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Article

Comparative Analysis of Pneumococcal Serotypes over a 10-Year Period (2014–2024) in the Comunidad Valenciana Region of Spain and Their Relationship with PCV13, PCV20 and PCV21

Laura Diab-Casares [1], Nuria Tormo-Palop [1], Rafael Medina-González [1], Sonia Cortés-Badenes [1], Francisco Javier Hernández-Felices [1], Violeta Artal-Muñoz [1], José Luis Martín-Rodríguez [1,2], Francisco Roig-Sena [3], José Manuel Marín [3], María Dolores Gómez-Ruiz [4], Francisco José Rodríguez Nortes [1], Mariana Lamas-Santángelo [1], Concepción Gimeno-Cardona [1,2,*,&] and Remedio Guna-Serrano [1,2,&]

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Abstract

Background/Objectives: This study analyzes the epidemiology of invasive pneumococcal disease (IPD) and the dynamics of Streptococcus pneumoniae serotypes in the Comunidad Valenciana (CV), Spain, over a 10-year period (2014–2024), with particular focus on vaccine coverage and effectiveness of PCV13 compared to the newer PCV20 and PCV21 formulations. Methods: A total of 2,014 isolates were collected from sterile clinical samples and characterized by serotype, patient demographics, and vaccination status. Results: Overall vaccination coverage was low (22.6%), with the highest rates observed in children under 10 years (78%) compared to only 16% in those aged 10–64 years and 22% in those over 64. Serotype distribution revealed 120 distinct serotypes, with serotype 8 (17.6%) and serotype 3 (14.7%) being the most frequent. Serotype 8 predominated among unvaccinated individuals, while serotype 3 remained highly prevalent despite inclusion in PCV13, reflecting limited vaccine effectiveness. Other relevant serotypes included 22F, 9N, 19A, 6C, and 23A. Temporal analysis showed increases in serotypes 8, 3, and 23A in recent years, while 9N, 19A, 15A, and 11A significantly declined. Among serotypes with <2% incidence, some such as 4, 12F, 16F, and 24F showed upward trends. Conclusions: The findings suggest that PCV20 currently provides broad coverage of dominant serotypes, but PCV21 may offer advantages should serotypes like 23A, 9N, or 15A increase further due to serotype replacement. Continuous epidemiological surveillance is essential to guide evidence-based vaccine policy and anticipate future vaccine reformulations.

Keywords: Comunidad Valenciana; invasive pneumococcal disease; serotypes; Streptococcus pneumoniae; PCV20; PCV21

1. Introduction

Streptococcus pneumoniae (SP) remains the leading bacterial cause of severe pneumonia and pneumonia-related mortality worldwide. It is an asymptomatic colonizer of the human nasopharynx, especially in children, and can spread to adjacent areas, causing infections such as otitis, aspiration

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pneumonia, or invading sterile body fluids, resulting in what is known as invasive pneumococcal disease (IPD). IPD can reach mortality rates of up to 20% for sepsis and 50% for meningitis, being more common in individuals under 5 years of age and over 65 [1,2]. In this regard, pneumococcal vaccines are of particular interest, as they are designed to cover the serotypes most frequently associated with severe pneumonia and IPD.

Currently, there are two categories of vaccines: the pneumococcal polysaccharide vaccine (PPV) and the pneumococcal conjugate vaccine (PCV). The former contains 23 different serotypes and is based on SP capsular polysaccharides (PPV23), promoting immunity by stimulating B lymphocytes, which differentiate into plasma cells responsible for antibody production. It induces only serum IgG secretion, not mucosal IgA. Developed and introduced in 1983, it failed to generate adequate immunity in children and older adults [3].

To overcome this limitation, conjugate vaccines were developed. These are based on the linkage of a weak polysaccharide molecule to a strong antigen, such as a protein, generating a T-cell–dependent immune response in addition to B-cell stimulation. This induces immune memory, a robust antibody response, and mucosal immunity [4–6]. The first conjugate vaccine was PCV7, introduced in 2000, followed sequentially by PCV10 and PCV13, with the aim of including additional serotypes and reformulating the antigenic content to increase specificity and serotype coverage [5,7].

The most recent vaccines developed are the 15-, 20-, and 21-valent conjugates (PCV15, PCV20, and PCV21). Their approval was based on solid clinical trials evaluating their safety and immunogenicity compared to previous vaccines [8–10]. Focusing on PCV20 and PCV21, the main differences lie in their serotype coverage. Both vaccines share the following serotypes: 3, 6A, 6B, 8, 10A, 11A, 12F, 15B, 19A, 19F, 22F, and 33F. Unique to PCV20 are serotypes 1, 4, 5, 7F, 9V, 14, 18C, and 23F, while PCV21 uniquely includes serotypes 9N, 16F, 17F, 20, 23A, 23B, 24F, 31, and 35B [11,12].

PCV20 was approved by the EMA in 2022 and made available in Spain in 2024 [13]. It is also the vaccine funded by the Comunidad Valenciana as of July, 2024 (CV) [14]. According to the Spanish Association of Paediatrics (AEP) Vaccine Committee, PCV20 is expected to replace PCV13 in the vaccination schedule for children born from January 1, 2025 [15].

In light of all this, the aim of this study was to evaluate the frequency in the Comunidad Valenciana region of uncommon serotypes included in PCV20, PCV21, and non-vaccine serotypes with the potential to cause IPD, identifying those that have shown a significant increase over the past 10 years (2014–2024, inclusive), in order to justify the preferential use of one vaccine over the other.

2. Materials and Methods

2.1. Study Design and Data Collection

This is a retrospective study including a sample of 2.014 *Streptococcus pneumoniae* isolates obtained from sterile clinical specimens collected in hospitals of the CV over a 10-year period (2014–2024). The dataset includes sociodemographic data (sex and age), comorbidities, serotype, and vaccination status.

Of the total sample, 41.5% were women and 58.5% were men. The mean age of the population was 61.24 years (±23.18), ranging from 0 to 100 years. When regrouping the sample into three age brackets (excluding 21 cases with age = 124), the distribution is shown in Figure 1.

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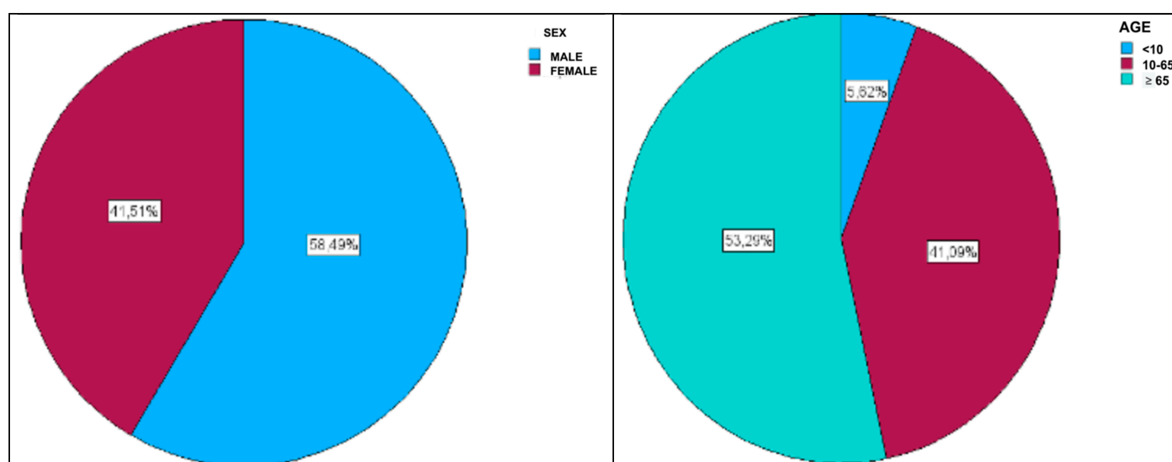

Figure 1. Distribution of the sample by sex and age.

2.2. Sociodemographic and Clinical Data

All case data were obtained from the Epidemiological Surveillance System ("AVE"), which integrates information from three primary sources: outpatient information systems, hospital preventive medicine departments, and the Microbiological Surveillance Network of the CV ("RedMIVA") [16]. RedMIVA provides real-time microbiological testing results from hospitals across the region. The following variables were collected and analyzed in this study: age, sex, serotype and vaccination status.

2.3. Statistical Analysis

For categorical variables, frequencies and percentages were calculated. Differences between groups were assessed using Pearson's chi-square test, and in the case of 2×2 tables, Fisher's exact test was applied. All multiple comparisons were adjusted using the Bonferroni correction. Effect sizes were reported to support the interpretation of results, using Cramer's V for categorical variables.

The classification of effect size magnitude based on Cramer's V was as follows [17]:

- 0.00–0.09: negligible
- 0.10–0.29: small
- 0.30–0.49: medium
- ≥0.50: large

A significance level of 5% ($\alpha = 0.05$) was used for all analyses.

3. Results

3.1. Vaccination Status Overview

The distribution of vaccination status in the sample was as follows: 77.4% were either unvaccinated or unknown, 6.2% had received PCV13, 11.3% had received PPV23, 1.4% had received both vaccines, and 3.7% were vaccinated, but the specific vaccine was unknown. This results in a total vaccination rate of 22.6%.

By age group, individuals over 10 years—particularly those aged 10 to 64 years—had the lowest vaccination rates (16%), as well as those over 64 years, with 22%. In contrast, children under 10 years had a vaccination rate of 78%, with 47% vaccinated with PCV13 ($p < 0.001$) and 27% with unknown vaccination status ($p < 0.001$). Post-hoc pairwise comparisons showed significant differences between the 10–64 and <10 age groups ($p < 0.001$), and between the >64 and <10 age groups ($p = 0.002$).

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Table 1. Vaccination rates by age group and type of pneumococcal vaccine.

	Age							
	Total		<10		10-64		>64	
	Count	N %	Count	N %	Count	N %	Count	N %
Total	1993	100.0%	112	100.0%	819	100.0%	1062	100.0%
Not vaccinated/Unknown status	1542	77.4%	25	22.3%	689	84.1%	828	78.0%
Vaccinated with PPV23	226	11.3%	3	2.7%	45	5.5%	178	16.8%
Vaccinated with PCV13	125	6.2%	53	47.4%	51	6.3%	21	2.0%
Vaccinated, but vaccine type unknown	73	3.7%	30	27%	26	3.2%	17	1.5%
Vaccinated with both PCV13 and PPV23	27	1.4%	1	0.9%	8	1.0%	18	1.7%

3.2. Pneumococcal Serotypes

A total of 120 different serotypes were identified in the sample. The most common was serotype 8, accounting for 17.6% of cases, followed by serotype 3 (14.7%). Serotypes 22F and 9N had incidences between 4–5%, while serotypes 14, 19A, 6C, and 23A showed incidences between 3–4%. The remaining serotypes each represented less than 3% of the cases.

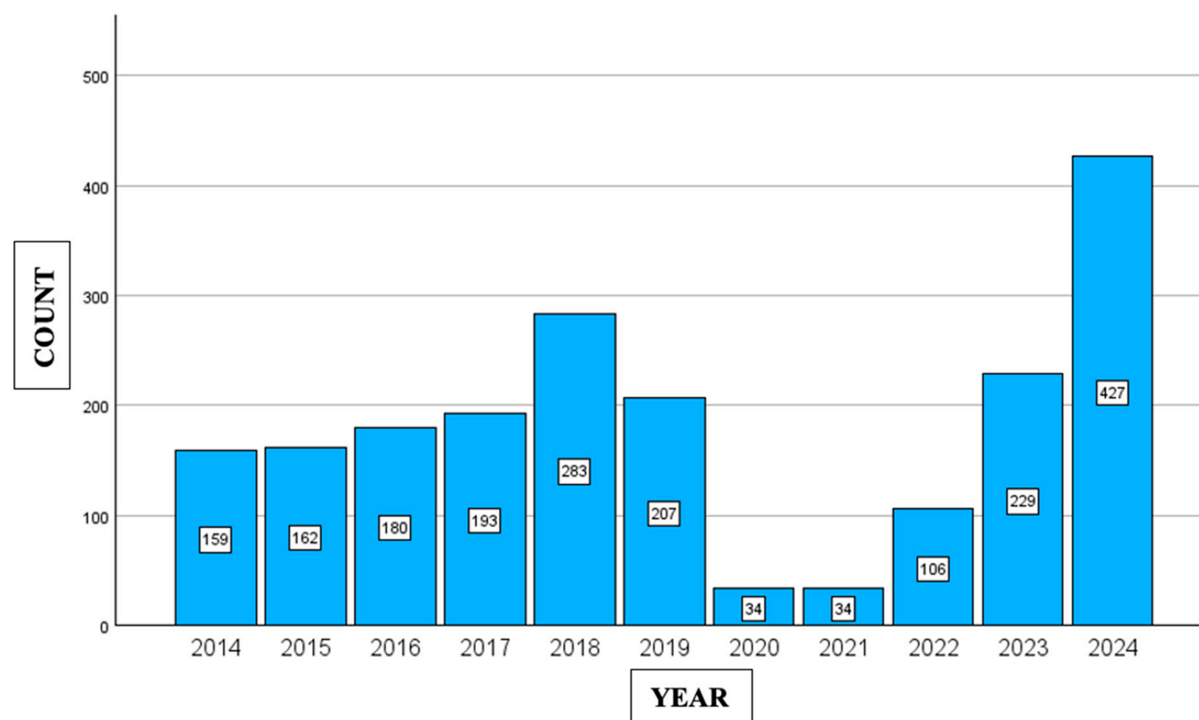

Figure 2. Distribution of IPD cases by year, showing a decrease between 2020–2021, coinciding with the COVID-19 period.

Working only with serotypes with an incidence greater than 2%, we observed the following according to:

Serotypes by Age (Table 2):

- Serotype 8 was more common in individuals over 10 years of age, particularly those aged 10–64 years.
- Serotype 3 was more frequent in individuals over 64 years than in those aged 10–64 years.
- Serotypes 10A and 23B were more frequent in children under 10 years of age (small effect size, Cramer's V = 0.186).

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Table 2. Correlation between serotypes and age groups.

		Age							
		Total		<10		10-64		>64	
		Count	N %	Count	N %	Count	N %	Count	N %
Serotype	**Total**	1319	100.0%	60	100.0%	532	100.0%	727	100.0%
	8	353	26.8%	5	8.3%	188	35.3%	160	22.0%
	3	293	22.2%	15	25.0%	99	18.6%	179	24.6%
	22F	99	7.5%	5	8.3%	40	7.5%	54	7.4%
	9N	79	6.0%	0	0.0%	35	6.6%	44	6.1%
	14	71	5.4%	5	8.3%	21	3.9%	45	6.2%
	19A	70	5.3%	5	8.3%	22	4.1%	43	5.9%
	6C	64	4.9%	1	1.7%	21	3.9%	42	5.8%
	23A	61	4.6%	3	5.0%	19	3.6%	39	5.4%
	10A	56	4.2%	10	16.7%	24	4.5%	22	3.0%
	15A	46	3.5%	3	5.0%	18	3.4%	25	3.4%
	31	44	3.3%	0	0.0%	15	2.8%	29	4.0%
	11A	43	3.3%	2	3.3%	13	2.4%	28	3.9%
	23B	40	3.0%	6	10.0%	17	3.2%	17	2.3%

3.3. Serotypes and Vaccination

There were statistically significant differences between serotype and vaccination status ($Chi^2(56) = 132.421$, $p < 0.001$, Cramer's $V = 0.147$), indicating a small effect size. Specifically, serotype 8 was more prevalent among unvaccinated individuals or those with unknown vaccination status than among those vaccinated with PCV13 or PPV23.

Additionally, serotype 10A was more frequently found in individuals vaccinated with PCV13 than in those vaccinated with PPV23 or in unvaccinated individuals.

Table 3. Serotypes with an incidence greater than 2% in relation to PCV13 and PPV23 vaccination status.

		Vaccination status											
		Total		Not vaccinated/Unknown		Vaccinated PCV13		Vaccinated PPV23		Vaccinated with PCV13 and PPV23		Vaccinated, but vaccine type unknown	
		Count	N %	Count	N %	Count	N %	Count	N %	Count	N %	Count	N %
Serotypes (>2% incidence)	**Total**	2014	100.0%	1563	100.0%	125	100.0%	226	100.0%	27	100.0%	73	100.0%
	Others	669	33.2%	472	30.2%	61	48.8%	87	38.5%	14	51.9%	35	47.9%
	8	354	17.6%	308	19.7%	11	8.8%	23	10.2%	5	18.5%	7	9.6%
	3	297	14.7%	243	15.5%	12	9.6%	32	14.2%	3	11.1%	7	9.6%
	22F	101	5.0%	86	5.5%	5	4.0%	8	3.5%	2	7.4%	0	0.0%
	9N	80	4.0%	71	4.5%	0	0.0%	8	3.5%	0	0.0%	1	1.4%
	14	71	3.5%	60	3.8%	3	2.4%	7	3.1%	0	0.0%	1	1.4%
	19A	70	3.5%	54	3.5%	5	4.0%	6	2.7%	0	0.0%	5	6.8%
	6C	66	3.3%	51	3.3%	2	1.6%	13	5.8%	0	0.0%	0	0.0%
	23A	61	3.0%	46	2.9%	1	0.8%	9	4.0%	1	3.7%	4	5.5%
	10A	56	2.8%	35	2.2%	12	9.6%	5	2.2%	1	3.7%	3	4.1%
	15A	46	2.3%	29	1.9%	6	4.8%	6	2.7%	0	0.0%	5	6.8%
	31	45	2.2%	36	2.3%	0	0.0%	7	3.1%	0	0.0%	2	2.7%
	11A	43	2.1%	28	1.8%	4	3.2%	9	4.0%	0	0.0%	2	2.7%
	23B	40	2.0%	31	2.0%	3	2.4%	5	2.2%	0	0.0%	1	1.4%
	Invalidated	15	0.7%	13	0.8%	0	0.0%	1	0.4%	1	3.7%	0	0.0%

3.4. Serotype Trends Over Time

Regarding serotype trends, no serotype showed a statistically significant increase in recent years when analyzed year by year. Serotype 8 increased in 2019 and then remained stable, although descriptively it appears to be declining slightly in the most recent years—this trend, however, was

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not confirmed statistically. Serotype 3 increased in 2022, followed by a downward trend that was also not statistically significant. The remaining serotypes did not show significant evolution over time. It is worth noting that small sample sizes reduce the statistical power of the tests performed (Figure 3).

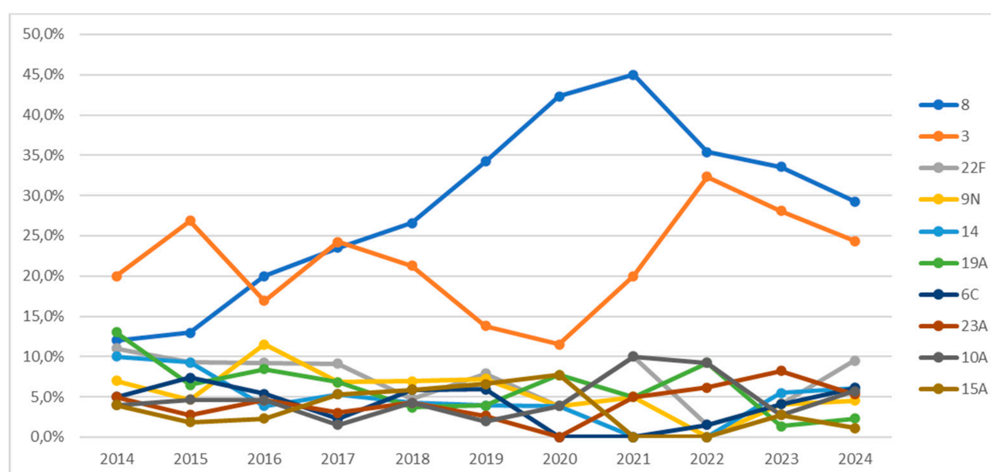

Figure 3. Incidence of IPD cases by responsible serotype.

A grouped-year analysis was conducted to increase the statistical power of the tests (Chi²(24) = 81.840, $p < 0.001$). Serotype 8 showed greater prevalence starting in 2020, particularly in the 2020–2022 ($p = 0.001$) and 2023–2024 ($p = 0.008$) periods, both compared to 2014–2019, even though no further increase was observed in the last two years.

Serotype 3 tended to be more prevalent in 2023–2024 compared to pre-2019 data ($p = 0.093$, marginally significant at <0.1). Conversely, serotype 19A significantly decreased in the last two years ($p = 0.002$ and $p = 0.004$, respectively), and serotypes 15A and 11A also showed reduced presence since 2019 ($p = 0.035$ and $p = 0.003$, respectively).

Table 4. Grouped-year periods and incidence of IPD-causing serotypes.

		Year							
		Total		**2014-2019**		**2020-2022**		**2023-2024**	
		Count	**N %**	**Count**	**N %**	**Count**	**N %**	**Count**	**N %**
Serotype	**Total**	1330	100.0%	810	100.0%	111	100.0%	409	100.0%
	8	354	26.6%	185	22.8%	43	38.7%	126	30.8%
	3	297	22.3%	164	20.2%	28	25.2%	105	25.7%
	22F	101	7.6%	66	8.1%	4	3.6%	31	7.6%
	9N	80	6.0%	60	7.4%	2	1.8%	18	4.4%
	14	71	5.3%	46	5.7%	1	0.9%	24	5.9%
	19A	70	5.3%	53	6.5%	9	8.1%	8	2.0%
	6C	66	5.0%	43	5.3%	1	0.9%	22	5.4%
	23A	61	4.6%	30	3.7%	5	4.5%	26	6.4%
	10A	56	4.2%	28	3.5%	9	8.1%	19	4.6%
	15A	46	3.5%	37	4.6%	2	1.8%	7	1.7%
	31	45	3.4%	32	4.0%	3	2.7%	10	2.4%
	11A	43	3.2%	37	4.6%	2	1.8%	4	1.0%
	23B	40	3.0%	29	3.6%	2	1.8%	9	2.2%

To enhance interpretability, the two pandemic years were grouped with the 2014–2019 period (Chi²(12) = 58.456, $p < 0.001$, Cramer's V = 0.210, small effect size). This analysis revealed that serotypes 8, 3, and 23A significantly increased in the last three years compared to the previous eight-year period ($p = 0.003$, 0.006, and 0.024, respectively).

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In contrast, serotypes 9N, 19A, 15A, and 11A showed a significant decrease (p = 0.011, 0.005, 0.003, and 0.000, respectively).

Table 5. IPD-causing serotypes grouped into three time periods.

		Year					
		Total		**2014-2021**		**2022-2024**	
		Count	**N %**	**Count**	**N %**	**Count**	**N %**
Serotype	**Total**	1330	100.0%	856	100.0%	474	100.0%
	8	354	26.6%	205	23.9%	149	31.4%
	3	297	22.3%	171	20.0%	126	26.6%
	22F	101	7.6%	69	8.1%	32	6.8%
	9N	80	6.0%	62	7.2%	18	3.8%
	14	71	5.3%	47	5.5%	24	5.1%
	19A	70	5.3%	56	6.5%	14	3.0%
	6C	66	5.0%	43	5.0%	23	4.9%
	23A	61	4.6%	31	3.6%	30	6.3%
	10A	56	4.2%	31	3.6%	25	5.3%
	15A	46	3.5%	39	4.6%	7	1.5%
	31	45	3.4%	33	3.9%	12	2.5%
	11A	43	3.2%	39	4.6%	4	0.8%
	23B	40	3.0%	30	3.5%	10	2.1%

Among the serotypes with less than 2% incidence, the following showed an increase in the last three years: 4, 38, 12F, 16F, 24F, 17F, and 15B/C (Table 6).

Table 6. IPD-causing serotypes by year groupings with less than 2% incidence.

		Year					
		Total		**2014-2021**		**2022-2024**	
		Count	**N %**	**Count**	**N %**	**Count**	**N %**
Serotype	**Total**	2014	100.0%	1252	100.0%	762	100.0%
	4	29	1.4%	12	1.0%	17	2.2%
	38	23	1.1%	9	0.7%	14	1.8%
	12F	22	1.1%	5	0.4%	17	2.2%
	16F	16	0.8%	3	0.2%	13	1.7%
	24	11	0.5%	1	0.1%	10	1.3%
	17F	11	0.5%	1	0.1%	10	1.3%
	15B/C	10	0.5%	1	0.1%	9	1.2%

4. Discussion

This study presents an analysis of the epidemiological distribution of *Streptococcus pneumoniae* serotypes responsible for invasive pneumococcal disease (IPD) over the past 10 years, comparing pneumococcal vaccines—particularly PCV13 *versus* PCV20 and PCV21—with the aim of determining which vaccine would provide the most appropriate coverage in the CV based on our results.

IPD continues to represent a high-burden disease globally and remains a major public health concern. Thanks to the inclusion of pneumococcal vaccines in immunization programs, there has been a substantial reduction in disease caused by vaccine-covered serotypes [18,19]. Regarding PCVs, PCV13 includes 7 fewer serotypes than PCV20 and 8 fewer than PCV21. The inclusion of serotype 8 in the newer vaccines is especially noteworthy, as it has been the most frequently isolated serotype globally over the last 7 years, both according to our data from the CV and ECDC reports across

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Europe over the past 6 years [20], in both vaccinated and unvaccinated individuals. This trend reflects an indirect effect of introducing PCV13 into the regional vaccination schedule in 2015 [21], the same year serotype 8 (not included in PCV13) began rising progressively, becoming the most prevalent. This occurred despite being included in PPV23, whose effectiveness against serotype 8 has been reported at 46% in a study conducted in Germany [22]. The lack of protection from PPV23 and the absence in PCV13 likely contributed to the dominance of serotype 8. Furthermore, its increased presence in Spain from 2015–2018 has been linked to the expansion of a single clone (ST53) [23], which was also demonstrated by our research group using whole genome sequencing (WGS), confirming ST53 as the dominant clone within serotype 8 in our setting [24].

Serotype 3 is more prevalent in children under 10 and adults over 64, and is the second most frequent in individuals aged 10–64, despite being included in PCV13. This is particularly concerning, as it is one of the most invasive serotypes, associated with older patients, comorbidities, and higher lethality [24,25]. The lack of PCV13 efficacy against serotype 3 in children is likely a key reason for its persistent incidence [26]. The inclusion of serotype 3 in both PCV20 and PCV21 has received much attention due to its evasiveness. A Phase 3 clinical trial by Essink *et al*. demonstrated that PCV20 elicited a non-inferior immunological response to PCV13 for this serotype, reaching adequate opsonophagocytic activity (OPA) across adult age groups [27]. PCV21 also showed robust immunogenicity against serotype 3, in some cases equal to or superior to PCV20 [28].

Serotype 23A, which has increased over the past three years in the region, is another serotype of epidemiological concern—only covered by PCV21. A study by Løchen *et al*. identified divergent serotype trends across regions, yet consistently reported a progressive rise in 23A, attributed to the replacement of non-PCV13 serotypes [29]. Although not highly virulent, its persistent increase could warrant future vaccine inclusion and closer surveillance.

Continuing along serotypes with >2% incidence, serotypes 9N and 15A have shown a statistically significant decrease, while 31 and 23B have also declined, though not significantly. In contrast, serotypes 14 and 6C have exhibited an upward trend over the past 10 years, although these increases have not yet reached statistical significance. Notably, 9N, 15A, 31, and 23B would no longer be covered if switching from PCV21 to PCV20. Conversely, serotype 14 is included in PCV20 but not in PCV21. Serotype 6C is not covered by any of the current vaccines, and its incidence in IPD in our study is around 3.3% in CV, a finding that supports considering its future inclusion in higher-valent conjugate vaccines, particularly if its prevalence continues to rise. Serotypes 19A and 11A have shown low attack rates and have decreased in incidence in recent years, likely as a response to immunization with PPV23 and PCV13.

Among serotypes with <2% incidence that have increased over the last three years, only serotype 4 is included in PCV20, whereas 16F, 17F, and 15C are only included in PCV21. Serotype 12F is covered by PCV13, PCV20, and PCV21. Serotype 38 is not covered by either PCV20 or PCV21. Serotype 24F would only be covered by PCV21, depending on exact classification. According to the 2023 Spain National Epidemiological Surveillance Network (RENAVE) report, the serotypes showing increased incidence were 22F, 38, 4, 12F, and 3 [30]. Notably, serotype 4 (included only in PCV20) should be monitored closely, as it has been responsible for recent occupational outbreaks in shipyard workers [31].

Regarding study limitations, the 10-year span includes data through 2024. It will be important to reassess the situation in the near future to determine whether serotypes excluded from PCV21 but on the rise continue to increase further, possibly due to serotype replacement under PCV20 use. Similarly, expanding the sample size will improve statistical power to assess whether increases in low-prevalence (<2%) serotypes are statistically meaningful.

5. Conclusions

- PCV20 covers the main circulating serotypes currently, including 8, 3, 22F, 14, 19A, 11A, and 10A.

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- PCV21 could be superior if serotypes such as 9N, 23A/B, 15A, and 31 increase in incidence as a consequence of serotype replacement following the introduction of PCV20. This study provides preliminary data that must be confirmed with further follow-up. At present, the rise in serotypes not included in PCV20 appears residual. It remains to be seen what collateral impact PCV20 inclusion in vaccination programs may have.
- Future research should assess immune responses to PCV20, especially against serotype 3, one of the most lethal and evasive serotypes not well controlled by PCV13.
- It is essential to select vaccines based on local epidemiological data.
- This study underscores the importance of ongoing epidemiological surveillance of IPD to evaluate the evolution of vaccine and non-vaccine serotypes, analyze vaccine effectiveness, and guide future evidence-based vaccine reformulation.

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References

1. World Health Organization. Pneumococcus. In: Vaccine-Preventable Diseases Surveillance Standards. Geneva: World Health Organization; 2018. Disponible en: https://cdn.who.int/media/docs/default-source/immunization/vpd_surveillance/vpd surveillance-standards-publication/who-surveillancevaccinepreventable-17_pneumococcus-r2.pdf
2. Drijkoningen JJ, Rohde GG. Pneumococcal infection in adults: burden of disease. Clin Microbiol Infect. 2014;20(Suppl 5):45–51. doi:10.1111/1469-0691.12461.
3. Musher DM, Anderson R, Feldman C. The remarkable history of pneumococcal vaccination: an ongoing challenge. Pneumonia (Nathan). 2022;14(1):5. doi:10.1186/s41479-022-00097-y.
4. World Health Organization. Pneumococcal disease: vaccine standardization. Geneva: World Health Organization; 2025. Disponible en: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/norms-and-standards/vaccine-standardization/pneumococcal-disease
5. Scelfo C, Menzella F, Fontana M, Ghidoni G, Galeone C, Facciolongo NC. Pneumonia and Invasive Pneumococcal Diseases: The Role of Pneumococcal Conjugate Vaccine in the Era of Multi-Drug Resistance. Vaccines (Basel). 2021;9(5):420. doi:10.3390/vaccines9050420.

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 August 2025 doi:10.20944/preprints202508.1704.v1

6. Davies LRL, Cizmeci D, Guo W, Luedemann C, Alexander-Parrish R, Grant L, *et al.* Polysaccharide and conjugate vaccines to *Streptococcus pneumoniae* generate distinct humoral responses. Sci Transl Med. 2022;14(656):eabm4065. doi:10.1126/scitranslmed.abm4065.
7. Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, Reingold A, Cieslak PR, Pilishvili T, Jackson D, *et al.* Decline in invasive pneumococcal disease after the introduction of protein–polysaccharide conjugate vaccine. N Engl J Med. 2003;348 (18):1737–1746. doi:10.1056/NEJMoa022823 .
8. Platt HL, Cardona JF, Haranaka M, Sugayama SM, Rausch D, Greenberg D, *et al.* A phase 3 trial of a 15-valent pneumococcal conjugate vaccine in healthy infants. Pediatr Infect Dis J. 2022;41(5):345–52. doi:10.1097/INF.0000000000003454.
9. Essink B, Sabharwal C, Cannon K, Frenck RW, Lal H, Xu X, *et al.* Pivotal Phase 3 randomized clinical trial of the safety, tolerability, and immunogenicity of 20-valent pneumococcal conjugate vaccine in adults aged ≥18 years. Clin Infect Dis. 2022;75(3):390–8. doi:10.1093/cid/ciab1014.
10. Jansen KU, Anderson AS, Scully IL, Tanimura D, Gruber WC. Safety, tolerability, and immunogenicity of V116, a 21-valent pneumococcal conjugate vaccine, in adults aged ≥50 years: a randomized, double-blind, phase 1/2 trial. Vaccine. 2022;40(43):6223–30. doi:10.1016/j.vaccine.2022.08.034.
11. Pfizer Inc. U.S. Food and Drug Administration. PREVNAR 20 (Pneumococcal 20-valent Conjugate Vaccine). Prescribing Information [Internet]. 2023 [cited 2025 Jun 8]. Disponible en: https://www.fda.gov/media/150386/download.
12. Merck & Co., Inc. CAPVAXIVE™ (Pneumococcal 21-valent Conjugate Vaccine): FDA Approval Press Release [Internet]. 2023 Nov 28 [cited 2025 Jun 8]. Disponible en: https://www.merck.com/news/u-s-fda-approves-capvaxive-pneumococcal-21-valent-conjugate-vaccine-for-adults/.
13. European Medicines Agency. *Prevenar 20 (pneumococcal polysaccharide conjugate vaccine—20 valent).* EMA/80599/2024 [Internet]. Amsterdam: EMA; 2022 Feb 14 [citado 2025 Jun 8]. Disposable in: EMA pfizer.com+15.
14. Generalitat Valenciana. El Consell adquiere 500.000 dosis de vacuna frente al neumococo para reforzar la protección infantil y de personas adultas. Comunica GVA [Internet]. 2024 Jul 28 [citado 2025 Jun 8]. Disponible en: https://comunica.gva.es/es/detalle?id=384988364&site=373422400.
15. Comité Asesor de Vacunas de la AEP. Calendario de vacunaciones de la Comunidad Valenciana. Vacunas AEP [Internet]. 2025 [cited 2025 Jun 8]. Disponible en: https://vacunasaep.org/profesionales/calendario-vacunas/comunidad-valenciana.
16. Muñoz,Isabel- Vanaclocha,Hermelinda- Martín-Sierra,Miguel y González,Francisco. Red de Vigilancia Microbiológica de la Comunidad Valenciana (RedMIVA). Enfermedades Infecciosas y Microbiología Clínica. 2008; 26(2): 77-81.
17. Diehl, J. M. / Kohr, H.U. (1999). Deskriptive Statistik. («Estadística descriptiva») 12ª edición. Klotz Eschborn, p.161.
18. Pilishvili T, Lexau C, Farley MM, *et al.* Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis 2010; 201:32–41. 15.
19. Wiese AD, Griffin MR, Grijalva CG. Impact of pneumococcal conjugate vaccines on hospitalizations for pneumonia in the United States. Expert Rev Vaccines 2019; 18:327–41.
20. ECDC. Invasive Pneumococcal Disease—Annual Epidemiological Report for 2018. 2018. Available online: https://www.ecdc.europa.eu/en/publications-data/invasive-pneumococcal-disease-annual-epidemiological-report-2018 (accessed on 1 June 2023).
21. Conselleria de Sanitat. Orden 3 Abr 2015, de inclusión de la vacuna frente al neumococo en los niños nacidos a partir del 1 de enero 2015. *Diari Oficial de la Generalitat Valenciana* [Internet]. 13 Abr 2015 [citado 2025 Jun 12]. Disponible en: https://dogv.gva.es/datos/2015/04/13/pdf/2015_3241.pd.
22. Perniciaro S, van der Linden M. *Pneumococcal vaccine uptake and vaccine effectiveness in older adults with invasive pneumococcal disease in Germany: a retrospective cohort study*. Lancet Reg Health Eur. 2021 Jun 3;7:100126. doi:10.1016/j.lanepe.2021.100126.
23. Suaya, J.A.; Mendes, R.E.; Sings, H.L.; Arguedas, A.; Reinert, R.-R.; Jodar, L.; Isturiz, R.E.; Gessner, B.D. *Streptococcus pneumoniae* serotype distribution and antimicrobial nonsusceptibility trends among adults with pneumonia in the United States, 2009–2017. J. Infect. 2020, 81, 557–566.

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 August 2025 doi:10.20944/preprints202508.1704.v1

24. Diab-Casares L, Tormo-Palop N, Hernández-Felices FJ, Artal-Muñoz V, Floría-Baquero P, Martin-Rodríguez JL, Medina-González R, Cortés-Badenes S, Fuster-Escrivá B, Gil-Bruixola A, *et al. Predominant pneumococcal serotypes in isolates causing invasive disease in a Spanish region: an examination of their association with clinical factors, antimicrobial resistance, and vaccination coverage*. J Clin Med. 2025;14(5):1612. doi:10.3390/jcm14051612.
25. Ciruela P, Broner S, Izquierdo C, Pallarés R, Muñoz-Almagro C, Hernández S, *et al.*; Catalan Working Group on Invasive Pneumococcal Disease. Indirect effects of paediatric conjugate vaccines on invasive pneumococcal disease in older adults [Erratum in: Int J Infect Dis.
26. Domínguez Á, Ciruela P, Hernández S, García-García JJ, Soldevila N, Izquierdo C, Moraga-Llop F, Díaz A, de Sevilla MF, González-Peris S, Campins M, Uriona S, Martínez-Osorio J. *Effectiveness of the 13-valent pneumococcal conjugate vaccine in preventing invasive pneumococcal disease in children aged 7–59 months: a matched case-control study*. PLoS One. 2017 Aug 14;12(8):e0183191. doi:10.1371/journal.pone.0183191.
27. Essink B, Sabharwal C, Cannon K, Pérez JL, Peng Y, Rupp R, *et al.* Pivotal phase 3 randomized clinical trial of the safety, tolerability, and immunogenicity of 20-valent pneumococcal conjugate vaccine in adults aged ≥18 years. Clin Infect Dis. 2022;75(3):390–8. doi:10.1093/cid/ciab1014.
28. Platt HL, Bruno C, Buntinx E, *et al. Safety, tolerability, and immunogenicity of an adult pneumococcal conjugate vaccine, V116 (PCV21), in healthy adults: phase 1/2, randomised, double-blind, active comparator-controlled, multicentre, US-based trial*. Lancet Infect Dis. 2024;24(11):1141–50. doi:10.1016/S1473-3099(24)00157-3.
29. Løchen A, Croucher NJ, Anderson RM. *Divergent serotype replacement trends and increasing diversity in pneumococcal disease in high income settings reduce the benefit of expanding vaccine valency*. Sci Rep. 2020 Nov 4;10(1):18977. doi:10.1038/s41598-020-75691-5.
30. Red Nacional de Vigilancia Epidemiológica (RENAVE). Situación de la enfermedad neumocócica invasiva en España. Año 2023. *Boletín Epidemiológico Semanal* [Internet]. 2024;32(2):17–31 [cited 2025 Jun 12]. Disponible en: https://revista.isciii.es/index.php/bes/article/view/1381/1685.
31. Paavilainen H, Rantala S, Lyytikäinen O, *et al. Second reported outbreak of pneumococcal pneumonia among shipyard employees in Turku, Finland, Aug–Oct 2023: a case–control study*. Epidemiol Infect. 2025;153:e32. doi:10.1017/S0950268824001870.

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