak of 6.6% in spring and the lowest rate of 2.2% occurring during winter. Antibiotic use was highest during periods of low colonisation.

Conclusions There is seasonal variation in *S. pneumoniae* colonisation among patients with COPD which may reflect antibiotic use in autumn and winter. The predominance of non-vaccine types suggests that PCV-13 may have limited

Strengths and limitations of this study

- A strength of this study is that it assessed the proportion of patients with COPD that is permanently or intermittently colonised with *Streptococcus pneumoniae*, which is an important prerequisite for invasive pneumococcal infections.
- Patients with COPD were monitored regularly for an extended period at quarterly intervals over 12 months and were assessed during periods of clinical stability which is indicative of colonisation.
- A limitation of the study is that molecular methods of detection of *S. pneumoniae* were not incorporated into the study protocol as they were not standard practice of the laboratory of the hospital. It has been shown that molecular methods of detection are more sensitive than culture methods. However, this was beyond the remit of our laboratory.
- This study correlates serotyped *S. pneumoniae* isolated from patients with COPD to currently available pneumococcal vaccines to determine the vaccine coverage of the cohort and how relevant the currently available pneumococcal vaccines are to the study cohort.
- The number of *S. pneumoniae* isolated was low at 16 from 417 samples and the population cohort was small with 133 people recruited, thus limiting the generalisability of the results. However, the colonisation incident rate of 3.8% is reflective of results from previous studies.

impact among patients with COPD in Ireland who currently receive PPV-23.

Trial registration number NCT02535546; post-results.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease

characterised by airflow obstruction and inflammation of the airways arising from exposure to inhaled irritants.¹ The patient's clinical course is often interrupted by infective exacerbations of their illness. Approximately 50%–70% of COPD exacerbations are infectious in origin and one of the most common aetiological agents is *Streptococcus pneumoniae*.^{2–6} *S. pneumoniae* colonises the nasopharyngeal niche and this colonisation precedes invasive pneumococcal disease (IPD).⁷ Therefore, vaccination to prevent colonisation and to reduce IPD in patients with COPD is a potentially valuable intervention.

There are over 90 identified serotypes of *S. pneumoniae* and two pneumococcal vaccines; the 23-valent pneumococcal polysaccharide vaccine (PPV-23) which targets 23 serotypes that are commonly associated with IPD and the 13-valent pneumococcal conjugate vaccine (PCV-13) which provides immunity from 13 serotypes associated with IPD.⁸

There is mounting evidence that PCV-13 may be of benefit to the adult population that currently receives PPV-23.^{9–12} These studies suggest that vaccination with PCV-13 after initial vaccination with PPV-23 rather than administration of a booster PPV-23 after 5 years has greater protection from subsequent infection. A recent large-scale study of 84,496 adults ≥ 65 years of age demonstrated the effectiveness of PCV-13 for preventing vaccine-type IPD and non-bacteraemia community-acquired pneumonia.¹²

We monitored Irish patients with COPD over 12 months to determine the colonisation rates of *S. pneumoniae* among this cohort, to investigate the vaccine coverage of this population and to determine if the serotypes carried within the population would be covered by PCV-13.

METHODS

Study design and aim

Patients with COPD were recruited from specialist respiratory clinics in Beaumont Hospital, Dublin to determine the colonisation rate of these patients with *S. pneumoniae*. Recruitment began in July 2014 and ceased in February 2015 and all patients provided informed consent. A target of 150 patients was chosen for the most feasible and convenient sampling. Patients were reviewed and assessed at quarterly intervals to determine the colonisation rate of *S. pneumoniae*. A sputum sample (or if not available, an oropharyngeal swab) was obtained from patients at each visit. Patients were provided with collection containers for gathering sputum samples. Oropharyngeal swabs were collected by a research worker who conducted study visits with participants. Exacerbations were recorded if patients reported receiving antibiotics and/or steroids for COPD-related complaints in the period preceding their study visit. Prescription of antibiotics and steroids was confirmed by telephone contact with the patients' general practitioners and/or reviewing hospital records. Diagnosis of pneumonia was confirmed by reviewing patient hospital records. Routine methods were used for the isolation of *S. pneumoniae* from sputum and

oropharyngeal swabs. Any *S. pneumoniae* isolates obtained were characterised for serotype and antibiotic resistance profiles using PCR¹³ and conventional typing using slide co-agglutination with pneumococcal antisera (Staten Serum Institute). Antimicrobial susceptibility was determined using the E-test method and the current European Committee on Antimicrobial Susceptibility Testing criteria.

Inclusion/exclusion criteria

Inclusion criteria were patients over 40 years of age with both a clinical diagnosis of COPD and a spirometry value indicating obstruction, FEV₁/FVC (forced expiratory volume 1/forced vital capacity) ratio of $<70\%$.¹⁴ Patients were excluded if they were considered to be too unwell to participate in a year-long study that would involve four clinic visits.

Data sources

Demographic data were collected at recruitment and at follow-up visits, that is, visits 2, 3 and 4, over each of the four seasons: between March and May (inclusive) was considered spring data, from June to August (inclusive) was recorded as summer, September to November (inclusive) was recorded as autumn data and December to February (inclusive) was recorded as winter data. Patients too sick to attend appointments sent sputum samples for analysis by courier and the questions for the visit were conducted by telephone.

Statistical analysis

Data are shown as mean \pm SD, median (IQR) or numbers with proportions where indicated. Patients were categorised into colonised and non-colonised status based on isolation of *S. pneumoniae* at any point during the study period. Comparisons between groups and within seasons were performed using an unpaired t-test for normally distributed continuous variables or Wilcoxon rank sum test for count data, where appropriate. For comparisons of proportional data, χ^2 analysis was used. Statistical significance was set at $p \leq 0.05$. Statistical analysis was performed using Stata V.13.1 (Stata Corporation, College Station, Texas, USA).

RESULTS

Demographic data of study participants

A total of 133 patients with COPD was recruited to the study as shown in figure 1 with high retention throughout the study period with 420 out of 532 (79%) predicted visits occurring. The most common reasons for missed visits and dropout from the study at each stage were illness and inability to commit to participation in the full duration of the study.

The average age of participants was 69 ± 8.5 years and the male to female ratio was almost equally balanced with 53% male (n=71) and 47% female (n=62). The study population on average had a diagnosis of moderate COPD, the mean FEV₁/FVC was $47 \pm 12.5\%$, 97% were

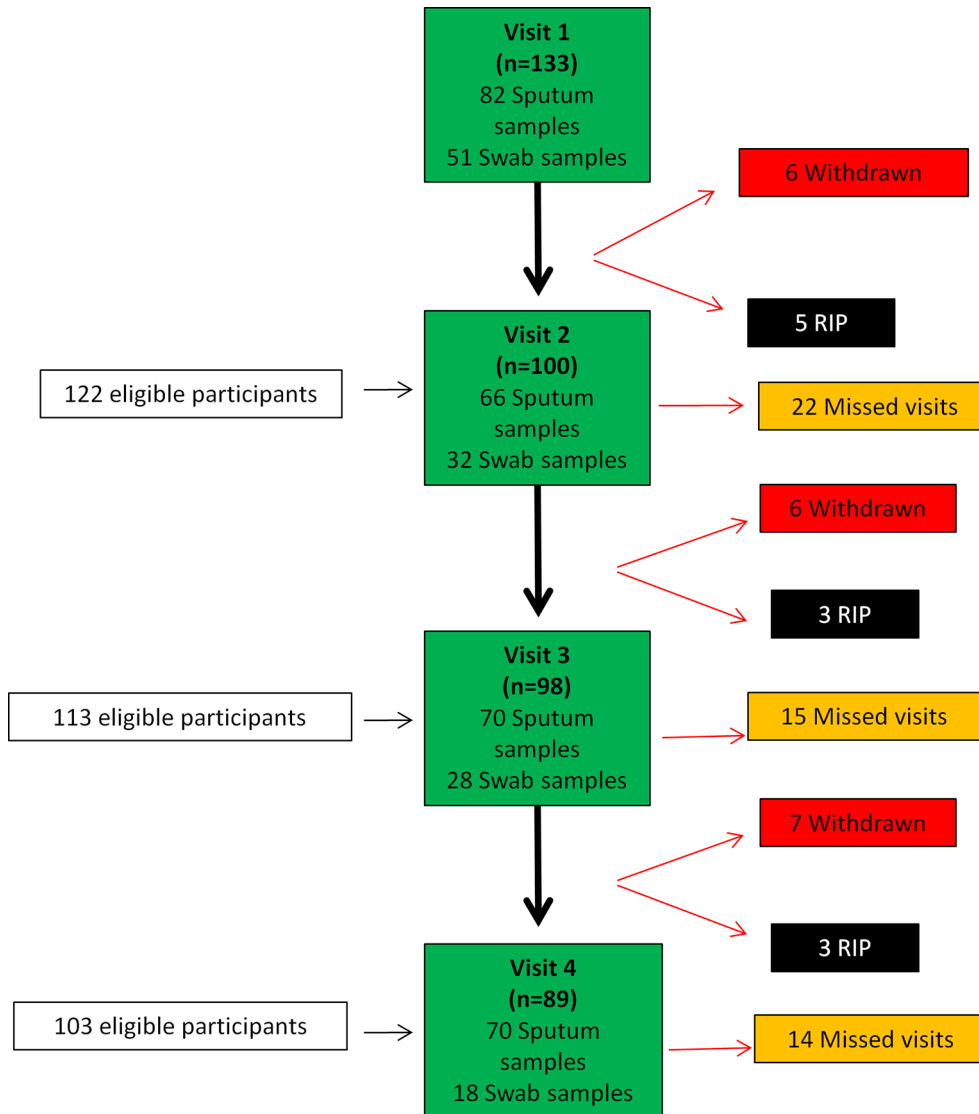


Figure 1 Study flow and patient retention. Rest in peace/deceased (RIP).

current or ex-smokers and 76% were reviewed as outpatients. The immunisation rate with the influenza vaccine for the season relevant to the study period was 81% but the pneumococcal vaccination rate was only 61%, that is, 61% of participants had received the PPV-23 vaccine within the 5 years prior to the study period. Cardiovascular disease was found to be the most prevalent relevant medical condition within the cohort at 39%, followed by radiologically defined coexisting bronchiectasis at 38%, and diabetes mellitus at 11%. Eleven study participants passed away during the study period. In three cases information on the cause of death could not be obtained. Where information could be obtained it was found that three participants died of cardiac-related issues while four died of pneumonia and one died of an exacerbation of COPD. There was no statistical significant difference in the baseline characteristics between patients subsequently confirmed as colonised with *S. pneumoniae* and those who were not (table 1).

Table 1 Baseline characteristics of the patient population based on colonisation status

	Colonised participants (n=11)	Non-colonised participants (n=122)	p Value
Mean age	68.2±6.4	68.8±8.7	0.82
Sex (% female)	45%	47%	0.94
Mean smoking pack years	48.7±25.8	56.0±42.0	0.57
Mean FEV ₁ /FVC ratio %	43.8±10.2	47.5±12.5	0.34
Influenza vaccine (% yes)	91%	80%	0.38
PPV-23 (% yes)	64%	61%	0.85

Data presented as mean±SD or %.

PPV-23, 23-valent pneumococcal polysaccharide vaccine.

S. pneumoniae isolates and characteristics of colonised participants

A total of 420 visits occurred throughout the study period and 288 sputum and 129 oropharyngeal swabs were collected. At each study visit, participants preferably provided a sputum sample or donated an oropharyngeal sample when a sputum sample could not be produced. Three participants failed to provide a sputum or an oropharyngeal sample during a visit. All isolates of *S. pneumoniae* were isolated from sputum samples (n=15 samples) and no *S. pneumoniae* were isolated from oropharyngeal samples. Sputum samples were the dominant sample type provided by non-colonised participants with 68% of the samples provided being sputum (n=273) and 32% of the samples being oropharyngeal swabs (n=129). Sixteen isolates of *S. pneumoniae* were isolated from 417 samples giving a colonisation-incident rate of 3.8%. A colonisation rate of 8% for patients was identified in the cohort with 11 participants out of 133 found to be colonised on at least one occasion. One participant was persistently colonised by *S. pneumoniae* and was colonised with the non-vaccine serotype 35B on all four study visits. This accounted for 0.8% of the study cohort. The remainder of colonised participants was intermittently colonised and did not have a positive sample at every visit. This accounted for 7.5% of the cohort.

Serotype analysis revealed that the majority of *S. pneumoniae* isolates, 63% (n=10/16), were non-vaccine type (NVT) and were not covered by any currently available pneumococcal vaccines (figure 2). PPV-23 covered 31% of the *S. pneumoniae* isolates (n=5/16). The smallest proportion of isolates, only 19% (n=3/16), was covered by PCV-13. All of the serotypes identified to be of PCV-13 type were also covered by PPV-23 (figure 2). No isolate was identified that was only covered by PCV-13. All isolates were susceptible to cefotaxime, tetracycline and levofloxacin while 69% were penicillin susceptible. Antimicrobial resistance was only detected for isolates classed as NVT.

There was no correlation between PPV-23 vaccination status and the serotypes of *S. pneumoniae* that these patients with COPD were colonised with. No incidents of pneumonia were reported during the study period for any of the participants colonised with *S. pneumoniae*. Colonised participants received a median of two (four) courses of antibiotics and two (three) courses of steroids over the study period, whereas non-colonised participants received a median of three (five) antibiotic courses and two (four) courses of steroids between study visits.

Relationship between COPD-related incidents and colonisation of *S. pneumoniae* within the study cohort

The colonisation-incident rate of *S. pneumoniae* fluctuated over the four seasons with a peak of 6.6% in spring and the lowest rate of 2.2% occurring during winter (figure 3). Antibiotic and steroid use among this cohort had a partially inverse correlation with the colonisation-incident rate; both peaked in the autumn when the colonisation-incident rate was dropping (figure 3). This peak in medication use correlated with a peak in exacerbations and COPD-related hospitalisations in autumn (figure 3).

Patients were categorised into colonised (n=11) or non-colonised (n=122) status if *S. pneumoniae* was isolated at any time period during the study. There was a trend in the number of exacerbations experienced during winter with colonised participants experiencing zero (one) exacerbation compared with one (two) experienced by non-colonised participants (p=0.06, table 2). Antibiotic use was significantly higher for non-colonised participants during winter with a median 1 (2) course in contrast to 0 (0.5) for colonised participants (p=0.01, table 2). This higher antibiotic usage in non-colonised participants might have contributed to the low colonisation rate of *S. pneumoniae*, particularly in winter. There were also significant differences in steroid use between the two groups. Non-colonised participants had a median of one (two) courses during winter whereas colonised participants had no courses (p=0.01, table 2).

Serotypes of *S. pneumoniae* isolated from COPD patients

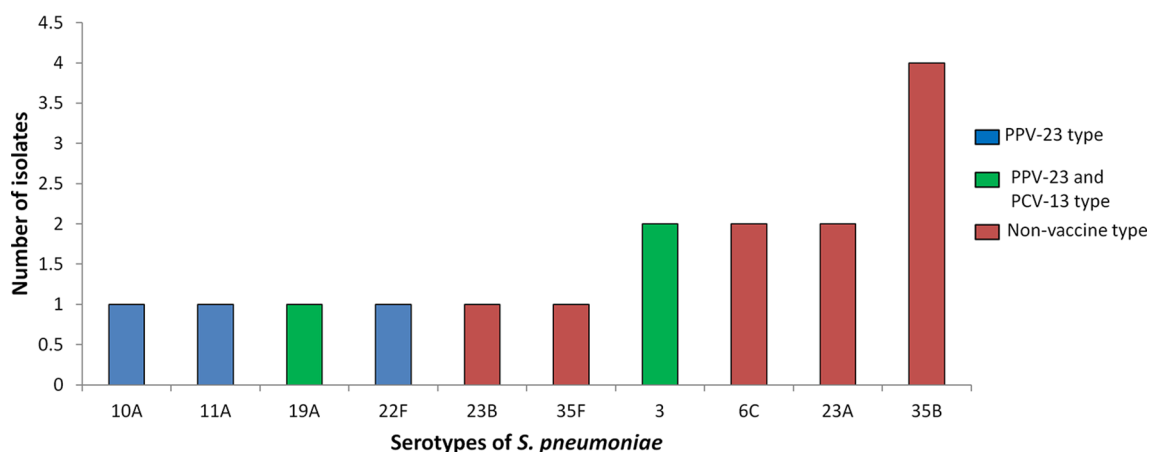


Figure 2 Serotypes of *Streptococcus pneumoniae* isolated from patients with COPD. COPD, chronic obstructive pulmonary disease. PCV-13, 13-valent pneumococcal conjugate vaccine; PPV-23, 23-valent pneumococcal polysaccharide vaccine.

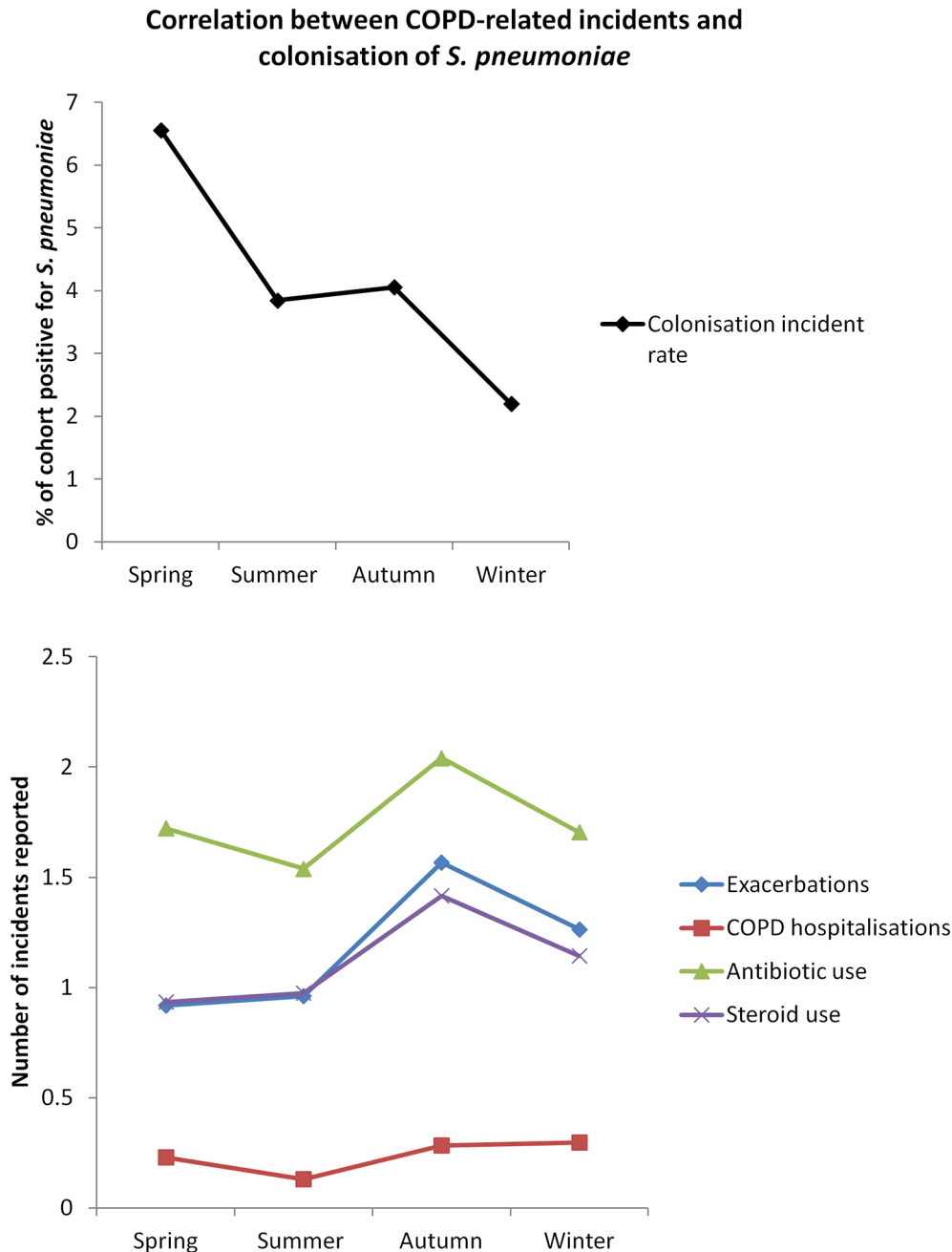


Figure 3 Correlations between colonisation-incident rate and mean number of antibiotic use, steroid use, exacerbations and COPD-related hospitalisations. Colonisation rate is presented as the percentage of isolates identified from all patients during each season.

DISCUSSION

Given the recent findings on the potential benefits of PCV-13 for preventing pneumococcal infection in adults as well as children¹⁵ we undertook a 12-month observational cohort study of 133 Irish patients with COPD. We sought to determine the colonisation rate of *S. pneumoniae* within this cohort, to identify the vaccine coverage of the cohort and to investigate if PCV-13 could potentially reduce infective exacerbation rates among these patients. Patients with COPD are at an increased risk of pneumococcal pneumonia.¹⁶ However, we identified a low colonisation-incident rate of 3.8% (n=16 *S. pneumoniae* isolates identified from 417 samples) within the study cohort and a low colonisation

rate of 8% (n=11/133 participants). No incidents of pneumonia were reported by those found to be colonised with *S. pneumoniae* during the study period.

Culture-based methods for detecting *S. pneumoniae* colonisation have previously reported similar rates of colonisation to those in this study, specifically a rate of 9% outside of periods of acute exacerbations.¹⁷ While the samples assessed by Rosell *et al* (2005) were bronchoscopic samples and not sputum samples, the methods of isolation of *S. pneumoniae* were similar to this study.¹⁷ Another study isolating *S. pneumoniae* by culture-based methods reported a carriage rate of 8% for adults ≥18 years of age which reflects the colonisation rate of this study.¹⁸ However, the

work of Hussain *et al* (2005) was conducted on a healthy adult population and *S. pneumoniae* was isolated from nasopharyngeal swabs.¹⁸ Colonisation rates that mirror the colonisation-incident rate of 3.8% observed in this study have been reported in other studies that used culture-based methods of detection of *S. pneumoniae*. Oropharyngeal samples assessed by culture methods revealed a colonisation rate of 4% with *S. pneumoniae* in adults >18 years of age¹⁹ while culture methods of detection also revealed colonisation rates of 2.3% for adults >60 years of age and a rate of nasal carriage in the elderly as low as 0.8%.²⁰ The work of Regev-Yochay *et al* (2004) however differs from this study in the cohort of adults assessed which was not specific to patients with COPD and in the samples used to detect *S. pneumoniae* which were nasopharyngeal.¹⁹ Furthermore, in contrast to our study design, Ridda *et al* (2010) used culture-based methods of detection to isolate *S. pneumoniae* from both oral and nasopharyngeal swabs whereas no *S. pneumoniae* was detected in oropharyngeal swabs during this study.²⁰ In addition, the study populations differ between this study and that of Ridda *et al* (2010); hospitalised elderly patients were assessed by Ridda *et al* (2010) whereas the majority of patients assessed in this study were recruited as outpatients (76%). Molecular-based methods of detection

were not incorporated into our sampling protocol as they are not part of the test schedule in our laboratory and this is a potential limitation of this study. While *S. pneumoniae* was not detected in any oropharyngeal swab samples and therefore all donors of oropharyngeal samples are classed as non-colonised participants based on the detection methods available, it is worth noting that evidence from studies conducted with molecular-based methods of detection indicate that this result could have been different had molecular-based detection methods been available. Several reports demonstrate the advantage of molecular methods for the detection of *S. pneumoniae* colonisation over conventional culture-based methods. Real time PCR has been reported to be superior to conventional culturing techniques for detecting *S. pneumoniae* in children and it has been reported that *S. pneumoniae* was detected at a rate of 54% by real time PCR in healthy children compared with 24% by culture-based methods.²¹ PCR-based methods of identification from pharyngeal swabs in broth enrichment culture yielded a colonisation rate of *S. pneumoniae* of 32.7% in adults >40 years of age.²² One study comparing the culture and molecular analysis of colonisation revealed that a colonisation rate of 10% could be detected in the elderly from culture methods but that qPCR analysis yielded rates

Table 2 Characteristics of patients with chronic obstructive pulmonary disease (COPD) for each season according to colonisation status for *Streptococcus pneumoniae* at any time period

	Colonised participants	Non-colonised participants	p Value
N	11	122	
Spring			
Number of COPD exacerbations	1 (1)	0.5 (2)	p>0.05
% with pneumonia	0%	9%	
Incidents of antibiotic use	0 (1)	1 (3)	
Incidents of steroid use	1 (1)	1 (2)	
Summer			
Number of COPD exacerbations	0 (1)	1 (2)	p>0.05
% with pneumonia	0%	3%	
Incidents of antibiotic use	0 (2)	1 (2)	
Incidents of steroid use	1 (1)	1 (2)	
Autumn			
Number of COPD exacerbations	1 (2)	1 (3)	p>0.05
% with pneumonia	0%	2%	
Incidents of antibiotic use	2 (2)	2 (2)	
Incidents of steroid use	1 (1)	1 (2)	
Winter			
Number of COPD exacerbations	0 (1)	1 (2)	0.06
% with pneumonia	0%	2%	p>0.05
Incidents of antibiotic use	0 (0.5)	1 (2)	*0.01
Incidents of steroid use	0 (0)	1 (2)	*0.01

Data presented as median (IQR) or % unless otherwise specified.
*significance at p=0.01 level.

of 28%, 11% and 5% from saliva, transoral and transnasal samples, respectively.²³

A very recent study describing *S. pneumoniae* colonisation in adults >65 years of age used molecular methods of detection and reported a predominance of PCV-13 serotypes within the cohort whereas this study revealed a predominance of NVT serotypes within the cohort.²⁴ The different findings between this study and that of Esposito *et al* (2016) could be due to regional variation and the lack of molecular detection methods used in this study. A factor that may be negatively impacting on the recovery of *S. pneumoniae* from the study volunteers could be the high rate of antibiotic use in this patient group; patients reported high antibiotic use between visits with the median number of courses of antibiotics prescribed in the period between visits ranging from one in the spring to two in the autumn.^{25–27} Antibiotic treatment has been shown to impair the diagnostic value of sputum for detecting *S. pneumoniae* by 34%.²⁸ A total of 72% of patients (n=302/420) reported antibiotic use during the period between their previous visit and their current visit when a sample for analysis was being taken.

In summary, the findings of this prospective cohort observational study demonstrate a low colonisation rate of *S. pneumoniae* among these Irish patients with COPD (3.8%). The majority of the isolates identified in the study cohort were NVT and the PCV-13 serotypes only accounted for 19% of isolates (n=3/16). The isolates identified as PCV-13 serotypes are also included in the PPV-23 which is the vaccine that is currently administered to this patient cohort.

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Contributors HH and RWC conceptualised the study and participated in its design with assistance from EMH and IS. HMC and MJ coordinated the study, conducted recruitment of patients and study visits and prepared samples for analysis. MC and MME conducted serotyping analysis. HMC analysed the data and IS and BC provided statistical analysis. HMC, HH and RWC drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests HH is in receipt of research funds from Astellas and has in the recent past received lecture or consultancy fees from Novartis, Astellas, Cepheid and Astra Zeneca. All other authors have no declarations to make.

Patient consent This is not required as all patients who consented to participation in the study were also consented to the study's publication.

Ethics approval Ethics (Medical Research) Committee of Beaumont Hospital in December 2013.

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Data sharing statement No additional data available.

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