Supplemental Materials

Pneumococcal pneumonia trends in adults hospitalised with community-acquired pneumonia over 10 years (2013-2023), and the role of serotype 3

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Methods

Acute admissions were screened for study eligibility each weekday and reviewed within 48 hours of admission by the study team. Eligibility for inclusion in the study was defined as patients aged ≥16 years presenting with ≥1 symptoms associated with a lower respiratory tract infection (cough, increasing dyspnoea, sputum production and/or fever), with acute abnormalities consistent with infection on a chest radiograph taken within 48 hours of admission, and treated as CAP. Exclusion criteria were hospitalisation within ten days of the index admission, or diagnosis of post-obstructive pneumonia secondary to lung cancer. For patients lacking capacity, proxy consent was sought from their personal representative. A standard proforma was used to collect demographic and clinical information through direct questioning of the patient or their representative, medical notes review, and electronic data resources for imaging, biochemistry and microbiology results. Readmission and mortality data were collected from electronic resources up to 30 days following discharge. Pneumonia severity was assessed using the CURB-65 mortality risk assessment score (low severity ≤1, moderate =2, high severity ≥3).

Multiple Imputation for missing data

The percentage of missing values ranged from 0% up to 4.2% for PPV23 receipt. 92.8%% of the 5186 patients in the cohort were available for analysis under the traditional listwise deletion method. To assess for evidence of bias in those who were excluded from whole case analysis due to incomplete records, we used multiple imputation including all analysis variables for each outcome under the assumption that data are missing at random. Stata 17's 'mi impute chained' command generated five imputed datasets. Analyses run on each

dataset were pooled according to Rubin's rules. Imputed result compared reasonably to observed values and results using listwise deletion.

Bioplex-24 assay

The Bio-Plex24 assay uses human monoclonal antibodies (mAbs) to detect the pneumococcal serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F and pneumococcal cell-wall polysaccharide.[i] As 16 of the mAbs used in the Bio-Plex24 assay exhibit a degree of cross-reactivity with non-targeted pneumococcal serotypes, a checkerboard system was used to interpret results and allow identification. Where alternative serotypes were reported, the final serotype designation was based upon the predominant serotype observed in national IPD surveillance data for the corresponding time-period using a probabilistic approach.

[i] Eletu SD, Sheppard CL, Rose S, Smith K, Andrews N, Lim WS, Litt DJ, Fry NK. Re-validation and update of an extended-specificity multiplex assay for detection of *Streptococcus pneumoniae* capsular serotype/serogroup-specific antigen and cell-wall polysaccharide in urine specimens. Access Microbiol. 2020 Jan 28;2(3):acmi000094. doi: 10.1099/acmi.0.000094. PMID: 32974571; PMCID: PMC7470314.

Serotypes in Pneumococcal Vaccines

PCV7: 4, 6B, 9V, 14, 18C, 19F, 23F

PCV13: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F

PCV15: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F

PCV20: 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F

PCV21: 3, 6A, 7F, 8, 9N, 10A, 11A, 12F, **15A, 15C, 16F**, 17F, 19A, 20, 22F, **23A**, **23B**, 24F, 31, 33F, 35B

PPV23: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F

	OR (95% CI)	p-value	aOR* (95% CI)	p-value
Age category **16-49 years** **50-64 years** **65-74 years** **75-84 years** **≥85 years**	Ref 1.09 (0.91-1.30) 1.18 (0.99-1.41) 0.90 (0.75-1.07) 0.98 (0.81-1.20)	**0.19**	Ref 1.07 (0.89-1.28) 1.14 (0.94-1.38) 0.91 (0.75-1.10) 1.05 (0.85-1.30)	0.61
Sex, male	1.07 (0.96-1.19)	**0.24**	1.06 (0.95-1.19)	0.28
PPV23 vaccine receipt	1.08 (0.96-1.21)	0.20	1.11 (0.98-1.26)	0.12
Smoking **Never** **Ex** **Current**	Ref 1.06 (0.93-1.21) 1.41 (1.21-1.65)	**<0.001**	Ref 1.01 (0.889-1.16) 1.36 (1.16-1.60)	**0.001**
Liver disease	0.96 (0.63-1.46)	0.85	0.90 (0.59-1.37)	0.61
Chronic kidney disease	1.08 (0.90-1.30)	**0.42**	1.14 (0.95-1.38)	0.16
Chronic cardiac failure	0.94 (0.76-1.18)	0.62	1.00 (0.80-1.26)	0.99
Ischaemic heart disease	0.87 (0.74-1.03)	**0.12**	0.89 (0.74-1.06)	0.19
COPD	1.21 (1.06-1.37)	**0.004**	1.18 (1.02-1.36)	0.02
Asthma	1.05 (0.88-1.24)	0.61	1.12 (0.94-1.33)	0.22
Diabetes	0.96 (0.83-1.11)	0.60	0.98 (0.84-1.14)	0.77
Cerebrovascular disease	1.00 (0,82-1.23)	0.96	1.05 (0.85-1.29)	0.66
Cognitive impairment	0.78 (0.58-1.07)	0.13	0.83 (0.60-1.14)	0.25
Immunosuppression	1.30 (1.00-1.69)	**0.05**	1.32 (1.01-1.72)	**0.04**
Number of clinical risk factors **0** **1** **2** **≥3**	 Ref 1.07 (0.94-1.22) 1.07 (0.91-1.26) 1.08(0.88-1.34))	 0.38	 Ref 1.01 (0.87-1.17) 1.06 (0.87-1.28 1.15 (0.88-1.51)	 0.34

Supplementary Table 1: Unadjusted and adjusted odds ratios with 95% confidence intervals from uni- and multivariable logistic regression models for patient characteristics for patients with pneumococcal pneumonia (N=2193) compared to patients with non-pneumococcal pneumonia (n=2993)

*Adjusted for age, PPV receipt, smoking, IHD, COPD, cognitive impairment and immunocompromise.

	OR (95% CI)	p-value	aOR (95% CI)	p-value
CURB-65 score	1.12 (1.02-1.24)	**0.02**	1.16 (1.08-1.26)*	**<0.001**
Critical care admission	1.40 (1.14-1.72)	**0.001**	1.36 (1.10-1.66)**	**0.004**
30–day mortality	0.68 (0.55-0.84)	**<0.001**	0.64 (0.52-0.79)**	**<0.001**
30-day readmission	0.86 (0.72-1.03)	0.10	0.84 (0.70-1.00)**	0.06

Supplementary Table 2: Unadjusted and adjusted odds ratios with 95% confidence intervals from uni- and multivariable logistic regression models for patient outcomes for patients with pneumococcal pneumonia (N=2193) compared to patients with non-pneumococcal pneumonia (n=2993)

*Adjusted for age

** Adjusted for age, CURB65

Patient characteristic	Unadjusted coefficient of slope (95% CI)	p-value	Adjusted coefficient of slope	p-value
Age	0.069 (-2.904 – 3.043)	0.96	2.082 (-0.705 – 4.869)[a]	0.14
Sex (% male)	-0.052 (-0.137 – 0.033)	0.23	-0.054 (-0.141 – 0.032)[a]	0.22
PPV23 receipt (%)	-0.118 (-0.202 – -0.034)	0.006	-0.097 (-0.180 – -0.015) [a]	**0.02**
Outcome				
30 day mortality (%)	0.042 (0.000 – 0.084)	0.05	0.054 (0.013 – 0.095) [b]	**0.02**
Readmission (%)	0.007 (-0.050 – 0.065)	0.80	0.015 (-0.044 – 0.073) [c]	0.63
Critical care (%)	0.000 (-0.029 – 0.029)	0.99	-0.010 (-0.039 – 0.020) [d]	0.52

Supplemental Table 3a: Unadjusted and adjusted regression of trends by study year in age, sex, PPV23 receipt and outcomes in patients with pneumococcal CAP admitted between September 2013 and May 2023. Patients admitted between 13 March 2020 and 31 August 2021 (the 'pandemic period') were excluded from the analysis. Variables were modelled by restricted cubic splines with five knots at 6 April 2014, 24 June 2016, 5 February 2018, 2 September 2019 and 25 December 2022 based on Harrell's recommended percentiles.

[a]Adjusted for sex, PPV23 receipt and COPD

[b]Adjusted for age, smoking status and CURB65

[c]Adjusted for age, PPV23 receipt and COPD

[d]Adjusted for age, PPV23 receipt and CURB65

Patient characteristic	Unadjusted coefficient of slope (95% CI)	p-value	Adjusted coefficient of slope	p-value
Age	0.555 (-2.891 – 4.001)	0.75	2.749 (-0.548 – 6.046)[a]	0.10
Sex (% male)	-0.087 (-0.105 – 0.010)	0.07	-0.093 (-0.193 – 0.006)[a]	0.06
PPV23 receipt (%)	-0.125 (-0.185 – -0.030)	0.01	-0.106 (-0.198 – -0.013) [a]	**0.03**
Outcome				
30 day mortality (%)	0.050 (0.003 – 0.098)	0.04	0.058 (0.011 – 0.104) [b]	**0.01**
Readmission (%)	0.036 (-0.029 – 0.101)	0.28	0.045 (-0.022 – 0.112) [c]	0.20
Critical care (%)	-0.003 (-0.062 – 0.055)	0.91	-0.021 (-0.080 – 0.039) [d]	0.50

Supplemental Table 3b: Unadjusted and adjusted regression of trends by study year in age, sex, PPV23 receipt and outcomes in patients with pneumococcal CAP admitted during the pre-pandemic period only (September 2013 to March 2020). Variables were modelled by restricted cubic splines with five knots at 12 March 2014, 5 January 2016, 9 August 2017, 12 November 2018 and 29 December 2019 based on Harrell's recommended percentiles.

[a]Adjusted for sex, PPV23 receipt and COPD

[b]Adjusted for age, smoking status and CURB65

[c]Adjusted for age, PPV23 receipt and COPD

[d]Adjusted for age, PPV23 receipt and CURB65

Serotype	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22	2022-23	p-value
PCV13 serotypes											
1	1.03	1.77	3.91	0.00	0.71	1.40	0.33	0.61	0.89	0.00	0.15
3	13.40	17.70	12.85	21.84	21.63	26.69	34.00	42.94	41.12	48.86	**0.001**
4	0.00	0.00	1.68	0.77	1.06	0.84	0.67	0.00	0.30	0.00	0.4
5	8.76	0.88	5.59	6.51	4.96	5.62	4.00	0.61	0.89	0.33	**0.02**
6A	2.06	1.77	3.35	3.45	1.77	0.00	0.00	0.00	0.00	0.33	0.08
6B	0.00	1.33	0.56	0.38	0.71	0.00	0.33	0.61	1.18	0.33	1
7F	6.19	1.33	1.12	1.15	0.00	2.25	0.67	0.00	0.00	0.98	**0.04**
9V	0.00	0.00	0.56	0.38	0.71	0.00	0.00	0.00	0.00	0.00	0.37
14	1.55	3.10	1.68	1.92	0.00	1.12	0.00	0.00	0.00	0.98	0.05
18C	0.00	0.88	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00	0.24
19A	3.09	2.65	1.68	1.53	5.67	3.37	2.67	0.61	1.18	2.28	0.24
19F	3.09	0.00	1.68	3.07	3.19	3.09	2.00	0.61	2.37	1.95	0.65
23F	0.52	0.88	0.56	1.15	1.77	0.56	1.33	0.00	0.30	0.33	0.53
Additional PCV15 serotypes											
22F	3.09	1.33	4.47	4.98	2.48	2.25	2.00	0.00	1.48	3.58	0.32
33F	4.64	3.98	1.12	1.53	0.71	2.81	1.67	4.91	5.92	1.95	0.53
Additional PCV20 serotypes											
8	17.01	19.03	15.64	17.62	17.38	19.66	24.00	37.42	28.11	19.87	**0.02**
10A	1.55	1.33	2.23	1.53	2.13	1.69	2.67	0.00	2.07	0.00	0.72
11A	2.58	3.10	2.23	2.30	5.67	4.49	2.00	3.68	0.89	1.95	0.24
12F	3.61	5.75	8.94	5.75	3.90	1.12	1.67	0.61	0.00	0.33	**0.006**
15B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.
Additional PCV21 serotypes											
15A	6.70	7.08	9.50	6.90	5.67	4.78	3.33	1.23	3.25	2.28	**0.003**
15C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.

16F	1.03	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.03**
23A	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12
23B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.
24F	0.52	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.09
31	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12
35B	0.00	0.00	0.00	0.00	0.71	0.00	0.00	0.00	0.00	0.00	0.86
6C	0.52	0.44	0.00	0.00	0.35	0.84	2.00	0.61	0.89	0.98	**0.05**
Additional PPV23 serotypes											
2	0.00	0.44	0.56	0.00	0.00	0.28	0.00	0.00	1.18	1.30	0.29
9N	3.09	3.10	2.79	4.98	1.42	5.62	5.00	3.07	5.03	4.56	0.32
17F	1.55	4.87	3.35	0.77	1.06	1.69	1.67	0.00	0.30	0.98	0.09
20	1.03	0.88	0.56	1.15	0.71	4.78	1.67	0.00	0.30	3.26	0.93
Serotypes not in above vaccines											
12B	0.00	0.00	0.00	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.86
33C/39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.22
38	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
47A/43	0.00	0.00	0.56	0.00	0.35	0.00	0.33	0.00	0.00	0.00	0.5
non-ssUAD	12.37	15.49	12.85	9.96	14.89	4.78	6.00	2.45	2.07	2.61	**0.01**

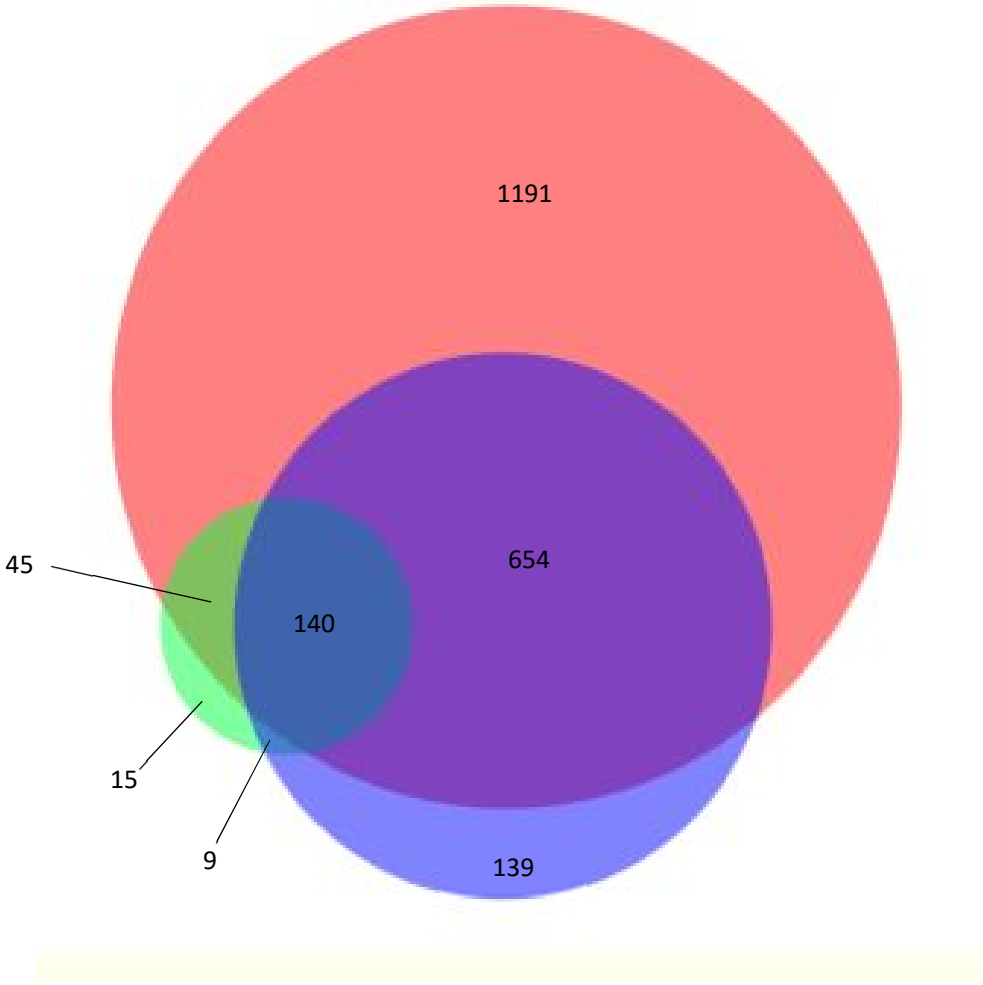
Supplemental Table 4: Trends in the proportion of individual serotypes by year in hospitalised pneumococcal CAP patients in Nottingham between 2013 and 2023.

p-value Jonckheer-Terpstra test for trend. Bold indicates p-value <0.05.

PCV class	Serotypes included	% of STs covered by vaccine
PCV13	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	44.2
PCV15	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, **22F,** 23F, **33F**	49.6
PCV20	1, 3, 4, 5,6A, 6B, 7F, **8** ,9V, **10A, 11A, 12F,** 14, **15B,** 18C, 19A, 19F, 22F, 23F, 33F	78.3
PCV21	3, 6A, 7F, 8, 9N, 10A, 11A, 12F, **15A, 15C, 16F**, 17F, 19A, 20, 22F, **23A, 23B, 24F, 31**, 33F, **35B**	80.8

Supplementary Table 5: Proportion of serotypes detected over the study period which were covered by different PCV formulations, including experimental PCV21

Supplementary figure 1



Venn diagram illustrating the overlap of diagnostic methods in patients with pneumococcal CAP (N=2193)

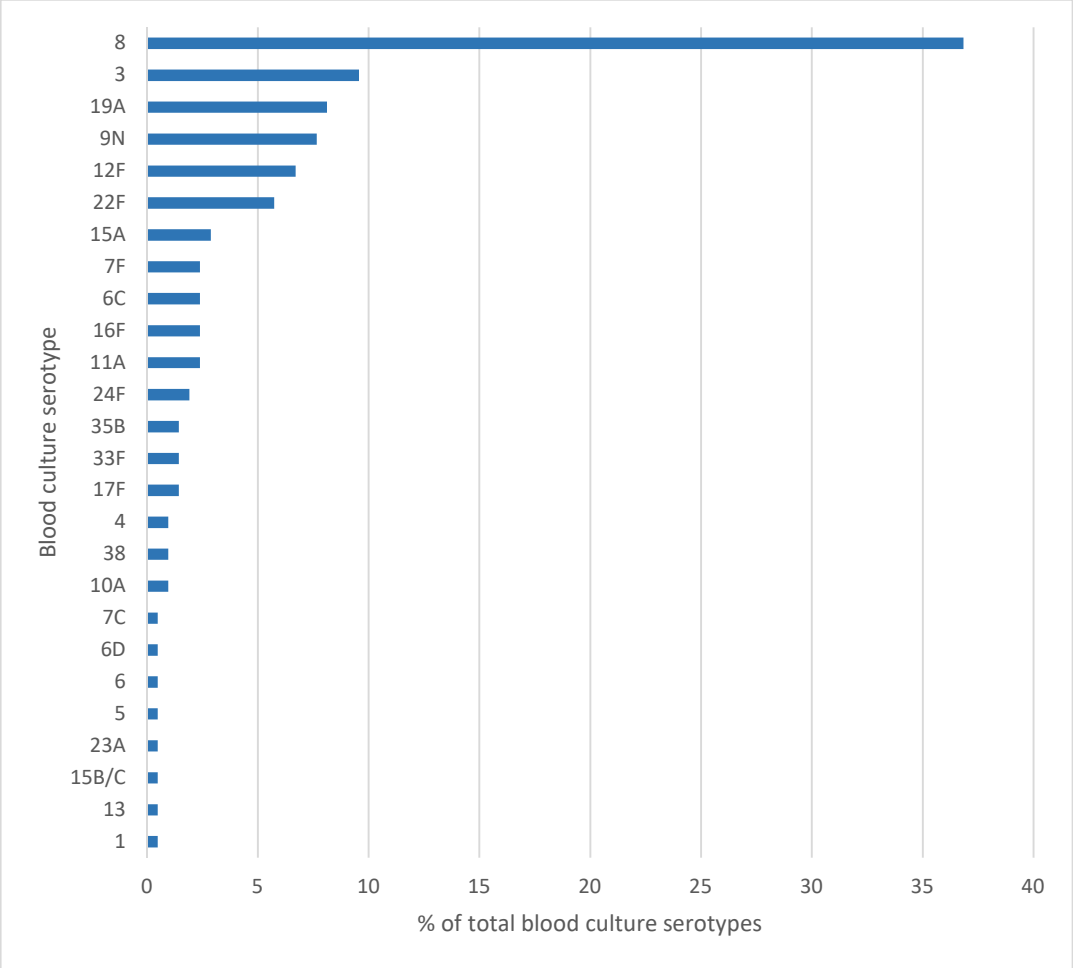
Key: Red = ssUAD; green = positive blood culture; Blue = BinaxNOW

Venn diagram generated using Biovenn web application. [1]

https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-9-488

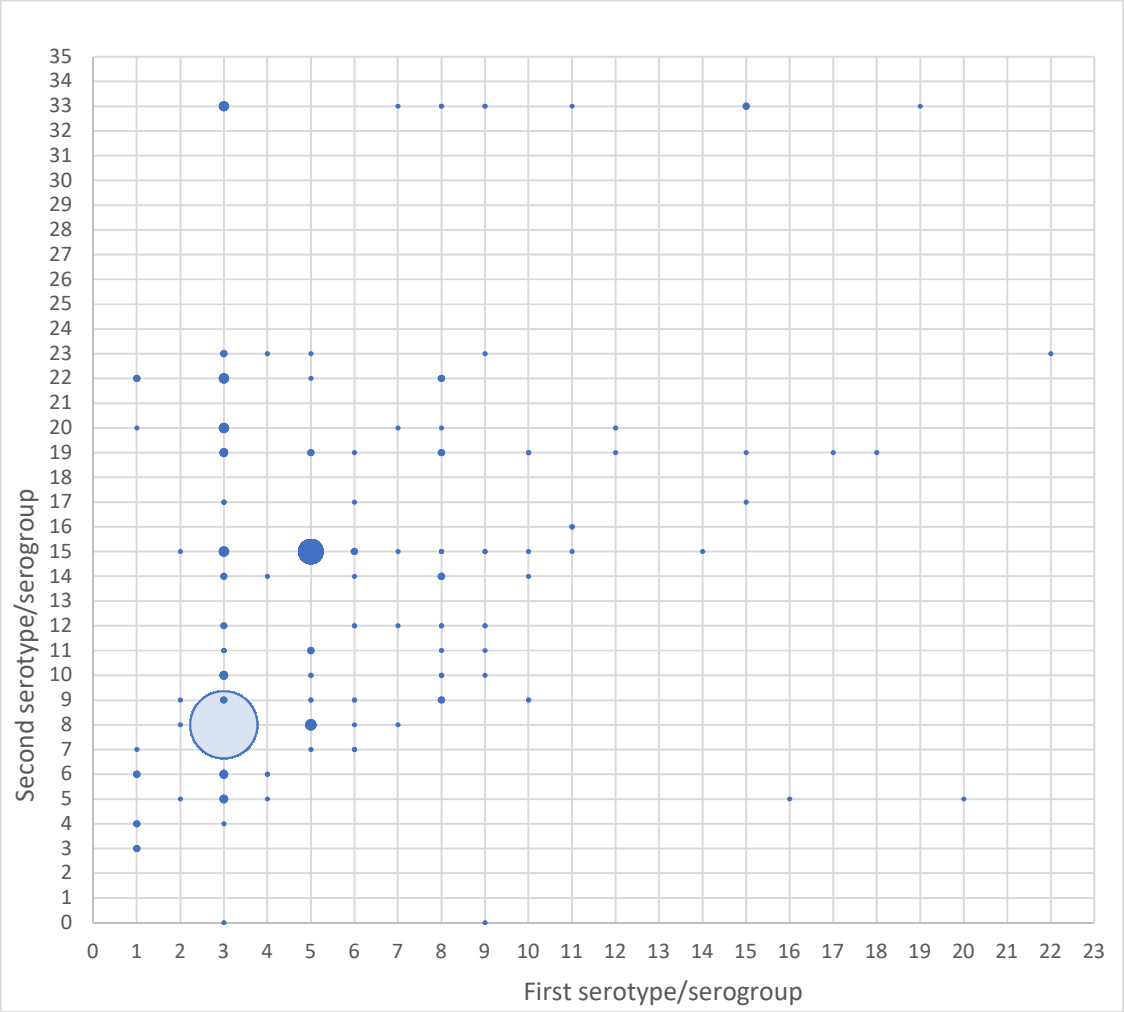
[1] Hulsen T, de Vlieg J, Alkema W. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. BMC Genomics 2008;**9**:488

Supplementary Figure 2



Pneumococcal serotypes identified in blood cultures as a percentage of the total number of blood culture serotypes

Supplementary Figure 3



Bubble plot of first and second pneumococcal serotypes/serogroups in 466 people with ≥2 serotypes identified by Bio-Plex24 assay. Area of the circle is proportional to the frequency of each serotype/serogroup combination