Journal of Infection 89 (2024) 106277

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Bacteria and Bacterial Diseases

Carriage of *Streptococcus pneumoniae* in adults hospitalised with community-acquired pneumonia

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ARTICLE INFO

Article history:
Accepted 13 September 2024
Available online 19 September 2024

Keywords:
Streptococcus pneumoniae
Carrier state
Pneumonia
Serogroup
Pneumococcal vaccines

SUMMARY

Objectives: We aimed to determine the prevalence of and risk factors for nasopharyngeal and oral pneumococcal carriage in adults with community-acquired pneumonia (CAP), and the relationship between carried and disease-causing serotypes.

Methods: Between 2016 and 2018, nasopharyngeal swabs, oral-fluid, and urine were collected from hospitalised adults recruited into a prospective cohort study of CAP. Pneumococcal carriage was detected by semi-quantitative real-time PCR of direct and culture-enriched nasopharyngeal swabs and culture-enriched oral-fluid. *LytA* and *piaB* positive/indeterminate samples underwent semi-quantitative serotype/serogroup-specific real-time-PCR. Serotypes in urine were identified using a 24-valent serotype-specific urinary-antigen assay.

Results: We included 465 CAP patients. Nasopharyngeal carriage was detected in 34/103 (33.0%) swabbed pneumococcal pneumonia patients and oral carriage in 18/155 (12%) of sampled pneumococcal pneumonia patients. Concordance between nasopharyngeal/urine serotypes and oral/urine serotypes was 70.6% and 50% respectively. Serotypes 3 (26%, 22.2%), 8 (19.7%, 19.4%), non-typeable (11.6%, 13.9%) and 19A/F (7.5%, 8.3%) were most prevalent in urine and nasopharyngeal swabs respectively, with non-typeable (35%) and 15A/F (17%) most prevalent in oral-fluid. Pneumococcal carriage was significantly associated with pneumococcal pneumonia (nasopharyngeal adjusted odds ratio [aOR] 8.1, 95% confidence interval [CI] 3.8–17.2; oral aOR 5.5, 95% CI 2.1–13.3). All-cause CAP patients ≥65 years had lower odds of nasopharyngeal carriage (aOR 0.47, 95% CI 0.24–0.91) and current smokers had higher odds of oral carriage (aOR 2.69, 95% CI 1.10–6.60).

Conclusions: The association between nasopharyngeal carriage and pneumococcal CAP was strong. Adult carriage and disease from serotypes 8 and 19A may support direct protection of adults with PCV vaccines.

Introduction

Pneumococcal colonisation of the upper respiratory tract is usually asymptomatic, but is considered to be a precursor of pneumococcal disease.[1] Most studies of pneumococcal carriage have focused on children, in whom prevalence has been shown to be very high, with young children thought to be major transmitters of pneumococcus to the rest of the population.[1] Carriage rates decrease

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https://doi.org/10.1016/j.jinf.2024.106277

with age[2,3] but the prevalence of pneumococcal carriage in adults has been less well characterised than in children. Very low carriage rates in adults (< 5%) have been reported from studies in which culture-based methods have been used to ascertain the presence of pneumococci.[4,5] These methods are highly specific but potentially lack sensitivity to detect the presence of pneumococci. Recent studies employing molecular methods targeting conserved pneumococcal DNA sequences to detect pneumococci have indicated that adult carriage may have been underestimated.[6,7]

Most pneumococcal carriage studies have been conducted on healthy asymptomatic volunteers, in whom pneumococcal acquisition, carriage and clearance may occur frequently and for a variable duration.[8] There are few carriage data from patients who actually have pneumococcal pneumonia. One study found that pneumonia severity, symptom duration and serum immunoglobulin titres against the patient's serotype were associated with a high pneumococcal density,[9] but this study did not examine the relationship between carriage and presumed disease-causing serotypes.

With the recent licensure of new higher-valency pneumococcal conjugate vaccines (PCVs), it is important to monitor the evolution of pneumococcal serotypes in both children and adults. Surveillance of pneumococcal colonisation in patients with pneumonia could provide a means to monitor trends in pneumococcal serotypes carried in the wider population if the relationship between colonising and disease-causing serotypes can be established.

In this study we employed molecular methods to detect and serotype pneumococci from nasopharyngeal swabs and oral-fluid from patients hospitalised with all-cause community-acquired pneumonia. We aimed to determine the prevalence of pneumococcal carriage in these patients, to determine risk factors associated with carriage, and to investigate the relationship between nasopharyngeal and oral samples for the detection of pneumococcal carriage.

Methods

This pneumococcal carriage study was conducted between September 2016 and July 2018 on a subset of consecutive patients recruited for an ongoing prospective cohort study of pneumococcal serotypes in adults aged ≥16 years old hospitalised with community-acquired pneumonia (CAP) and who gave their consent to participate in this carriage study. Study details have been published separately[10] and are outlined in the supplement.

Microbiology

Nasopharyngeal swabs and oral-fluid were collected from consented patients within 48 h of admission for molecular detection of pneumococcal carriage and serotyping. Urine samples were obtained within 48 h of admission for pneumococcal antigen detection using the Binax-NOW® (Alere, Stockport, UK) assay for pneumococcal C-polysaccharide urinary antigen detection (UAD) and for pneumococcal serotype-specific urinary antigen testing (Bio-Plex24 assay). (Full details of methods in online supplement.) Blood cultures were conducted at the discretion of the clinical team.

Patients were considered to have pneumococcal CAP if they met ≥1 criteria: a) positive pneumococcal Binax-NOW or b) *Streptococcus pneumoniae* in blood culture or c) detection of a pneumococcal serotype or cell-wall polysaccharide in urine by the Bio-Plex24 assay.

Statistical analysis

Baseline characteristics were compared for nasopharyngeal and oral pneumococcal carriers versus non-carriers, and for pneumococcal versus non-pneumococcal CAP. Categorical variables were compared using Pearson's $\chi 2$ or Fisher's exact tests, and the Mann-Whitney U-test was used for non-parametric continuous variables. The independent association between demographic and risk factors for pneumococcal disease with carriage and pneumococcal pneumonia was compared using multivariable logistic regression, adjusted for age, sex, and smoking status. Serotypes were classified according to the serotype content of pneumococcal vaccines: PCV13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), PCV20non13 serotypes (8, 10A, 11A, 12F, 15B, 22F, 33F), PPV23non20 serotypes (2, 9N, 17F, 20), non-vaccine types (NVT)(any non-PCV13/20, non-PPV23 serotypes, or non-typeable serotype in which cell-wall polysaccharide was detected but the Bio-Plex24 assay was not able to generate a serotype-specific result, or the serotype was not targeted on PCR of nasopharyngeal or oral samples), PCV21 serotypes (3, 6A, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19A, 20, 22F, 23A, 23B, 24F, 31, 33F, 35B), or 'untyped' disease in which Binax-NOW was positive without subsequent serotype identification.

Statistical analyses were conducted using Stata/SE V.17.0.

Ethics approval

The work was approved by Nottingham Research Ethics Committee (REC reference 08/H0403/80).

Results

Study population

We included 465 patients hospitalised with CAP between September 2016 and July 2018. The median age of the cohort was 58.3 years (IQR 54.5–78.7) and 228 (49.2%) were male. A diagnosis of pneumococcal pneumonia was made in 182 (39.1%) patients.

Fifteen patients (8.2%) with pneumococcal pneumonia were bacteraemic. A serotype was determined from 13 blood culture isolates, with serotype 8 being most frequent (7/13, 53.8%). Other serotypes identified were 3, 11A, 12F, 19A, 22F and 35B (1 each, 7.7%).

Urinary Bio-Plex24 assay was performed on samples from 362 of 465 patients (77.8%), of which 156 (43.1%) were positive. Two serotypes were detected in 13 (8.3%) and 3 serotypes in 2 (1.3%) positive samples. Serotype 3 was most frequent (45 of 173 total serotypes, 25%), followed by serotype 8 (34/173, 19.6%).

Pneumococcus was detected in either or both nasopharyngeal swab and oral-fluid in 61/465 (13.1%) patients with all-cause pneumonia; in 45/182 (24.7%) of those with pneumococcal CAP versus 16/283 (5.6%) of those with non-pneumococcal CAP ($p < 0.001$).

Nasopharyngeal carriage

Nasal carriage was significantly associated with pneumococcal pneumonia compared to non-pneumococcal CAP (adjusted OR (aOR) 8.09, 95% confidence intervals (CI) 3.81–17.2, $p < 0.001$).

A nasopharyngeal swab was obtained from 103 of 182 (56.5%) pneumococcal pneumonia patients, with detection of nasal carriage in 34 (33.0%) (supplemental figure 1). Serotypes 3, 8, non-typeable and 19A/F accounted for the greatest proportion of serotypes in both urine and nasopharyngeal swabs (ST3: 26%, 22.2%; ST8: 19.7%, 19.4%; non-typeable 11.6%, 13.9%; 19A/F 7.5%, 8.3% respectively)(Fig. 1).

Concordance was 91.7% between blood culture and urine serotypes, and 42.9% between blood culture and nasopharyngeal serotypes. The same serotypes were identified in urine and nasopharyngeal swab in 70.6% (24/34) of patients in whom a serotype was detected in both samples. (Table 1) The association between blood culture isolates and those detected in nasopharyngeal, oral-fluid and urine samples is also shown in Fig. 1 and online supplemental figures 2 and 3).

PCV13-serotypes accounted for 30.6% of nasopharyngeal pneumococci, with a further 38.8% being a PCV20-serotype not included

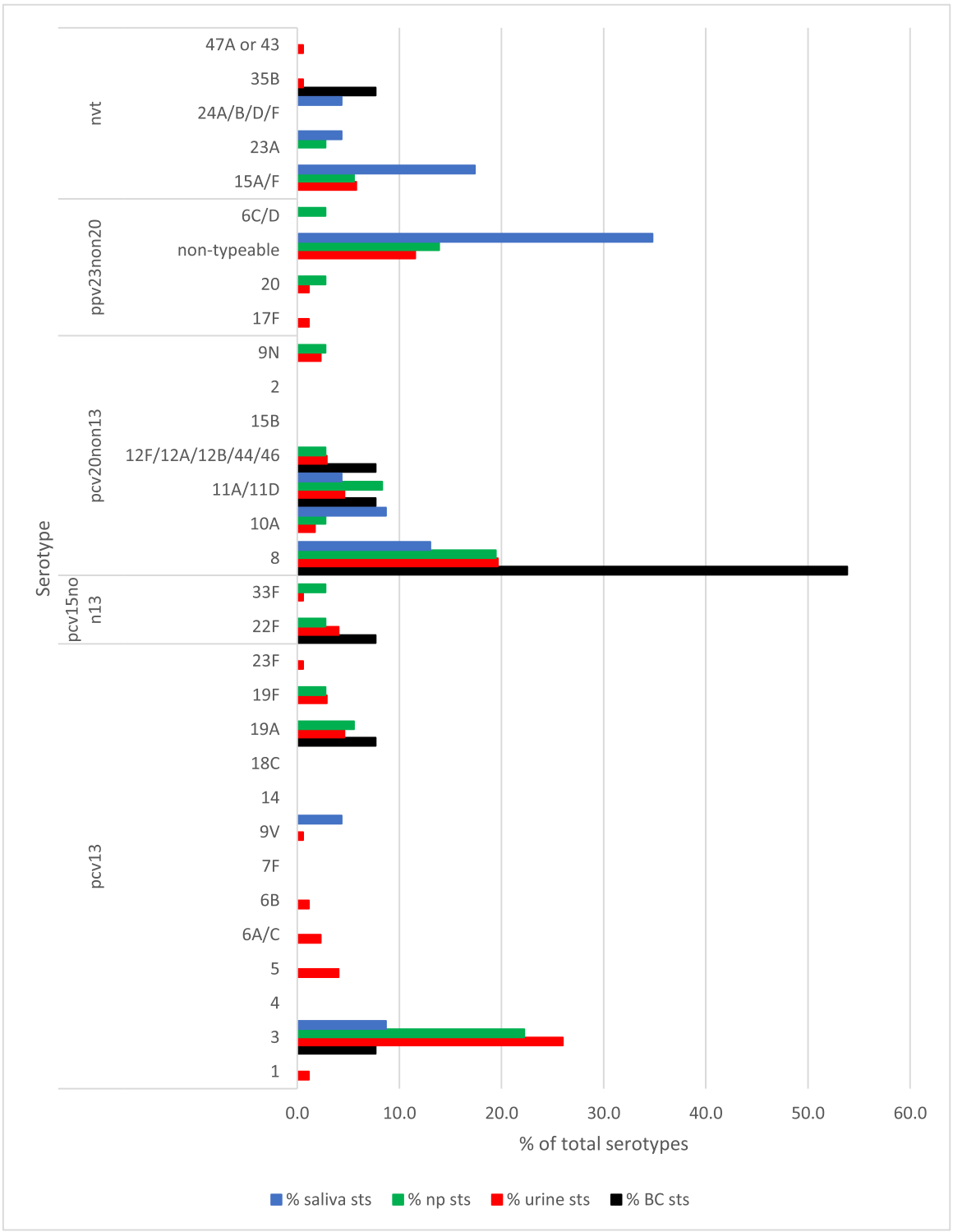

Fig. 1. Distribution of pneumococcal serotypes in blood culture, urine, nasopharyngeal swabs and oral-fluid in patients with pneumococcal pneumonia (N = 182), grouped according to pneumococcal vaccine group. Each column represents the % of each serotype in positive samples.

in PCV13, 5.6% an additional PPV23-serotype, and 25% a non-PCV13/20-serotype type. PCV21 serotypes made up 80.6% of nasopharyngeal serotypes (Table 2).

Twelve of 188 swabbed patients with non-pneumococcal pneumonia were nasal carriers (6.4%), two of whom also had *S. pneumoniae* detected in their oral-fluid. Four patients (1.4%) with non-pneumococcal pneumonia were oral carriers only.

Risk factors for nasopharyngeal pneumococcal carriage

The median age of nasopharyngeal carriers with all-cause pneumonia was 62.6 years (IQR 50.5–74.4 years), compared to 68.8 years (IQR 54.4–78.9 years, p = 0.08) in non-carriers. Older people aged ≥65 years had lower odds of nasopharyngeal carriage compared to younger people (aOR 0.47, 95% CI 0.24–0.91, p = 0.02). There was

Table 1
Concordance between pneumococcal serotypes detected in blood culture, urine, nasopharyngeal swabs and oral-fluid.

	BioPlex24	Nasopharyngeal	Oral fluid
Blood culture	11/12[a] (91.7%)	3/7 (42.9%)	0/13 (0%)
BioPlex24		24/34 (70.6%)	9/18 (50%)
Nasopharyngeal			5/6 (83.3%)

The denominator in each cell is the number of patients in whom a serotype was detected in both sample types, and the numerator is the number of patients with a concurrent serotype.
[a] One 'non-concurrent' serotype 35B in blood culture and non-BioPlex24 serotype in urine.

no significant difference between current smokers and non-smokers for nasopharyngeal carriage (aOR 1.38, 95% CI 0.67–2.82, p = 0.38) (Table 3). Excluding patients with a negative urine BioPlex24 assay (n = 130) did not significantly affect the observed associations.

See online supplement for results comparing microbiological methods for nasopharyngeal swabs.

Oral carriage and risk factors

Oral-fluid was collected from 461 patients with all-cause pneumonia. Culture-enrichment was performed on 410 (88.9%) samples, with pneumococcus detected in 24 (5.8%). Two serotypes were detected in one sample, and five in another. Non-typeable pneumococci were most prevalent and detected in 11 (37.9%) positive culture-enriched samples, followed by serotype 15A/F (3/29, 17.2%), serotypes 8 and 10A (3/29, 10.3% each), and serotype 3 (2/29, 6.9%) (Fig. 1).

Culture-enrichment was performed in 155 oral–fluid samples from 182 patients with pneumococcal pneumonia (85.2%), with detection of oral carriage in 18 samples (11.6%). Oral carriage was significantly associated with pneumococcal pneumonia compared to non-pneumococcal pneumonia (11.6% versus 2.4%; aOR 5.48, 95% CI 2.09–14.4, p = 0.001), with non-typeable pneumococci most prevalent (34.8%), followed by serotypes 15A/F (17.4%), 8 (13.0%), 10A and 3 (8.7% each) (Fig. 1). Oral carriage was not detected in any bacteraemic patients. Concordance between serotypes detected in oral fluid and urine was 50% (9/18), and 83.3% (5/6) between oral and nasopharyngeal serotypes (Table 1).

The median age of oral carriers with all-cause pneumonia was 67.7 years (IQR 58.3–75.4) versus 66.9 years (IQR 53.0–77.6 years, p = 0.74) in non-carriers. Compared to non-smokers, current smokers were significantly more likely to be oral carriers (aOR 2.69, 95% CI 1.10–6.60, p = 0.03) (Table 4). Excluding patients with a negative urine BioPlex24 assay (n = 189) did not significantly affect the observed associations.

The relationship between nasopharyngeal and oral samples is outlined in the online supplemental results.

Discussion

To our knowledge, this is the first study of pneumococcal carriage in adults admitted to hospital with CAP in which carriage serotypes from nasopharyngeal and oral samples have been compared to presumed disease-causing serotypes identified using a serotype-specific urinary antigen detection (BioPlex24). Using PCR methods to detect pneumococcal serotypes in nasopharyngeal and oral-fluid samples we noted that nasopharyngeal carriage was detected in a third of adults admitted with pneumococcal pneumonia who were swabbed, and that there was a strong association between carriage and pneumococcal pneumonia, especially for younger adults.

Notably we found good concurrence between the serotypes detected in blood cultures, urine and nasopharyngeal carriage. Conversely, the association between oral serotypes and those causing clinical disease was less apparent and serotypes in bacteraemic disease were not detected in any of the corresponding culture-enriched oral samples.

Our oral carriage rate is lower than the 18% rate reported in a study of older community-dwelling adults with influenza-like illness,[11] and our combined prevalence of 25% was lower than the 36% carriage at ILI onset in an earlier study.[7] This may be due to differences in sampling techniques and detection methods, or population differences. We only included hospitalised patients so antibiotics given at admission may have affected carriage prevalence. Alternatively, patients in the ILI studies may have been infected with respiratory viruses, and viral co-colonisation has been associated with increased pneumococcal acquisition and density.[12]

The majority of carriage studies have been conducted in children, in whom carriage rates have generally been shown to be high, particularly in infants and young children (40–90%).[13,14] Studies in adults which relied on conventional culture for detection of pneumococcus have reported much lower carriage rates compared to children (< 1%–5%).[3,4,15] The addition of molecular methods has been shown to increase the number of pneumococcal detections,[7,16] with a further significant increase when culture-enriched samples are tested compared to direct testing of samples only.[17] Determination of which samples will provide the best data for surveillance of carriage in the future requires further clarification; oral-fluid is easier to obtain than a properly taken nasopharyngeal swab, which can be uncomfortable for the individual, and trans-orally collected samples have been reported to have superior sensitivity for the detection of pneumococcal carriage compared to trans-nasal samples by some.[17] However, other studies have found higher carriage rates in the nasopharynx than oral-fluid,[8] which concurs with our findings. This discrepancy may be due to our use of oral-fluid rather than

Table 2
Percentage of serotypes for each sample type grouped by potential coverage in PCV and PPV pneumococcal vaccines.

Vaccine serotypes	Sample type					
	Urine/blood culture STs (N = 173)		NP STs (N = 36)		Oral fluid STs (N = 23)	
	n	% coverage (95% CI)	n	% coverage (95% CI)	n	% coverage (95% CI)
PCV13[a]	75	43.3 (43.1–43.5)	11	30.6 (30.1–31.1)	3	13.0 (12.6–13.4)
PCV20-nonPCV13[b]	58	33.5 (33.3–33.7)	14	38.8 (38.4–39.4)	6	26.1 (25.5–26.7)
PPV23-nonPCV[c]	8	4.6 (4.5–4.7)	1	2.8 (2.6–3.0)	0	0.0
PCV21[d]	134	77.5 (77.3–77.7)	29	80.6 (80.2–81.0)	14	60.9 (60.3–61.5)
Non-vaccine serotypes[e]	32	18.4 (18.3–18.7)	9	25.0 (24.5–25.4)	14	60.9 (60.3–61.5)

[a] PCV13 serotypes: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F.
[b] PCV20-nonPCV13 serotypes (additional serotypes in PCV20 but not in PCV13): 8, 10A, 11A, 12F, 15B, 22F, 33F.
[c] PPV23- nonPCV serotypes (additional serotypes in PPV23 but not PCV13/PCV20: 2, 9N, 17F, 20).
[d] PCV21 serotypes: 3, 6A, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19A, 20, 22F, 23A, 23B, 24F, 31, 33F, 35B (N.B. includes serotypes which are classified as 'non-vaccine serotypes' using the classification below).
[e] Non-vaccine serotypes: any non-PCV13/20, non-PPV23 serotype and non-typeable serotypes.

Table 3
Table of association between demographic and clinical factors with nasopharyngeal pneumococcal carriage.

	Nasopharyngeal Carriage, n = 46 (%)	Nasopharyngeal Carriage not detected, n = 245 (%)	OR (95%CI)	p-value	aOR[a] (95% CI)	p-value
Median age (IQR)	62.6 (50.5–74.4)	68.8 (54.4–78.9)	-	0.08	-	-
Age:						
≥65 y	17 (37.0)	139 (56.7)	0.45 (0.23–0.86)	**0.02**	0.47 (0.24–0.91)	**0.02**
Sex:						
Male	23 (50.0)	121 (49.4)	0.98 (0.52–1.85)	0.96	1.07 (0.56–2.03)	0.84
Current Smoker	14 (30.4)	56 (23.1)	1.45 (0.72–2.91)	0.29	1.38 (0.67–2.82)	0.38
Child contact	22(47.8)	128 (52.9)	0.82 (0.43–1.53)	0.53	0.72 (0.37–1.38)	0.32
Received PPV23	14 (30.4)	80 (34.2)	0.87 (0.44–1.73)	0.69	0.99 (0.47–2.12)	0.99
Number of co-morbidities[b]						
0	24 (52.3)	130 (53.3)	Ref	0.64[c]	Ref	0.32[c]
1	14 (30.4)	59 (24.2)	1.28 (0.62–2.66)		1.51 (0.62–3.36)	
2	6 (13.0)	37 (15.2)	0.88 (0.33–2.31)		1.16 (0.39–3.40)	
≥3	2 (4.3)	18 (7.4)	0.60 (0.13–2.76)		0.81 (0.16–4.01)	
Pneumococcal Clinical Risk group[d]	20 (43.5)	102 (41.6)	1.08 (0.57–2.04)	0.82	1.33 (0.64–2.78)[e]	0.44
CURB-65						
0–1 (low)	29 (63.0)	133 (55.0)	Ref	0.28	Ref	0.35
2 (moderate)	12 (26.1)	71 (29.3)	0.78 (0.37–1.61)		0.82 (0.39–1.72)	
≥3 (high)	5 (10.9)	38 (15.7)	0.60 (0.22–1.66)		0.61 (0.71–2.95)	

[a] Adjusted for age, sex and smoking status.
[b] Number of co-morbidities includes malignancy, liver disease, renal disease, cerebrovascular disease, chronic lung disease, chronic heart disease and immunosuppression (immunosuppressive medications, previous solid organ transplant, previous bone marrow transplant, splenic dysfunction, neoplastic disease with active treatment in last 6 months, haematological malignancy, primary immunodeficiency, HIV).
[c] *P*-value for trend.
[d] Pneumococcal clinical risk group includes everyone aged ≥65 years, plus anyone aged 16–64 years with one or more of the following clinical risk factors: chronic respiratory disease, chronic heart disease, chronic liver disease, chronic kidney disease, diabetes requiring treatment, immunosuppression as defined above), CSF leak or cochlear implant).
[e] Adjusted for sex and smoking status.

oropharyngeal swabs and we only included the results of culture-enriched detection as we found high background noise and low specificity for direct oral-fluid PCR. The concern of non-specific results from molecular methods due to the presence of streptococci which may carry capsular pneumococcal genes has been noted by others, particularly from oral samples. No genetic target is universally present in *S pneumoniae*, but the use of a dual-target qPCR approach can improve the accuracy of carriage detection and the presence of multiple serotypes which is challenging using only culture.[18,19]

Carriage is considered to be the primary reservoir and a pre-requisite for pneumococcal disease.[1] Increased carriage density has been found to be a risk factor for pneumococcal pneumonia in adults with radiologically confirmed CAP,[9] and in patients co-infected with influenza or HIV.[20,21] Although such studies are not proof of causality, high pneumococcal carriage density in the upper respiratory tract may facilitate invasion and micro-aspiration of bacteria to the lower respiratory tract and increase the likelihood of disease.[22]

The burden of pneumococcal pneumonia in adults is recognised to be greatest in the elderly. However, we found lower nasopharyngeal

Table 4
Table of association between demographic and clinical factors with oral pneumococcal carriage.

	Oral Carriage, n = 24 (%)	Oral Carriage not detected, n = 386 (%)	OR (95%CI)	p-value	aOR[a] (95% CI)	p-value
Median age (IQR)	67.7 (58.3–75.4)	66.9 (53.0–77.6)	-	0.74	-	-
Age:						
≥65 y	14 (58.3)	207 (53.6)	1.21 (0.52–2.79)	0.65	1.38 (0.58–3.24)	0.46
Sex:						
Male	11 (45.8)	192 (50.0)	1.18 (0.52–2.70)	0.69	1.33 (0.57–3.11)	0.51
Current Smoker	10 (41.7)	91 (23.8)	2.28 (0.98–5.32)	0.06	2.69 (1.10–6.60)	**0.03**
Child contact	8 (33.3)	192 (50.9)	0.48 (0.20–1.15)	0.10	0.46 (0.19–1.15)	0.10
Received PPV23	4 (17.4)	121 (33.2)	0.42 (0.14–1.28)	0.13	0.41 (0.13–1.30)	0.13
Number of co-morbidities[b]						
0	10 (41.7)	191 (49.7)	Ref	0.93[c]	Ref	0.90[c]
1	10 (41.7)	106 (27.6)	1.81 (0.73–4.49)		1.58 (0.59–4.25)	
2	3 (12.5)	61 (15.9)	0.93 (0.25–3.50)		0.78 (0.19–3.23)	
≥3	1 (4.2)	28 (7.3)	0.68 (0.08–5.50)		0.64 (0.07–5.71)	
Pneumococcal Clinical Risk group[d]	10 (41.7)	172 (44.6)	1.11 (0.46–2.67)	0.81	1.28 (0.52–3.13)[e]	0.59
CURB-65						
0–1 (low)	12 (50.0)	213 (55.9)	Ref	0.70[c]	Ref	0.75[c]
2 (moderate)	8 (33.3)	105 (27.6)	1.35 (0.54–3.41)		1.44 (0.56–3.69)	
≥3 (high)	4 (16.7)	63 (16.5)	1.13 (0.35–3.62)		1.21 (0.37–3.95)	

[a] Adjusted for age, sex and smoking status.
[b] Number of co-morbidities includes malignancy, liver disease, renal disease, cerebrovascular disease, chronic lung disease, chronic heart disease and immunosuppression (immunosuppressive medications, previous solid organ transplant, previous bone marrow transplant, splenic dysfunction, neoplastic disease with active treatment in last 6 months, haematological malignancy, primary immunodeficiency, HIV).
[c] *P*-value for trend.
[d] Pneumococcal clinical risk group includes everyone aged ≥65 years, plus anyone aged 16–64 years with one or more of the following clinical risk factors: chronic respiratory disease, chronic heart disease, chronic liver disease, chronic kidney disease, diabetes requiring treatment, immunosuppression as defined above), CSF leak or cochlear implant).
[e] Adjusted for sex and smoking status.

carriage in people aged ≥65 years, which concurs with other studies which have found only infrequent colonisation in the older age group.[23] With age, the number and/or functionality of pneumococcal receptors in the nasopharyngeal epithelium declines[3,24]; the apparently paradoxical positive association of pneumonia with older age could be due to immunosenescence in older people[25] resulting in disease being instigated at a lower carriage density than in younger people.

Almost one-third of nasopharyngeal events, and nearly 45% of combined urine and blood culture serotypes were attributed to PCV13-vaccine serotypes, despite the introduction of the vaccine into the national immunisation programme for children several years prior to the study period. An additional 40% of nasopharyngeal serotypes would be covered by PCV20, and PCV21 vaccine would cover almost 81% of nasopharyngeal serotypes. We observed that serotype 8 was the second most prevalent nasopharyngeal carriage serotype, although interestingly this serotype is rarely carried in children.[14,26] This implies that the use of carriage data from children may be limited in forecasting changes in the epidemiology of pneumococcal disease in adults, and that serotype 8 may be an example of a serotype transmitted directly among adults, bypassing children.[27] Despite PCV13 immunisation in children, two PCV13 serotypes, 3 and 19A, were found to persist in adult nasopharyngeal carriage. Direct and indirect protection against serotype 3, the most prevalent nasopharyngeal and urine serotype, is already recognised to be lower than for other serotypes.[28–30] Carriage of persistent serotype 19A in children in the post-PCV era has been described in children,[26] and we have now confirmed carriage of this serotype in adults. The clonal distribution of serotype 19A has been found to be diverse among countries and regions, regardless of PCV used. Persistence of serotype 19A may therefore be related to clonal switching events and the emergence of clones with diverse antibiotic resistance, highlighting the need for active surveillance and molecular studies to understand its genetic changes and evolution.[31]

A strength of our study is that using both molecular methods and the Bio-plex24 assay permitted us to study the relationship between carriage serotypes and those implicated in disease in a large cohort of hospitalised patients. We acknowledge that there are some limitations to our study. Firstly, we only collected samples from a single city and at one time point so our results may not be generalizable to other locations or to patients who are in the recovery phase of their illness. Also, due to logistical reasons, not all included patients were able to provide urine or nasopharyngeal swabs, although the majority provided oral-fluid. We made the assumption that carriage was the precursor to infection, as is commonly assumed,[1] although we cannot exclude the possibility that we were detecting carriage secondary to lower respiratory tract infection as we did not sample patients prior to their admission. Also, all our patients were hospitalised and unwell so we did not have any healthy controls with whom to compare carriage rates and serotype distribution. The current urinary Bioplex24 assay has been shown to have a sensitivity of 94.3% and specificity of 93.6% based on isolation of *S pneumoniae* from a normally sterile site,[32] so it is possible that some patients were misclassified as pneumococcal CAP if we were detecting carriage serotypes in urine rather than those genuinely causing pneumonia. Misclassification would have been a greater concern had the study been conducted in children, in whom carriage rates are potentially much higher[13,14]; also the concordance between blood cultures and the Bio-Plex24 suggest that this is probably not the case, and additionally not all patients in whom carriage was detected had a positive Bio-Plex24 assay. Finally, as we only included the results of culture-enriched oral-fluid it is possible that we underestimated the true prevalence of oral carriage.

Implications

First, the use of higher-valency PCVs in adults to provide direct protection should be considered, especially for serotype 8 which has a large disease burden in adults and is possibly not related to carriage in children reducing the prospect of indirect herd protection from a childhood vaccination programme.

Secondly, we suggest that using nasopharyngeal swabs to detect carriage together with the urinary Bio-plex24 assay to detect disease-causing serotypes may be useful tools to monitor the changing epidemiology of carriage and pneumococcal pneumonia and the impact of new vaccines in adults. The detection of a greater range of serotypes from nasopharyngeal swabs is an additional advantage for post-vaccine surveillance. Based on our results, we believe that oral-fluid samples may be less reliable for this purpose and that further work is required to explore their feasibility.

In conclusion, nasopharyngeal and oral pneumococcal carriage were associated with pneumococcal CAP and the serotypes detected in nasopharyngeal swabs followed a similar pattern to that seen in patients with pneumococcal pneumonia. Surveillance of nasopharyngeal carriage may therefore be a useful means of monitoring trends in serotypes implicated in pneumococcal disease. In contrast, the usefulness of oral-fluid sampling for determination of pneumococcal carriage requires further exploration.

Funding

This study is independent research supported by the Nottingham National Institute for Health Research Biomedical Research Centre (NIHR BRC) arising from an unrestricted investigator-initiated research grant from Pfizer (Grant award number: 53232135). The study concept was developed and agreed by the authors with no input from the funding bodies; Pfizer had no part in the design or execution of the study, the analysis and interpretation of the results, the writing of this manuscript or the decision to submit for publication. The data are the sole responsibility of the authors and the sponsor for the study was Nottingham University Hospitals NHS Trust.

Author contributions

WSL and CS were responsible for study conception and design. HL, HP, VB, RCE-P, DA, PD, TB, CR, DL, SE, HP and SL were responsible for data acquisition and analysis. TM, LL and CT were responsible for the statistical analysis. LL and WSL drafted the initial version of the article. All authors contributed to data interpretation and read, commented on and approved the final version of the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: Wei Shen Lim reports unrestricted investigator-initiated research funding from Pfizer to WSL's institution for an unrelated study of pneumonia in which WSL is the Chief Investigator.WSL reports research funding from NIHR for a multi-centre clinical trial of aspirin in community acquired pneumonia in which WSL is a co-applicant. WSL is Deputy Chair of the Joint Committee of Vaccination and Immunisation (JCVI)(unpaid), and unpaid Chair of the NIHR Respiratory-Translational Research Centre's Acute Respiratory Infection National Strategy Research Group. Caroline Trotte participated in a CMV vaccine advisory board meeting in May 2022, unrelated to the topic of this paper. Seyi Eletu declares participation in a Virtual Advisory Board for a pneumococcal project organised by

Sanofi Pasteur SA unrelated to the submitted work. The UK Health Security Agency (UKHSA) received monies from the University of Nottingham from an unrestricted grant from Pfizer for this work. The Vaccine Preventable Bacteria Section of UKHSA has received grants from Pfizer for investigator led research unrelated to the current project. The UKHSA provides vaccine manufacturers (GlaxoSmithKline (GSK), MSD, Pfizer) with post marketing surveillance reports on vaccine-preventable disease, including pneumococcal infections for which a cost recovery charge is made and which is unrelated to the submitted work.

No other authors declare competing interests.

Acknowledgements

The authors would like to thank Gemma Clarke and colleagues from the Department of Clinical Microbiology, Nottingham University Hospitals NHS Trust for processing urine samples using the BinaxNOW assay; all the clinicians, research nurses and staff of Nottingham University Hospitals NHS Trust who recruited patients, collected samples and collected and entered data; the Nottingham Pneumonia Patients and Public Involvement group for their ongoing input into respiratory infection research, and all participants who gave their consent for the study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106277.

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