Effect of pneumococcal conjugate vaccines and SARS-CoV-2 on antimicrobial resistance and the emergence of *Streptococcus pneumoniae* serotypes with reduced susceptibility in Spain, 2004–20: a national surveillance study

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Summary

Background Epidemiological studies are necessary to explore the effect of current pneumococcal conjugate vaccines (PCVs) against antibiotic resistance, including the rise of non-vaccine serotypes that are resistant to antibiotics. Hence, epidemiological changes in the antimicrobial pattern of *Streptococcus pneumoniae* before and during the first year of the COVID-19 pandemic were studied.

Methods In this national surveillance study, we characterised the antimicrobial susceptibility to a panel of antibiotics in 3017 pneumococcal clinical isolates with reduced susceptibility to penicillin during 2004–20 in Spain. This study covered the early and late PCV7 periods; the early, middle, and late PCV13 periods; and the first year of the COVID-19 pandemic, to evaluate the contribution of PCVs and the pandemic to the emergence of non-vaccine serotypes associated with antibiotic resistance.

Findings Serotypes included in PCV7 and PCV13 showed a decline after the introduction of PCVs in Spain. However, an increase in non-PCV13 serotypes (mainly 11A, 24F, and 23B) that were not susceptible to penicillin promptly appeared. A rise in the proportion of pneumococcal strains with reduced susceptibility to β-lactams and erythromycin was observed in 2020, coinciding with the emergence of SARS-CoV-2. Cefditoren was the β-lactam with the lowest minimum inhibitory concentration (MIC_{50} or MIC_{90} values, and had the highest proportion of susceptible strains throughout 2004–20.

Interpretation The increase in non-PCV13 serotypes associated with antibiotic resistance is concerning, especially the increase of penicillin resistance linked to serotypes 11A and 24F. The future use of PCVs with an increasingly broad spectrum (such as PCV20, which includes serotype 11A) could reduce the impact of antibiotic resistance for non-PCV13 serotypes. The use of antibiotics to prevent co-infections in patients with COVID-19 might have affected the increased proportion of pneumococcal-resistant strains. Cefotaxime as a parenteral option, and cefditoren as an oral choice, were the antibiotics with the highest activity against non-PCV20 serotypes.

Introduction

Invasive pneumococcal disease and community-acquired bacterial pneumonia are infectious diseases of high priority for prevention as they are associated with high morbidity and mortality rates.[1,2] *Streptococcus pneumoniae* (also known as pneumococcus) is the most common cause of community-acquired bacterial pneumonia and is one of the most frequent causes of bacterial meningitis and sepsis.[1,2] Pneumococcal conjugate vaccine (PCV) use is the best prophylactic strategy to prevent invasive pneumococcal disease and community-acquired bacterial pneumonia in children,[2] although several clinical trials have also shown great effectiveness in the adult population.[3,4] In Spain, PCV7 was first used in 2001,

although mainly in private practice, and vaccine coverage was less than 50% before 2006.[5] PCV10 was authorised for use in 2009, but promptly replaced by PCV13 in 2010. PCV13 was highly prescribed by paediatricians and in 2016 was included in the national immunisation schedule of the Spanish public health system, leading to high vaccine coverage rates. In adults, pneumococcal vaccine coverage rates are not made public, although in 2018 they were 22% for Spanish regions that used PCV13, and 26% for those that used pneumococcal polysaccharide vaccine (PPV)23.[5] A marked reduction in the incidence of invasive pneumococcal disease caused by PCV13 serotypes has been reported in Spain, not only in children but also in adults, owing to the herd-

Research in context

Evidence before this study

We searched PubMed on Jan 2, 2021 for studies in children and adults published between Jan 1, 2004, and Dec 31, 2020, using the terms "invasive pneumococcal disease" and/or "serotypes", and "pneumococcal conjugate vaccines", and "antibiotic resistance", and "SARS-CoV-2", with no language restrictions. We screened the search results, which included population-based studies and observational studies related to the epidemiology of invasive pneumococcal disease caused by antimicrobial-resistant strains affecting adults before and after the introduction of pneumococcal conjugate vaccines (PCVs). Overall, studies that included countries that had introduced PCVs in childhood immunisation programmes reported a reduction in overall invasive pneumococcal disease, and a decline in incidence by vaccine serotypes, including serotypes with antibiotic resistance. Herd-immunity protection in adults has been observed in countries with long-term use of PCVs in children, upholding the importance of indirect protection conferred by PCVs. The replacement of serotypes after PCV13 has been subject to geographical discrepancies globally.

Added value of this study

Different PCVs have been used since 2001 (with the introduction of PCV7, followed by PCV13) and it is important to determine the effect of these vaccines on the epidemiology of resistant strains. Such knowledge is especially important given the COVID-19 pandemic, in which many antibiotics have been prescribed at hospital and community level to prevent the potential risk of co-infection by bacterial pathogens, and the

resulting threat of increased resistance is of concern. In this national-level longitudinal study in Spain, 2004–20, we evaluated the evolution of pneumococcal strains resistant to a range of antibiotics, including penicillin, amoxicillin, cefotaxime, erythromycin, levofloxacin, and third-generation oral cephalosporins such as cefixime, cefpodoxime, and cefotaxime. We also analysed the patterns of antibiotic resistance before and during the first year of the COVID-19 pandemic, to see if there were variations that might be attributable to the use of antibiotics to prevent co-infections in patients infected by SARS-CoV-2.

Implications of all the available evidence

The study shows a reduction in vaccine-serotypes displaying antibiotic resistance after the introduction of PCV7 and PCV13, confirming the importance of these vaccines in controlling the problem of antibiotic resistance. However, a rise in non-PCV13 serotypes that harbour resistance (including 11A, 24F, and 23B), has been observed in the past 5 years. Future vaccines that contain additional serotypes associated with antibiotic resistance will partly solve this problem by increasing potential coverage against some of these emerging non-vaccine serotypes. Our data suggest that the increased proportion of resistant strains during the first year of the COVID-19 pandemic should be taken into consideration regarding the use of antibiotics as a routine strategy to prevent bacterial co-infections, as such use could exacerbate the problem of antibiotic resistance.

immunity effects of paediatric vaccination.[5] Another important benefit of using PCVs is their contribution to lowering the burden of antimicrobial resistance, by controlling serotypes that have reduced susceptibility.[6] However, an increase in non-PCV13 serotypes, mainly in adults, might jeopardise the effectiveness of this vaccine.[5,7]

There have been constant increases in serotypes associated with antimicrobial resistance, with declines in susceptibility rates after the introduction of PCVs and increases in non-PCV serotypes after PCVs were implemented in the paediatric population.[8] In addition, the emergence of multidrug-resistant serotype 19A isolates was reported shortly after the introduction of PCV7 globally.[8] This occurrence is consistent with a 2011 report[9] that explored antimicrobial resistance rates in *S pneumoniae* globally, which showed that susceptibility rates had decreased throughout the years in particular regions. Therefore, we did a national longitudinal study to characterise the evolution of antibiotic susceptibility throughout 16 years (2004–20), with a special focus on third-generation oral cephalosporins, because in Spain these antibiotics are widely used to treat patients with pneumonia who have not been hospitalised. Another major goal of our study was to evaluate the contributions of PCV7, PCV13,

and the COVID-19 pandemic to the emergence of non-vaccine serotypes that are associated with antibiotic resistance. Studies published in the past 2 years suggested that *S pneumoniae* could interact with SARS-CoV-2.[10,11] Vaccination with PCV13 has been associated with a reduced risk of COVID-19 diagnosis, hospitalisation, and mortality in patients infected by SARS-CoV-2.[10] Furthermore, pneumococcal carriage has been linked with impaired anti-SARS-CoV-2 immune responses, affecting mucosal IgA concentrations in individuals with mild or asymptomatic infection and the cellular memory responses in most patients who are infected.[11] Hence, vaccination using PCVs that reduces both the duration of and the number of people in the carrier state could preserve the immune response against SARS-CoV-2 and be the reason for a lowered risk of COVID-19.[10,11]

Methods

Study design

In this national surveillance study, we characterised 3017 clinical isolates that were non-susceptible to penicillin, which we received at the Spanish Pneumococcal Reference Laboratory (Madrid, Spain) in 2004–20. These isolates were from adult patients hospitalised with invasive pneumococcal disease or non-bacteraemic

pneumococcal pneumonia. We did not include strains from adults with meningitis. We also analysed the effect of PCVs in the epidemiology of *S pneumoniae* strains with reduced susceptibility to penicillin assessed at different time periods. We compared 2019 (pre-COVID-19) and 2020 (COVID-19) to analyse the effect of SARS-CoV-2 in the antimicrobial susceptibility of *S pneumoniae* (appendix 2, pp 2–3).

We included around 500 clinical isolates that were non-susceptible to penicillin and had a minimum inhibitory concentration (MIC) of at least 0·12 μg/mL from 2004 (early PCV7 period), 2008 (late PCV7 period), 2012 (early PCV13 period), 2016 (middle PCV13 period), 2019 (late PCV13 and pre-COVID-19 period), and 2020 (COVID-19 period; appendix 2, p 3). These strains were obtained using our collection programme at the Spanish Pneumococcal Reference Laboratory for strains from hospitals distributed throughout the entire country. To avoid possible bias, we did a random selection using the RAND function in Microsoft Excel (2016 [Windows]) from our database of collected strains to ensure a general distribution from around the country.

This study was done as a public health investigation with internal approval from Instituto de Salud Carlos III (Madrid, Spain) for the characterisation of pneumococcal strains; as such, external ethics committee approval was not required.

Characterisation of pneumococcal serotypes and antibiotic susceptibility

Serotyping was done by Quellung reaction and dot blot assay using specific antisera, or PCR sequencing.[5,12] For antimicrobial susceptibility, we analysed different β-lactam antibiotics that included penicillin, amoxicillin, cefotaxime, cefditoren, cefixime, and cefpodoxime. We also analysed other antibiotic groups, such as erythromycin as a representative macrolide and levofloxacin to represent fluoroquinolones. PCV7 vaccine contains serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. PCV13 vaccine contains PCV7 serotypes plus 1, 3, 5, 6A, 7F, and 19A. PCV15 vaccine contains PCV13 serotypes plus 22F and 33F, and PCV20 contains PCV15 serotypes plus 8, 10A, 11A, 12F, and 15B.

Antibiotic susceptibility was evaluated by the test diffusion method and the MIC values were determined by the agar dilution technique in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, using EUCAST breakpoint recommendations for data interpretation.[8] For those antibiotics without a defined breakpoint by EUCAST or the Clinical and Laboratory Standards Institute such as cefixime and cefditoren, we used the same breakpoints as cefotaxime (appendix 2, p 4).

Statistical analysis

Statistical analysis was done by using a two-tailed Student's *t*-test (for two-group comparisons), and ANOVA followed by Dunnett's post-hoc test was used for multiple

comparisons. The effect of vaccination against resistant serotypes was calculated by comparing the incidence rates of resistant strains during the different periods and calculating the incidence rate ratio (IRR) with 95% CI using Poisson regression models. The effect of SARS-CoV-2 in the rise of pneumococcal-resistant strains was measured using Fisher's exact test. GraphPad InStat (version 8.0; GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Differences were considered significant if p<0·05 and highly significant if p<0·01 or p<0·001.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

To characterise the evolution of the clinical isolates by their susceptibility pattern 2004–20, we used EUCAST criteria to categorise the strains as fully susceptible, susceptible with increased exposure, or resistant (figure 1; appendix 2, p 4). With cefotaxime, more than 40% of strains were susceptible with increased exposure,
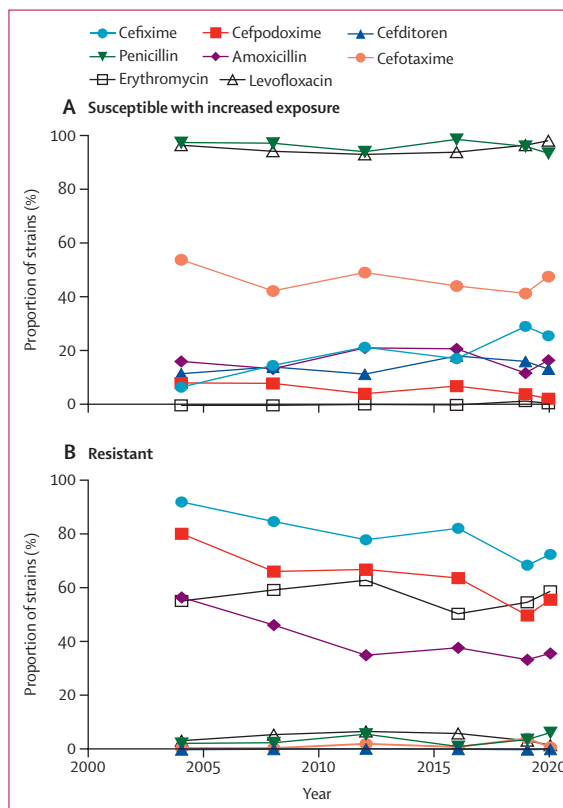
Figure 1: Evolution of *Streptococcus pneumoniae* strains based on their susceptibility to antibiotics, 2004–20
Strains were categorised using European Committee on Antimicrobial Susceptibility Testing criteria as susceptible with increased exposure to antibiotics (A) or resistant to antibiotics (B).
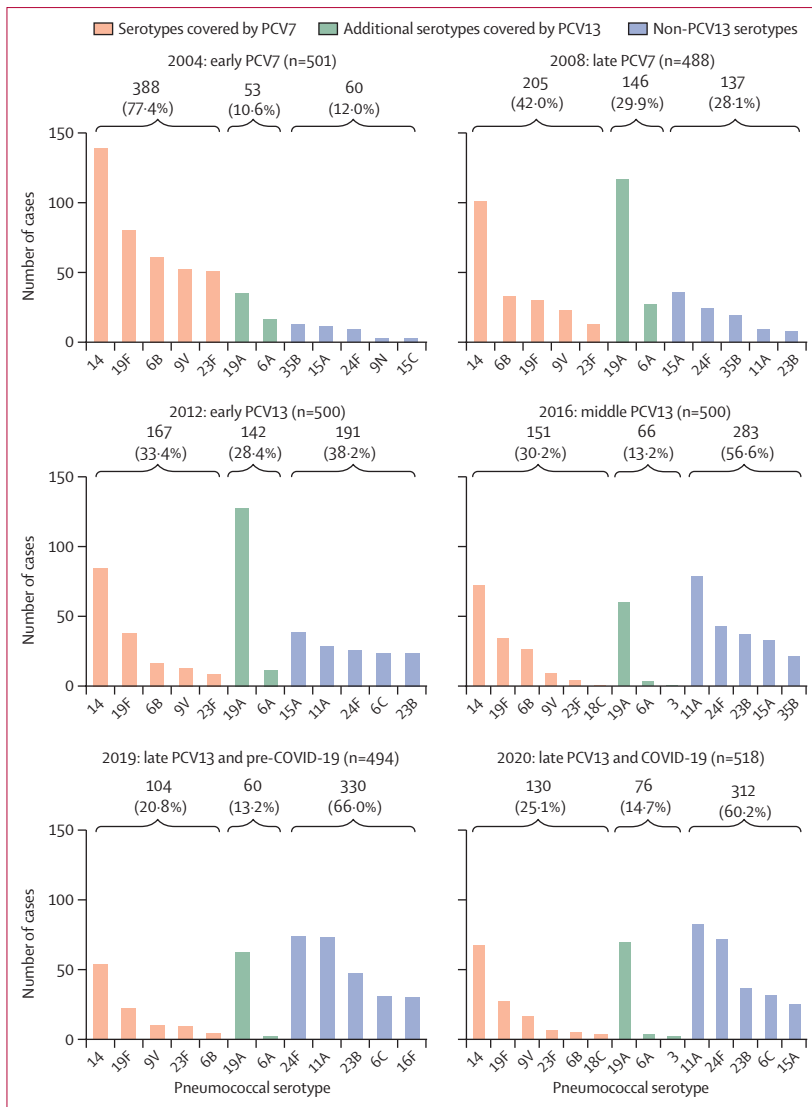
Figure 2: Evolution of pneumococcal serotypes with reduced susceptibility to penicillin, 2004–20
The number and proportion of pneumococcal cases caused by serotypes covered by PCV7, additional serotypes covered by PCV13, or not included in PCV13 (the top five or five most frequent) that were either susceptible with increased exposure or resistant to penicillin. Reduced susceptibility was defined as a minimum inhibitory concentration of 0·12 μg/mL or higher. PCV=pneumococcal conjugate vaccine.

periods), suggesting that these vaccines were effective in controlling the emergence of resistant strains. However, a trend towards a moderate increase in the proportion of resistant strains was observed for some antibiotics in the late PCV13 period (figure 1B). A comparison of the 2019 (pre-COVID-19) and 2020 (COVID-19) periods showed an increase (p<0·05) in the proportion of strains that were resistant to various antibiotics, such as penicillin (3% in 2019 vs 6% in 2020), amoxicillin (33% vs 36%), cefixime (68% vs 72%), cefpodoxime (50% vs 56%), and erythromycin (55% vs 59%), but showed no differences for cefditoren or levofloxacin (figure 1B). For cefotaxime, which is widely used in Spanish hospitals as a parenteral antibiotic against respiratory and systemic infection, we also found an increase in the proportion of strains with reduced susceptibility during the first COVID-19 pandemic year (42% in 2019 vs 48% in 2020; figure 1A). Cefditoren was the antibiotic showing the highest proportion of susceptible strains (>81%), followed by cefotaxime (>45%) and erythromycin (>37%; appendix 2, p 5). By contrast, cefixime, followed by cefpodoxime, had the lowest proportion of susceptible strains (appendix 2, p 5). We evaluated the contribution of pneumococcal vaccination (PCV7 until 2009 and PCV13 since 2010) to the national epidemiology of pneumococcal strains with reduced susceptibility to penicillin and either reduced susceptibility with increased exposure or resistance to erythromycin (figures 2, 3). The susceptibility with increased exposure or resistance of pneumococcal serotypes included in PCV7 and PCV13 decreased in the middle and late periods after the introduction of these vaccines (PCV7: IRR 0·31 [95% CI 0·26–0·38] for penicillin vs 0·35 [0·27–0·46] for erythromycin; PCV13: 0·37 [0·32–0·43] for penicillin vs 0·38 [0·31–0·47] for erythromycin). Serotype 14 accounted for the highest proportion of non-susceptible strains, showing a constant and steady trend in the last 5 years of the study period, especially for penicillin (figures 2 and 3). A reduction between 2004 and 2020 in PCV13 strains with susceptibility with increased exposure or resistance to penicillin (88% in 2004 vs 40% in 2020) and erythromycin (88% vs 46%) was obtained after the introduction of these PCVs, strengthening the importance of these vaccines in the fight against antibiotic resistance (figures 2 and 3). In the case of erythromycin, because we selected strains that had penicillin susceptibility with increased exposure or resistance, a limitation of our study was that we did not measure the effect of PCVs against strains that were fully susceptible to penicillin, but resistant to erythromycin. Also, an increase in non-susceptible strains belonging to serotype 19A was observed from 2008, coinciding with the late-PCV7 period (figures 2 and 3). Hence, the use of PCV13 enabled the control of serotype 19A strains that had reduced susceptibility to penicillin and

followed by (in order of declining percentages) cefixime, amoxicillin, cefditoren, cefpodoxime and erythromycin (figure 1A). Among resistant strains, the antibiotic associated with the highest proportion of resistant strains was cefixime (>68%), followed by cefpodoxime (>50%), erythromycin (>50%), and amoxicillin (>33%; figure 1B). In addition, the antibiotics showing the lowest proportion of resistant strains during the study period were cefditoren (<0·4%), cefotaxime (<5%), penicillin (<6·5%), and levofloxacin (<7%; figure 1B). A decrease in the proportion of resistant strains was observed after the introduction of PCVs (ie, in the late PCV7 and early-to-middle PCV13

erythromycin, although in the past 5 years, a situation of stability was observed for both penicillin and erythromycin (figures 2 and 3). We found an increase in non-PCV13 strains that had susceptibility with increased exposure or resistance since the introduction of both of these PCVs (12% in 2004 vs 54% in 2020 for erythromycin and 12% vs 60% for penicillin; figures 2 and 3). With non-PCV13 serotypes, we observed an increase of serotype 11A strains that were not susceptible to penicillin and an increase of serotype 24F strains that were not susceptible to penicillin or erythromycin (figures 2 and 3). With penicillin resistance, currently serotype 11A, followed by serotype 24F, are the two most frequent causes of pneumococcal disease caused by non-susceptible strains, and account for 30% of all cases associated with reduced susceptibility to penicillin. For simultaneous resistance to penicillin and erythromycin, epidemiological data suggested that serotype 24F was responsible for 24% of all cases, with a secondary role for serotype 11A, as only 5% of cases caused by this serotype had resistance to both antibiotics.

To evaluate the effect of PCVs and SARS-CoV-2 in the MIC values to β-lactams, we explored the evolution of MIC_{50} and MIC_{90}, analysing the three most prevalent PCV13 serotypes (19A, 14, and 19F) and non-PCV13 serotypes (11A, 24F, and 23B) associated with reduced susceptibility (ie, susceptibility with increased exposure or resistance; table). Among third-generation oral cephalosporins, cefixime had the highest MIC_{50} and MIC_{90} values, irrespective of the serotype, followed by cefpodoxime, whereas cefditoren was the most active cephalosporin showing the lowest MIC_{50} or MIC_{90} value, which was even lower than for cefotaxime—one of the most widely used parenteral cephalosporins against invasive pneumococcal disease (table). Overall, these MIC_{50} or MIC_{90} values indicated that cefotaxime, and cefditoren to a greater extent, were the β-lactam antibiotics with the highest activity against the most frequent serotypes with susceptibility with increased exposure or resistance to penicillin. Hence, our results showed that cefditoren achieved the lowest MIC values between 2004 and 2020, which was significant compared with each β-lactam antibiotic, including cefotaxime (p<0·001, two-tailed Student *t*-test) and even if multiple comparisons were done with oral cephalosporins such as cefixime and cefpodoxime (p<0·01 for one-way ANOVA followed by Dunnett´s post-hoc test). In addition, PCV13 serotypes (19A, 14, and 19F), and serotype 11A as a non-PCV13 serotype, had higher MIC_{50} or MIC_{90} values than all of these cephalosporins compared with serotypes 24F and 23B.

For penicillin and amoxicillin, the three most frequent PCV13 serotypes (19A, 14, and 19F) had higher MIC_{50} or MIC_{90} values than the non-PCV13 serotypes 24F and 23B. However, serotype 11A (which is not included in PCV13 but is included in PCV20 and PPV23) was the serotype with the highest MIC_{50} or MIC_{90} values since 2008, being even higher than the three PCV13 serotypes studied
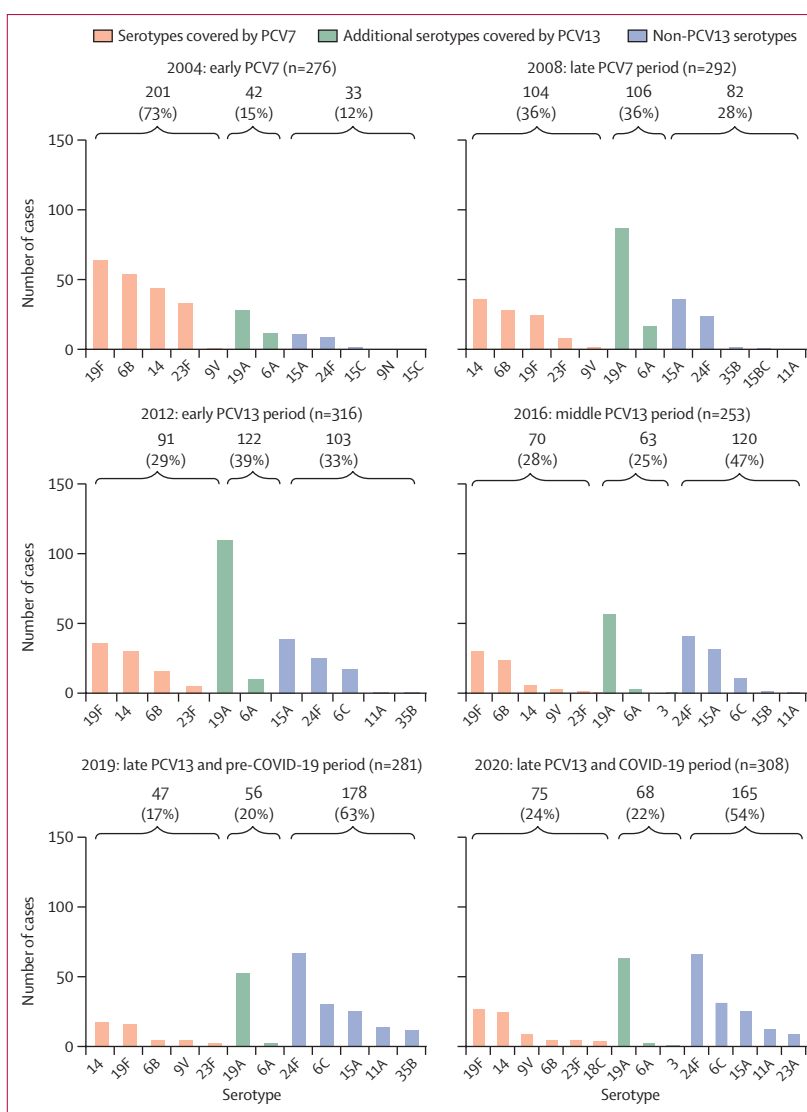

Figure 3: Evolution of pneumococcal serotypes with reduced susceptibility to erythromycin among strains that are either susceptible with increased exposure or resistant to penicillin, 2004–20
The number and proportion of pneumococcal cases caused by serotypes covered by PCV7, additional serotypes covered by PCV13, or not included in PCV13 (the top five or five most frequent) that were either susceptible with increased exposure or resistant to penicillin or erythromycin. Reduced susceptibility was defined as a minimum inhibitory concentration of 0·5 μg/mL or higher. PCV=pneumococcal conjugate vaccine.

(table). In terms of antibiotic resistance and SARS-CoV-2, we found an increase in the MIC_{90} values to penicillin for serotype 11A, which changed the interpretation from susceptible with increased exposure to resistant. Hence, the MIC_{90} value for serotype 11A increased from 2 μg/mL in 2016–19 to 4 μg/mL in 2020 (table).

In this study, we explored the proportion of pneumococcal disease caused by strains with reduced susceptibility to different antibiotics that are potentially covered by different PCVs and PPV23 (figure 4). During the late PCV7 and early PCV13 periods (2008–12), the majority of pneumococcal cases associated with reduced susceptibility were caused by PCV13 serotypes (figure 4).

	2004		2008		2012		2016		2019		2020	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Serotype 19A, mg/L												
Cefixime	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00
Cefpodoxime	2·00	2·00	2·00	4·00	2·00	4·00	4·00	4·00	4·00	4·00	2·00	4·00
Cefditoren	0·50	0·50	0·50	1·00	0·50	1·00	1·00	1·00	1·00	1·00	0·50	1·00
Penicillin	0·50	1·00	1·00	2·00	2·00	4·00	1·00	2·00	2·00	2·00	2·00	2·00
Amoxicillin	0·50	2·00	0·50	4·00	2·00	4·00	2·00	4·00	4·00	4·00	2·00	4·00
Cefotaxime	0·50	1·00	0·50	1·00	1·00	2·00	1·00	2·00	2·00	4·00	2·00	2·00
Serotype 14, mg/L												
Cefixime	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00
Cefpodoxime	2·00	4·00	2·00	4·00	2·00	4·00	2·00	4·00	2·00	4·00	2·00	4·00
Cefditoren	0·50	1·00	0·50	1·00	0·50	1·00	1·00	1·00	1·00	2·00	0·50	1·00
Penicillin	1·00	2·00	1·00	2·00	1·00	2·00	1·00	2·00	2·00	4·00	2·00	2·00
Amoxicillin	2·00	8·00	2·00	8·00	1·00	4·00	1·00	4·00	1·00	8·00	1·00	2·00
Cefotaxime	1·00	2·00	1·00	2·00	1·00	2·00	1·00	2·00	2·00	4·00	1·00	2·00
Serotype 19F, mg/L												
Cefixime	8·00	16·00	8·00	16·00	16·00	16·00	16·00	16·00	8·00	16·00	16·00	16·00
Cefpodoxime	1·00	2·00	1·00	2·00	2·00	4·00	2·00	4·00	1·00	2·00	2·00	4·00
Cefditoren	0·25	0·50	0·25	0·50	0·50	0·50	0·50	1·00	0·25	0·50	0·50	1·00
Penicillin	0·50	1·00	0·5	2·00	1·00	2·00	1·00	2·00	0·50	2·00	1·00	2·00
Amoxicillin	1·00	2·00	1·00	4·00	1·00	2·00	1·00	4·00	0·50	2·00	1·00	4·00
Cefotaxime	0·25	1·00	0·25	1·00	1·00	1·00	1·00	2·00	0·50	2·00	1·00	2·00
Serotype 11A, mg/L												
Cefixime	8·00	8·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00	16·00
Cefpodoxime	0·50	0·50	2·00	2·00	2·00	2·00	2·00	2·00	2·00	2·00	2·00	2·00
Cefditoren	0·25	0·50	0·50	0·50	0·50	0·50	0·50	0·50	0·50	1·00	0·50	0·50
Penicillin	0·25	0·25	2·00	2·00	2·00	4·00	2·00	2·00	2·00	2·00	2·00	4·00
Amoxicillin	0·50	0·50	4·00	8·00	4·00	8·00	4·00	4·00	4·00	8·00	4·00	8·00
Cefotaxime	0·25	0·25	1·00	1·00	1·00	2·00	1·00	1·00	1·00	2·00	1·00	2·00
Serotype 24F, mg/L												
Cefixime	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00
Cefpodoxime	0·25	0·50	0·25	0·25	0·25	0·25	0·25	0·50	0·25	0·25	0·25	0·25
Cefditoren	0·12	0·12	0·12	0·12	0·12	0·12	0·12	0·25	0·12	0·25	0·12	0·25
Penicillin	0·50	0·50	0·50	1·00	0·50	1·00	0·50	0·50	0·50	1·00	0·50	1·00
Amoxicillin	0·12	0·25	0·12	0·50	0·06	0·12	0·06	0·12	0·06	0·12	0·06	0·12
Cefotaxime	0·12	0·25	0·25	0·25	0·25	0·50	0·25	0·25	0·25	0·50	0·25	0·25
Serotype 23B, mg/L												
Cefixime	4·00	4·00	2·00	2·00	2·00	4·00	2·00	2·00	2·00	4·00	2·00	2·00
Cefpodoxime	0·25	0·25	0·12	0·12	0·12	1·00	0·12	0·25	0·12	1·00	0·12	0·12
Cefditoren	0·12	0·12	0·06	0·06	0·06	0·25	0·06	0·12	0·06	0·25	0·06	0·06
Penicillin	0·25	0·25	0·12	0·25	0·25	0·25	0·25	0·25	0·25	0·50	0·25	0·50
Amoxicillin	0·12	0·12	0·12	0·25	0·06	0·25	0·06	0·06	0·06	0·50	0·06	0·12
Cefotaxime	0·12	0·12	0·06	0·25	0·12	0·25	0·12	0·25	0·12	0·50	0·12	0·25
MIC=minimum inhibitory concentration.												

Table: MIC_{50} and MIC_{90} values for the three most prevalent PCV13 and non-PCV13 serotypes against different β-lactam antibiotics, 2004–20

Our results suggest that in comparison with PCV13 or PCV15, PCV20 would increase by up to 30% the potential coverage of cases by strains with reduced susceptibility to β-lactams (figure 4). Overall, the use of PPV23, despite containing three more serotypes than PCV20, offered similar protection against resistant strains (figure 4).

From the antibiotic perspective, for cefditoren and cefotaxime (which were the cephalosporins showing the best antimicrobial activity), the use of PCV20 would prevent more than 92% of all cases produced by pneumococcal strains that have reduced susceptibility to those antibiotics (figure 4).

Discussion

Antibiotic treatment with β-lactam antibiotics, including the use of third-generation cephalosporins, is one the first options for the management of pneumococcal infections.[13,14] A major threat in public health is the rise of resistant strains that can increase mortality rates by reducing the efficacy of antibiotic treatment.[15] The use of PCVs in children and adults has been shown to be an effective intervention to control the burden of invasive and non-invasive disease and a great measure to reduce the effect of antimicrobial resistance.[16,17]

In this study, we analysed the evolution of antimicrobial resistance in *S pneumoniae* in strains not susceptible to penicillin, including the contribution of different PCVs, to ameliorate the problem of antibiotic resistance. One of the main mechanisms for reduced susceptibility to β-lactam antibiotics (including penicillins and cephalosporins) is the mutation in penicillin-binding proteins.[18] Our results showed that the cephalosporin with the highest activity in terms of MIC_{50} or MIC_{90} values was cefditoren, which showed the greatest proportion (>80%) of susceptible strains during 2004–20. These results are in agreement with previous reports[19,20] that suggested a marked activity of this cephalosporin against penicillin-resistant pneumococcal strains, because of its high affinity to penicillin-binding protein 2X (PBP2X). Owing to its high antimicrobial activity, the proportion of strains resistant to cefditoren in our study was extremely low (<0·4%), despite the long-term use of this oral antibiotic in Spain since 2004.[21] These results were substantially different to those for the other oral cephalosporins tested, which had far higher proportions of resistant strains (68% for cefixime and 50% for cefpodoxime). Cefditoren, followed by cefotaxime, were the cephalosporins with the highest activity against serotypes during the study period. This activity is important against respiratory infections, because cefditoren has a similar bacterial spectrum to cefotaxime or ceftriaxone, and can be used as an oral treatment against community-acquired bacterial pneumonia in patients who have not been hospitalised or after intravenous treatment with parenteral cephalosporins.[21–23] Another benefit of using cefditoren is that, because its intrinsic activity is higher than for other cephalosporins, it could help to reduce the length of hospital stay and thereby the risk of hospital-acquired infection by multidrug-resistant strains.[24] Levofloxacin was one of the antibiotics with the lowest proportion of resistant strains. This result was in agreement with a 2016 surveillance study[6] that compared different countries, which found that levofloxacin was one of the most active agents against multidrug-resistant pneumococcal strains.[6]

Our data show the effectiveness of the different PCVs for controlling the dissemination of pneumococcal-resistant strains, and suggest that the use of these vaccines in national immunisation schedules is a cost-effective countermeasure to antibiotic resistance.[16] In the pre-PCV period, the majority of cases were caused by serotypes
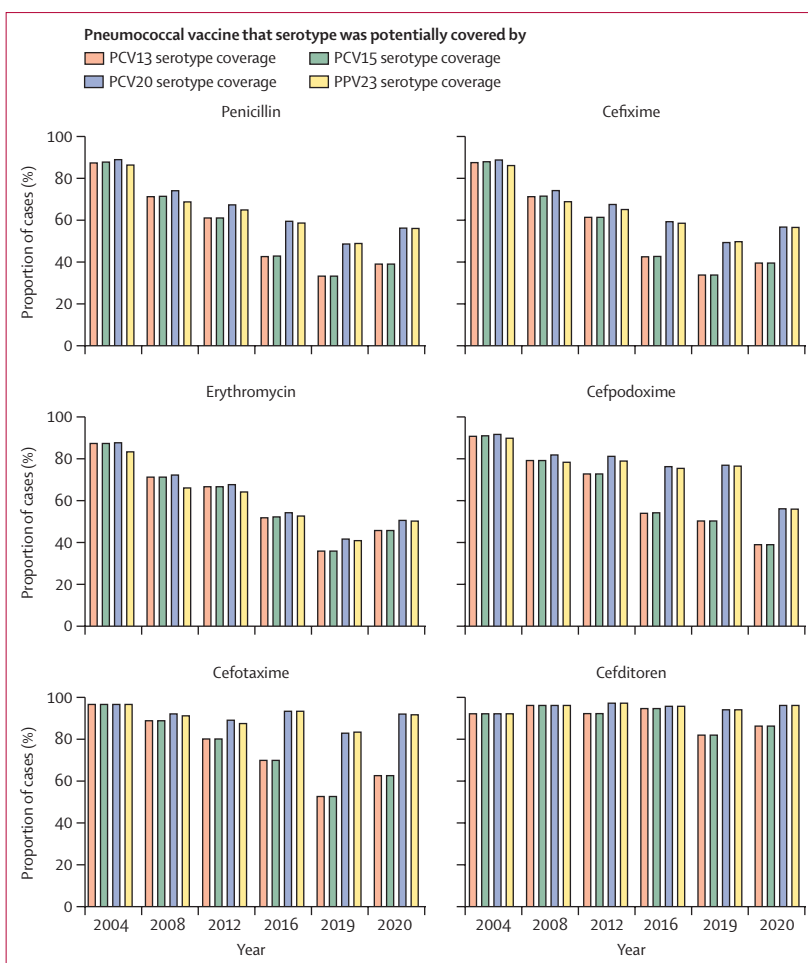

Figure 4: **Proportion of cases of pneumococcal disease caused by strains potentially covered by PCVs and PPV23 but which had reduced susceptibility to different antibiotics, 2004–20**
PCV=pneumococcal conjugate vaccine. PPV=pneumococcal polysaccharide vaccine.

included in the vaccines and were associated with multidrug resistance.[8,15,25] Our results reinforce this relationship, but also show a clear benefit in reducing vaccine-serotypes after the use of PCV7 and PCV13, although for serotype 19A, a situation of stability was observed in the past 5 years. This stability is intriguing, because PCV13 was included in the national paediatric immunisation schedule, which has had high coverage rates since 2016 and which led to the expectation of a more profound effect. This plateau perhaps therefore indicates the maximum benefit that can be achieved after several years of use. However, the emergence in our study of non-vaccine serotypes that harbour antibiotic resistance shows that this issue is a global threat, given that many other countries have reported similar replacement in pneumococcal serotypes and lineages.[5,25–27]

The emergence of penicillin-resistant strains of sero-type 11A is concerning from a pathogenesis perspective. This serotype contains a particular clone (ST6521[11A]) that has become one of the most prevalent among serotype

11A, with an increased ability to produce biofilms and invasive disease by very efficiently diverting the host immune response.[15] Hence, the profound potential of this serotype to produce infection might explain why serotype 11A was the serotype with the second highest fatality rate in a lethality study.[28] Another non-PCV13 serotype that has emerged is serotype 24F. This serotype is also alarming, because it displays resistance to penicillin and erythromycin, and its prevalence in the paediatric and adult population is increasing in various countries.[5]

A limitation of our study is that from each year we selected around 500 strains with penicillin susceptibility with increased exposure or resistance, rather than all pneumococcal strains, and therefore our results might underestimate the potential effect of PCVs in reducing the burden of disease caused by resistant serotypes. We did not include paediatric strains and, although the majority of serotypes affecting children are similar to those in adults,[5] our results might not be generalisable to children.

During the first year of the COVID-19 pandemic, generic use of antibiotics to avoid co-infections with bacterial pathogens might explain the increased proportion of pneumococcal strains resistant to different antimicrobial drugs.[29] This idea is consistent with a clinical trial[30] published in 2021 that advised against the routine use of azithromycin in people with suspected COVID-19 in the community because it might exacerbate the antimicrobial resistance problem. The increased resistance to penicillin for serotype 11A in Spain during the COVID-19 pandemic is worrying and deserves further attention, because it changes the consideration from a serotype with reduced susceptibility to a serotype that is resistant, according to MIC_{90} values in 2016–2019 and 2020.

The introduction of newer PCVs with a broader spectrum of covered serotypes might help to resolve the problem of non-PCV13 serotypes with antibiotic resistance. The difference in the effect of PCV15 compared with PCV13 was minimal in terms of increased coverage against non-susceptible strains to antibiotics, whereas PCV20 markedly enhanced the potential coverage against non-susceptible strains, as PCV20 could prevent 92% of strains not susceptible to cefotaxime.

In the context of using PCVs that have a higher spectrum, such as PCV20 (with the potential risk of replacement by non-vaccine serotypes after their implementation), the antibiotics with the highest activity against non-PCV20 strains were cefotaxime as a parenteral option, and cefditoren as an oral option. This finding might be important in helping to avoid the selection of resistant strains after massive use of this vaccine in the general population. For resistance to erythromycin, the potential coverage of PCV20 and PPV23 is more limited than for penicillin because they did not prevent cases caused by serotype 24F, which was the most frequent cause of infection associated with erythromycin resistance.

Overall, our results support the potential of cefditoren as an oral administration option for pneumococcal disease, based on its high antimicrobial activity, and highlight the increase in non-PCV13 serotypes, especially serotype 11A, which can be further prevented by the use of PCV20 or PPV23.

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