A national surveillance study exploring the impact of pneumococcal conjugate vaccines and SARS-CoV-2 on antimicrobial resistance during 2004-2020 alerts the emergence of *Streptococcus pneumoniae* serotypes with reduced susceptibility

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Summary

Background Epidemiological studies are necessary to explore the impact of current pneumococcal conjugate vaccines (PCVs) against antibiotic resistance including the rise of non-vaccine resistant serotypes. Hence, epidemiological changes in the antimicrobial pattern of *S. pneumoniae* before and during the first SARS-CoV-2 pandemic year were studied.

Methods We characterized the antimicrobial susceptibility to a panel of antibiotics in 3017 pneumococcal clinical isolates with reduced susceptibility to penicillin during 2004-2020. This study covered early/late PCV7 period, early/middle/late PCV13 periods and the first year of COVID-19 to evaluate the contribution of PCVs and the recent pandemic SARS-CoV-2 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 these vaccines in Spain. However, an increase of non-PCV13 serotypes, mainly 11A, 24F, and 23B, with non-susceptibility 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 MIC_{90} / MIC_{50} levels and had the highest proportion of susceptible strains throughout 2004-2020.

Interpretation The rise of non-PCV13 serotypes associated with antibiotic resistance is worrisome, especially the increase of penicillin resistance linked to serotypes 11A and 24F. The future use of PCVs with broader spectrum such as PCV20 that includes serotype 11A could partially ameliorate the impact of antibiotic resistance by non-PCV13 serotypes. The use of antibiotics to prevent co-infections in SARS-CoV-2 patients might have influenced 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.

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Research in context

Evidence before this study

Antimicrobial resistance is a global problem worldwide and of great concern in Public Health. We searched Pubmed using the terms "invasive pneumococcal disease" and/or "serotypes", as well as "pneumococcal conjugate vaccines" and "antibiotic resistance", "antibiotic resistance" and "SARS-CoV-2" with no language restrictions including data from children and adults. We screened the studies published between the periods 2004 and 2020 including population-based studies and observational studies related to the epidemiology of IPD caused by resistant strains affecting adults before and after the introduction of pneumococcal conjugate vaccines (PCVs). Overall, studies including countries that have introduced PCVs in childhood immunization programs, report a reduction in global IPD and decline in incidence by vaccine serotypes including those with antibiotic resistance. Herd protection to adults has also been observed in countries with long-term use of PCVs in children, upholding the importance of indirect protection conferred by PCVs. Replacement of serotypes after PCV13 has suffered geographical discrepancies.

Added value of this study

In the context of using different PCVs since 2001 with the introduction of PCV7 followed by PCV13 it is important to know the impact of these vaccines on the epidemiology of resistant strains. This is important in the current situation of a pandemic by SARS-CoV-2 when many antibiotics have been prescribed at the hospital and community level to prevent the potential risk of co-infections with bacterial pathogens. In this sense, a threat of increased resistance during the pandemic period is worrisome and needs to be explored. In this national longitudinal study during the period 2004-2020, we have evaluated the evolution of pneumococcal resistant strains to a panel of antibiotics including penicillin, amoxicillin, cefotaxime, erythromycin, levofloxacin and 3rd generation oral cephalosporins such as cefixime, cefpodoxime, and cefotaxime. In addition, we have analyzed the patterns of antibiotic resistance before and during the first pandemic year by SARS-CoV-2 to see variations that might be attributable to the use of antibiotics to prevent co-infections in patients infected by SARS-CoV-2.

Implication of all available evidence

The study demonstrates the reduction of vaccine-serotypes displaying antibiotic resistance after the introduction of PCV7 and PCV13, confirming the importance of these vaccines to control the antibiotic resistance problem. However, a prompt rise of non-PCV13 serotypes including 11A, 24F and 23B, harboring resistance, is observed in the last years. The contribution of future vaccines containing additional serotypes to antibiotic resistance will partially solve the problem by increasing the potential coverage against some of these emerging non-vaccine serotypes. Our data demonstrating an increased proportion of resistant strains during the first pandemic year of SARS-CoV-2 may be useful to reconsider the protocol of using antibiotics as a routine strategy to prevent bacterial co-infections as this can exacerbate the antibiotic resistance problem.

Introduction

Invasive pneumococcal disease (IPD) and community-acquired bacterial pneumonia (CABP) are infectious diseases of high priority to be prevented because they are associated with high morbidity and mortality rates.[1,2] *Streptococcus pneumoniae*, also termed pneumococcus, is the most common etiologic agent of CABP and one of the most frequent causes of bacterial meningitis and sepsis.[1,2] Pneumococcal conjugate vaccines (PCVs) are the best prophylactic strategies to prevent IPD and CABP in children, although in the last years several clinical trials have shown great effectiveness in the adult population.[3,4] In Spain, PCV7 was first used in 2001, although it was mainly attributed to private practice with a coverage below 50% before 2006.[5] PCV10 was authorized in 2009 but it was promptly replaced by PCV13 in 2010. Although PCV13 was mainly administered for private use, it had a good acceptance and it was in 2016 when this vaccine was included in the pediatric calendar of the public health system with high coverage rates. In adults pneumococcal vaccine coverage rates are unavailable publicly although in 2018 it was 22% for Spanish regions using PCV13 and 26% for Spanish regions using PPV23.[5] A marked reduction in the incidence of IPD by PCV13 serotypes has recently been reported in Spain not only in children but also in adults due to herd effects by the pediatric vaccination.[5] Another important benefit after the use of PCVs is their contribution reducing the burden of antimicrobial resistance by controlling serotypes with reduced susceptibility.[6] However, the increase of non-PCV13 serotypes in the last years, mainly in adults, might jeopardize the effectiveness of this vaccine.[5,7]

The rise of serotypes associated with antimicrobial resistance has been constant with declines after the introduction of PCVs and increases of non-PCV serotypes after their implementation in the pediatric population.[8] In addition, the emergence of multidrug resistance (MDR) isolates of serotype 19A isolates was reported shortly after the introduction of PCV7 worldwide.[8] This is consistent with a recent report exploring the global antimicrobial resistance rates in *S. pneumoniae* worldwide showing that susceptibility rates have decreased throughout the years in particular regions.[9] Hence, we have performed a national longitudinal study to characterize the evolution of antibiotic susceptibility during the last 16 years (period 2004-2020) with a special focus on third oral cephalosporins as they are widely used antibiotics in Spain to treat non-hospitalized pneumonia cases. In addition, another major goal of the study was to evaluate the contribution of PCV7, PCV13 and the recent pandemic SARS-CoV-2 to the emergence of non-vaccine serotypes associated with antibiotic resistance. In this sense, recent studies suggest that *S. pneumoniae* may interact with SARS-CoV-2.[10,11] Vaccination with PCV13 has been associated with a reduced risk of COVID-19 diagnosis, hospitalization and mortality in patients infected by SARS-CoV-2.[10] Moreover, pneumococcal carriage has been linked with impaired anti-SARS-CoV-2 immune responses, affecting mucosal IgA levels among individuals with mild or asymptomatic infection and cellular memory responses in infected patients.[11] Hence, vaccination with PCVs that reduces the carrier state may preserve the immune response against SARS-CoV-2 and might be the reason for the lower risk of COVID-19.[10 11]

Methods

Study design

In this study, we have characterized 3017 non-susceptible clinical isolates to penicillin received at the Spanish Pneumococcal Reference Laboratory (SPRL) during the period 2004-2020. These isolates were from adult population hospitalized with IPD or with non-bacteraemic pneumococcal pneumonia. We did not include strains from adults with meningitis. We also analyzed the impact of PCVs in the epidemiology of *S. pneumoniae* strains with reduced susceptibility to penicillin assessing at different periods. A comparison of 2019 (pre-COVID-19) and 2020 (COVID-19) years was performed to analyze the impact of SARS-CoV-2 in the antimicrobial susceptibility of *S. pneumoniae* **(Figure S1 and Table S1).**

We included around 500 clinical isolates (non-susceptible to penicillin; minimum inhibitory concentration MIC ≥0·12 µg/ml) from the years 2004 (early PCV7), 2008 (late PCV7), 2012 (early PCV13), 2016 (middle PCV13), 2019 (late PCV13 and pre-COVID-19), and 2020 (COVID-19) **(Table S1).** These strains were selected using our strain collection at the SPRL from hospitals distributed throughout the entire country. To avoid possible bias, we performed a random selection from our database to assure a generic distribution around the country.

Characterization of pneumococcal serotypes and antibiotic susceptibility

Serotyping was performed by Quellung reaction, dot blot assay using specific antisera, and/or by PCR-sequencing.[5,12] For antimicrobial susceptibility, we analyzed different β-lactam antibiotics including penicillin, amoxicillin, cefotaxime, cefditoren, cefixime, and cefpodoxime. In addition, we analyzed other, antibiotic groups such as erythromycin and levofloxacin as representatives for macrolides and fluoroquinolones respectively. PCV7 vaccine contains serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. PCV13 vaccine contains PCV7 plus 1, 3 and 6A. PCV15 vaccine contains PCV13 serotypes plus 22F and 33F whereas PCV20 contains PCV15 serotypes plus 8, 10A, 11A, 12F and 15A.

Antibiotic susceptibility was evaluated by the test diffusion method and the MICs were determined by the agar dilution technique in accordance with the EUCAST criteria, following their breakpoints recommendations for data interpretation.[8] For those antibiotics without a defined breakpoint by EUCAST or CLSI such as cefixime and cefditoren, we used the same breakpoints as cefotaxime **(Table S2).**

Statistical analysis

Statistical analysis was performed by using a two-tailed Student's *t*-test (for two group's comparisons), whereas analysis of variance (ANOVA) followed by a Dunnett's post hoc test was chosen for multiple comparisons. The impact of vaccination against resistant serotypes was calculated by comparing the rates during the different periods and calculating the incidence rate ratio (IRR) with 95% confidence interval (CI) using Poisson regression models. The impact of SARS-CoV-2 in the rise of pneumococcal resistant strains was measured using a Fisher´s exact test. GraphPad InStat version 8.0 (GraphPad Software, San Diego, CA) was used for statistical analysis. Differences were considered statistically significant with $P < 0.05$ (*) and highly significant with $P < 0.01$ (**) and $P < 0.001$ (***).

Results

To characterize the evolution during 2004-2020 of the clinical isolates depending on their susceptibility pattern, we categorized the strains following EUCAST criteria as fully susceptible (S)**,** susceptible with increased exposure (I), and resistant (R) **(Figure 1 and S2)**. In the case of cefotaxime, more than 40% of strains were susceptible with increased exposure followed by cefixime, amoxicillin, cefditoren, cefpodoxime and erythromycin **(Figure 1A)**. Among resistant strains, cefixime was the antibiotic associated with a higher proportion of resistant strains (>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%), followed by cefotaxime (<5%), penicillin (<6·5%) and levofloxacin (<7%) **(Figure 1B)**. Interestingly, a decrease in the proportion of resistant strains was observed after the introduction of PCVs (late PCV7 and early/middle-PCV13 periods) confirming that these vaccines have been effective to control the emergence of resistant strains. However, a moderate increasing trend to certain antibiotics was observed in the late-PCV13 period **(Figure 1B).** Comparison of 2019 (Pre-COVID-19) with 2020 (COVID-19) periods, also showed a rise (P <0·05) in the proportion of resistant strains to different antibiotics such as penicillin (3% vs 6%), amoxicillin (33% vs 36%), cefixime (68% vs 72%), cefpodoxime (50% vs 56%), and erythromycin (55% vs 59%) with no differences in the case of cefditoren and levofloxacin **(Figure 1B)**. For cefotaxime, which is widely used in hospitals as a parenteral antibiotic against respiratory and systemic infection, we also found an increase in the proportion of strains with reduced susceptibility (42% vs 48%) during the first SARS-CoV-2 pandemic year **(Figure 1A)**. Cefditoren was the antibiotic showing the highest proportion of susceptible strains (>81%) followed by cefotaxime (>45%) and erythromycin (>37%) **(Figure S2)**. In contrast, cefixime followed by cefpodoxime had the lowest proportion of susceptible strains **(Figure S2)**.

The contribution of pneumococcal vaccination using PCV7 until 2009 followed by PCV13 since 2010 to the national epidemiology of pneumococcal strains with reduced susceptibility to penicillin (PEN) and erythromycin (ERY) strains (I/R) was evaluated **(Figures 2 and 3)**. Pneumococcal serotypes I/R included in PCV7 and PCV13 decreased in the middle and late periods after the introduction of these vaccines. For PCV7 (IRR, 0·31; CI 0·26-0·38 for PEN-I/R and IRR, 0·35; CI 0·27-0·46 for ERY-I/R). For PCV13 (IRR, 0·37; CI 0·32-0·43 for PEN-I/R and IRR, 0·38; CI 0·31-0·47 for ERY I/R). Serotype 14 accounted for the highest proportion of non-susceptible strains with a constant and steady trend in the last years, especially for penicillin **(Figures 2 and 3)**. A reduction of PCV13 strains (I/R) from 88% in 2004 for both antibiotics to 40-46% in the year 2020 to penicillin and erythromycin respectively was obtained after the introduction of these PCVs confirming the importance of these vaccines against antibiotic resistance **(Figures 2 and 3).** In the case of erythromycin, as we selected PEN-I/R strains, a limitation of our study is that we did not measure the impact of PCVs against PEN-S strains that are ERY-R. In addition, an increase of 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 allowed the control of serotype 19A strains with reduced susceptibility to penicillin and erythromycin although, in the last years, a situation of stability is observed for both antibiotics **(Figures 2 and 3)**. Interestingly, we found an increase of non-PCV13 strains (I/R) since the introduction of both PCVs, ranging from 12% in 2004 to 54-60% in the year 2020 for erythromycin and penicillin respectively **(Figures 2 and 3).** In terms of non-PCV13 serotypes, we have observed an increase of

strains PEN I/R associated with serotype 11A and an increase of serotype 24F strains with non-susceptibility to PEN and ERY **(Figures 2 and 3).** In the case of penicillin resistance, serotype 11A followed by serotype 24F, are currently the two most frequent causes of pneumococcal disease caused by non-susceptible strains, accounting for 30% of all cases associated with reduced susceptibility to this antibiotic. For simultaneous resistance to penicillin and erythromycin, epidemiological data from the last years confirmed that serotype 24F was responsible for 24% of all cases with a secondary role for serotype 11A as only 5% of cases by this serotype had resistance to both antibiotics.

To evaluate the impact of PCVs and SARS-CoV-2 in the MIC values to β-lactams, we explored the evolution of MIC_{50} **(Table 1)** and MIC_{90} **(Table 1)**, analyzing the three most prevalent PCV13 serotypes (19A, 14 and 19F) and non-PCV13 serotypes (11A, 24F and 23B) associated to reduced susceptibility (I/R). Among third-generation oral cephalosporins, cefixime had the highest $MIC_{50/90}$ values irrespective of the serotype, followed by cefpodoxime, whereas cefditoren was the most active cephalosporin showing the lowest $MIC_{50/90,}$ being even lower than cefotaxime, which is one of the most widely used parenteral cephalosporins against IPD **(Table 1).** Overall, these $MIC_{50\text{-}90}$ results indicate that cefotaxime and cefditoren to a higher extent were the β-lactam antibiotics with the highest activity against the most frequent (I/R) serotypes. Hence, our results showing that cefditoren achieved the lowest MIC levels along the period 2004-2020 were statistically significant in comparison to each β-lactam antibiotic including cefotaxime (P <0·001, two-tailed Student *t*-test) and even when multiple comparisons were performed with oral cephalosporins such as cefixime and cefpodoxime (P <0·01, one-way ANOVA followed by a Dunnett´s post hoc test). In addition, PCV13 serotypes (19A, 14 and 19F) and serotype 11A as non-PCV13 serotype had higher $MIC_{50/90}$ values to all these cephalosporins compared to serotypes 24F and 23B.

For penicillin and amoxicillin, the three most frequent PCV13 serotypes (19A, 14 and 19F) had higher $MIC_{50/90}$ levels 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 $MIC_{50/90}$ values since 2008 being even higher than the three PCV13 serotypes studied **(Table 1).** In terms of antibiotic resistance and SARS-CoV-2, we found an increase of MIC_{90} to penicillin for serotype 11A that changes the interpretation from susceptible with increased exposure (I) to resistant (R). Hence, the MIC_{90} value for serotype 11A increased from 2 µg/ml in the period 2016-2019 to 4 µg/ml in 2020 **(Table 1).**

In this study, we explored the fraction 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 (years 2008 to 2012), the majority of pneumococcal cases associated with reduced susceptibility were caused by PCV13 serotypes **(Figure 4).** Our results show that PCV20 would increase up to 30% the potential coverage of cases by strains with reduced susceptibility to β-lactams in comparison to PCV13 or PCV15 **(Figure 4)**. Overall, the use of PPV23 despite containing three additional serotypes than PCV20 offered similar protection against resistant strains **(Figure 4).**

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

Discussion

Antibiotic treatment with β-lactam antibiotics including the use of 3rd generation cephalosporins is one the first options to manage pneumococcal infections.[13,14] A major threat in public health is the rise of resistant strains that can increase the mortality rates by limiting the efficacy of the antibiotic treatment.[15] The use of PCVs in children and adults has been demonstrated as an effective intervention to control the burden of invasive and non-invasive disease and a great measure to reduce the impact of antimicrobial resistance.[16,17]

In this study, we have analyzed the evolution of antimicrobial resistance in *S. pneumoniae* in penicillin non-susceptible strains 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 (PBPs).[18] Our results showed that the cephalosporin with the highest activity in terms of $MIC_{50/90}$ was cefditoren, showing the greater proportion of susceptible strains (>80%) during the last 16 years. These results are in agreement with previous reports confirming a marked activity of this cephalosporin against penicillin-resistant pneumococcal strains due to its high affinity to PBP2x.[19,20] Due to its high antimicrobial activity, the proportion of resistant strains to cefditoren in our study was dramatically low (<0·4%), despite the long-term use of this oral antibiotic in Spain since 2004.[21] These results are substantially different from those obtained with the other oral cephalosporins tested (cefixime and cefpodoxime) with more than 68% and 50% of resistant strains respectively. In addition, cefditoren followed by cefotaxime were the cephalosporins with higher activity during the study period. This is important against respiratory infections because cefditoren has a similar bacterial spectrum to cefotaxime or ceftriaxone, and can be used as oral treatment against non-hospitalized CABP or after intravenous treatment with parenteral cephalosporins.[21-23] Additional benefits to de-escalate the treatment using cefditoren is because the intrinsic activity of cefditoren is higher and it may help to reduce the hospitalization duration, preventing therefore, the risk of acquiring hospital-associated infections by MDR strains.[24] Levofloxacin was one of the antibiotics with the lowest proportion of resistant strains. This is in agreement with a recent surveillance study comparing different countries being levofloxacin one the most active agents against MDR pneumococcal strains.[6]

Our data demonstrate the effectiveness of the different PCVs to control the dissemination of pneumococcal resistant strains, confirming that the implementation of these vaccines in national immunization calendars is a cost-effective measure against the antibiotic resistance problem.[16] In the pre-PCV period, the majority of cases were caused by serotypes included in the vaccines and were associated with MDR resistance.[8,15,25] Our results, confirmed this phenomenon but also demonstrate a clear benefit in reducing vaccine-serotypes after the use of PCV7 and PCV13, although for serotype 19A, a situation of stability is observed in the last years. This is intriguing because PCV13 was included in the national immunization pediatric calendar with high coverage rates since 2016 and therefore, a more profound impact would have been expected, suggesting that this plateau is maybe the maximum benefit that can be achieved after several years of use. However, the emergence of non-vaccine serotypes harboring antibiotic resistance in our

study, confirms that this is a global threat as many other countries have reported similar replacement in pneumococcal serotypes and lineages.[5,25-27]

The emergence of penicillin-resistant strains of serotype 11A is worrisome from the 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 diverting very efficiently the host immune response.[15] Hence, the profound potential of this serotype to produce infection might explain why serotype 11A was the second serotype with the highest fatality rate in a recent lethality study.[28] Another non-PCV13 serotype that is emerging in the last years is serotype 24F. This serotype is also alarming because displays resistance to penicillin and erythromycin and its prevalence in the pediatric and adult population is increasing in different countries.[5]

As limitations for our study, we selected around 500 PEN-I/R strains in each year and not all the pneumococcal PEN/IR strains and therefore, our results may underestimate the potential impact of PCVs reducing the burden of disease caused by resistant serotypes. We did not include pediatric strains and although the majority of serotypes affecting children are similar than those in adults[5], our results might not be applied for children.

During the first year of the SARS-CoV-2 pandemic, the generic use of antibiotics to avoid co-infections with bacterial pathogens might explain the increased proportion of pneumococcal resistant strains to different antimicrobial drugs.[29] This is consistent with a recent clinical trial advising against the routine use of azithromycin for people with suspected COVID-19 in the community because it may exacerbate the antimicrobial resistance problem.[30] The increased resistance to penicillin for serotype 11A in Spain during the SARS-CoV-2 pandemic is worrisome and deserves further attention because it changes the consideration from being a serotype with reduced susceptibility to resistant according to the MIC_{90} values between 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 impact of PCV15 compared to 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 non-susceptible strains to cefotaxime.

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

Overall, our results confirm the potential of cefditoren as one of the best options for oral administration based on its high antimicrobial activity and alarm the increase of non-PCV13 serotypes, especially serotype 11A that can be prevented by the use of PCV20.

Contributors

JY is the scientific leader of the national reference laboratory and was responsible for the management of the epidemiological surveillance data. JY wrote the first draft of the paper.

ML, JS, BLR, IDR, CPG, DL, MG, PC, FGC, MD and JY provided technical support for the study. MG, PC, MD, and JY contributed to the study conception, design, data analysis and interpretation. All authors contributed to the review of the different drafts, and approved all versions of the manuscript.

Declaration of interests

JY has received grants from MSD-USA (MISP Call), and Pfizer that are not related to this work. JY has participated in advisory boards organized by MSD, and Pfizer. MG and PC are members of the Medical Department of Meiji Pharma Spain. The other authors declare no competing interests.

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Figure legends

Figure 1: Evolution of the different strains based on their susceptibility pattern to different antibiotics during the period 2004-2020.

The strains were categorized as susceptible with increased exposure (A) and resistant (B). CFX (cefixime), CPD (cefpodoxime), CDN (cefditoren), PEN (penicillin), AMX (amoxicillin), CTX (cefotaxime), ERY (erythromycin), and LVX (levofloxacin).

Figure 2: Evolution of pneumococcal serotypes with reduced susceptibility to penicillin (MIC ≥0·12 µg/ml) during the period 2004-2020.

Number of IPD cases by PCV7 serotypes (red bars), by additional serotypes included in PCV13 (green bars), and by Non-PCV13 serotypes (blue bars). The values on top of the bars on the charts indicate the number and proportion of pneumococcal PEN I/R cases caused by serotypes covered by PCV7, PCV13 or not included in PCV13.

Figure 3: Evolution of pneumococcal serotypes with reduced susceptibility to erythromycin (MIC ≥0·5 µg/ml) among PEN-I/R strains during the period 2004-2020.

Number of IPD cases by PCV7 serotypes (red bars), by additional serotypes included in PCV13 (green bars), and by Non-PCV13 serotypes (blue bars). The values on top of the bars on the charts indicate the number and proportion of pneumococcal PEN I/R and ERY-I/R cases caused by serotypes covered by PCV7, PCV13 or not included in PCV13.

Figure 4: Fraction of pneumococcal disease caused by strains with reduced susceptibility to different antibiotics that is potentially covered by different PCVs and PPV23 during the period 2004-2020.

Proportion of pneumococcal cases caused by serotypes included in PCV13 (red bars), PCV15 (green bars), PCV20 (blue bars) or PPV23 (black bars). Penicillin (A), Erythromycin (B), Cefotaxime (C), Cefixime (D), Cefpodoxime (E), and Cefditoren (F).

References

1. Aliberti S, Dela Cruz CS, Amati F, Sotgiu G, Restrepo MI. Community-acquired pneumonia. *Lancet* 2021; **398**(10303): 906-19.
2. Collaborators GLRI. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* 2018. *18* (11):1191-210.
3. Bonten MJ, Huijts SM, Bolkenbaas M, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *N Engl J Med* 2015; **372**(12): 1114-25.
4. Torres A, Menendez R, Espana PP, et al. The evolution and distribution of pneumococcal serotypes in adults hospitalized with community-acquired pneumonia in Spain using a serotype-specific urinary antigen detection test: The CAPA Study, 2011-2018. *Clin Infect Dis* 2021; **73**(6): 1075-85.
5. de Miguel S, Domenech M, González-Camacho F, et al. Nationwide trends of invasive pneumococcal disease in Spain from 2009 through 2019 in children and adults during the pneumococcal conjugate vaccine era. *Clin Infect Dis* 2021. 73(11):e3778-e3787.
6. Sader HS, Mendes RE, Le J, Denys G, Flamm RK, Jones RN. Antimicrobial susceptibility of *Streptococcus pneumoniae* from North America, Europe, Latin America, and the Asia-Pacific Region: results from 20 years of the SENTRY antimicrobial surveillance program (1997-2016). *Open Forum Infect Dis* 2019; **6**(Suppl 1): S14-S23.
7. Sempere J, de Miguel S, González-Camacho F, Yuste J, Domenech M. Clinical relevance and molecular pathogenesis of the emerging serotypes 22F and 33F of *Streptococcus pneumoniae* in Spain. *Front Microbiol* 2020; **11**(309).
8. Fenoll A, Granizo JJ, Giménez MJ, Yuste J, Aguilar L. Secular trends (1990-2013) in serotypes and associated non-susceptibility of *S. pneumoniae* isolates causing invasive disease in the pre-/post-era of pneumococcal conjugate vaccines in Spanish regions without universal paediatric pneumococcal vaccination. *Vaccine* 2015; **33**(42): 5691-9.
9. Rosen JB, Thomas AR, Lexau CA, et al. Geographic variation in invasive pneumococcal disease following pneumococcal conjugate vaccine introduction in the United States. *Clin Infect Dis* 2011; **53**(2): 137-43.
10. Lewnard JA, Bruxvoort KJ, Fischer H, et al. Prevention of COVID-19 among older adults receiving pneumococcal conjugate vaccine suggests interactions between Streptococcus pneumoniae and SARS-CoV-2 in the respiratory tract. *J Infect Dis* 2021.
11. Mitsi E, Reine J, Urban BC, et al. *Streptococcus pneumoniae* colonization associates with impaired adaptive immune responses against SARS-CoV-2. *J Clin Invest* 2022; **132**(7).
12. Bornstein DL, Schiffman G, Bernheimer HP, Austrian R. Capsulation of pneumococcus with soluble C-like (Cs) polysaccharide. I. Biological and genetic properties of Cs pneumococcal strains. *J Exp Med* 1968; **128**(6): 1385-400.
13. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007; **44 Suppl 2**: S27-72.
14. Tomczyk S, Jain S, Bramley AM, et al. Antibiotic prescribing for adults hospitalized in the etiology of pneumonia in the community study. *Open Forum Infect Dis* 2017; **4**(2): ofx088.
15. Aguinagalde L, Corsini B, Domenech A, et al. Emergence of amoxicillin-resistant variants of spain^{9v}-ST156 pneumococci expressing serotype 11A correlates with their ability to evade the host immune response. *PLoS One* 2015; **10**(9): e0137565.
16. Atkins KE, Flasche S. Vaccination to reduce antimicrobial resistance. *Lancet Glob Health* 2018; **6**(3): e252.

17. Andrejko K, Ratnasiri B, Hausdorff WP, Laxminarayan R, Lewnard JA. Antimicrobial resistance in paediatric *Streptococcus pneumoniae* isolates amid global implementation of pneumococcal conjugate vaccines: a systematic review and meta-regression analysis. *Lancet Microbe* 2021; **2**(9): e450-e60.
18. Nagai K, Davies TA, Jacobs MR, Appelbaum PC. Effects of amino acid alterations in penicillin-binding proteins (PBPs) 1a, 2b, and 2x on PBP affinities of penicillin, ampicillin, amoxicillin, cefditoren, cefuroxime, cefprozil, and cefaclor in 18 clinical isolates of penicillin-susceptible, -intermediate, and -resistant pneumococci. *Antimicrob Agents Chemother* 2002; **46**(5): 1273-80.
19. Yamada M, Watanabe T, Miyara T, et al. Crystal structure of cefditoren complexed with Streptococcus pneumoniae penicillin-binding protein 2X: structural basis for its high antimicrobial activity. *Antimicrob Agents Chemother* 2007; **51**(11): 3902-7.
20. Yang Q, Xu Y, Chen M, et al. In vitro activity of cefditoren and other comparators against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* causing community-acquired respiratory tract infections in China. *Diagn Microbiol Infect Dis* 2012; **73**(2): 187-91.
21. Granizo JJ, Giménez MJ, Barberan J, Coronel P, Gimeno M, Aguilar L. The efficacy of cefditoren pivoxil in the treatment of lower respiratory tract infections, with a focus on the per-pathogen bacteriologic response in infections caused by *Streptococcus pneumoniae* and *Haemophilus influenzae*: a pooled analysis of seven clinical trials. *Clin Ther* 2006; **28**(12): 2061-9.
22. Di Marco F, Braido F, Santus P, Scichilone N, Blasi F. The role of cefditoren in the treatment of lower community-acquired respiratory tract infections (LRTIs): from bacterial eradication to reduced lung inflammation and epithelial damage. *Eur Rev Med Pharmacol Sci* 2014; **18**(3): 321-32.
23. Monmaturapoj T, Montakantikul P, Mootsikapun P, Tragulpiankit P. A prospective, randomized, double dummy, placebo-controlled trial of oral cefditoren pivoxil 400mg once daily as switch therapy after intravenous ceftriaxone in the treatment of acute pyelonephritis. *Int J Infect Dis* 2012; **16**(12): e843-9.
24. Blasi F, Concia E, Del Prato B, et al. The most appropriate therapeutic strategy for acute lower respiratory tract infections: a Delphi-based approach. *J Chemother* 2017; **29**(5): 274-86.
25. Cassiolato AP, Almeida SCG, Andrade AL, Minamisava R, Brandileone MCC. Expansion of the multidrug-resistant clonal complex 320 among invasive *Streptococcus pneumoniae* serotype 19A after the introduction of a ten-valent pneumococcal conjugate vaccine in Brazil. *PLoS One* 2018; **13**(11): e0208211.
26. Lo SW, Gladstone RA, van Tonder AJ, et al. Pneumococcal lineages associated with serotype replacement and antibiotic resistance in childhood invasive pneumococcal disease in the post-PCV13 era: an international whole-genome sequencing study. *Lancet Infect Dis* 2019; **19**(7): 759-69.
27. Gladstone RA, Lo SW, Lees JA, et al. International genomic definition of pneumococcal lineages, to contextualise disease, antibiotic resistance and vaccine impact. *EBioMedicine* 2019; **43**: 338-46.
28. De Miguel S, Latasa P, Yuste J, et al. Age-dependent serotype-associated case-fatality rate in invasive pneumococcal disease in the autonomous community of madrid between 2007 and 2020. *Microorganisms* 2021; **9**(11): 2286.
29. Langford BJ, So M, Raybardhan S, et al. Antibiotic prescribing in patients with COVID-19: rapid review and meta-analysis. *Clin Microbiol Infect* 2021; **27**(4): 520-31.

30. Group PTC. Azithromycin for community treatment of suspected COVID-19 in people at increased risk of an adverse clinical course in the UK (PRINCIPLE): a randomised, controlled, open-label, adaptive platform trial. *Lancet* 2021; **397**(10279): 1063-74.

A national surveillance study exploring the impact of pneumococcal conjugate vaccines and SARS-CoV-2 on antimicrobial resistance during 2004-2020 alerts the emergence of *Streptococcus pneumoniae* serotypes with reduced susceptibility

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Summary

Background Epidemiological studies are necessary to explore the impact of current pneumococcal conjugate vaccines (PCVs) against antibiotic resistance including the rise of non-vaccine resistant serotypes. Hence, epidemiological changes in the antimicrobial pattern of *S. pneumoniae* before and during the first SARS-CoV-2 pandemic year were studied.

Methods We characterized the antimicrobial susceptibility to a panel of antibiotics in 3017 pneumococcal clinical isolates with reduced susceptibility to penicillin during 2004-2020. This study covered early/late PCV7 period, early/middle/late PCV13 periods and the first year of COVID-19 to evaluate the contribution of PCVs and the recent pandemic SARS-CoV-2 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 these vaccines in Spain. However, an increase of non-PCV13 serotypes, mainly 11A, 24F, and 23B, with non-susceptibility 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 MIC_{90} / MIC_{50} levels and had the highest proportion of susceptible strains throughout 2004-2020.

Interpretation The rise of non-PCV13 serotypes associated with antibiotic resistance is worrisome, especially the increase of penicillin resistance linked to serotypes 11A and 24F. The future use of PCVs with broader spectrum such as PCV20 that includes serotype 11A could partially ameliorate the impact of antibiotic resistance by non-PCV13 serotypes. The use of antibiotics to prevent co-infections in SARS-CoV-2 patients might have influenced 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.

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Research in context

Evidence before this study

Antimicrobial resistance is a global problem worldwide and of great concern in Public Health. We searched Pubmed using the terms "invasive pneumococcal disease" and/or "serotypes", as well as "pneumococcal conjugate vaccines" and "antibiotic resistance", "antibiotic resistance" and "SARS-CoV-2" with no language restrictions including data from children and adults. We screened the studies published between the periods 2004 and 2020 including population-based studies and observational studies related to the epidemiology of IPD caused by resistant strains affecting adults before and after the introduction of pneumococcal conjugate vaccines (PCVs). Overall, studies including countries that have introduced PCVs in childhood immunization programs, report a reduction in global IPD and decline in incidence by vaccine serotypes including those with antibiotic resistance. Herd protection to adults has also been observed in countries with long-term use of PCVs in children, upholding the importance of indirect protection conferred by PCVs. Replacement of serotypes after PCV13 has suffered geographical discrepancies.

Added value of this study

In the context of using different PCVs since 2001 with the introduction of PCV7 followed by PCV13 it is important to know the impact of these vaccines on the epidemiology of resistant strains. This is important in the current situation of a pandemic by SARS-CoV-2 when many antibiotics have been prescribed at the hospital and community level to prevent the potential risk of co-infections with bacterial pathogens. In this sense, a threat of increased resistance during the pandemic period is worrisome and needs to be explored. In this national longitudinal study during the period 2004-2020, we have evaluated the evolution of pneumococcal resistant strains to a panel of antibiotics including penicillin, amoxicillin, cefotaxime, erythromycin, levofloxacin and 3rd generation oral cephalosporins such as cefixime, cefpodoxime, and cefotaxime. In addition, we have analyzed the patterns of antibiotic resistance before and during the first pandemic year by SARS-CoV-2 to see variations that might be attributable to the use of antibiotics to prevent co-infections in patients infected by SARS-CoV-2.

Implication of all available evidence

The study demonstrates the reduction of vaccine-serotypes displaying antibiotic resistance after the introduction of PCV7 and PCV13, confirming the importance of these vaccines to control the antibiotic resistance problem. However, a prompt rise of non-PCV13 serotypes including 11A, 24F and 23B, harboring resistance, is observed in the last years. The contribution of future vaccines containing additional serotypes to antibiotic resistance will partially solve the problem by increasing the potential coverage against some of these emerging non-vaccine serotypes. Our data demonstrating an increased proportion of resistant strains during the first pandemic year of SARS-CoV-2 may be useful to reconsider the protocol of using antibiotics as a routine strategy to prevent bacterial co-infections as this can exacerbate the antibiotic resistance problem.

Introduction

Invasive pneumococcal disease (IPD) and community-acquired bacterial pneumonia (CABP) are infectious diseases of high priority to be prevented because they are associated with high morbidity and mortality rates.[1,2] *Streptococcus pneumoniae*, also termed pneumococcus, is the most common etiologic agent of CABP and one of the most frequent causes of bacterial meningitis and sepsis.[1,2] Pneumococcal conjugate vaccines (PCVs) are the best prophylactic strategies to prevent IPD and CABP in children, although in the last years several clinical trials have shown great effectiveness in the adult population.[3,4] In Spain, PCV7 was first used in 2001, although it was mainly attributed to private practice with a coverage below 50% before 2006.[5] PCV10 was authorized in 2009 but it was promptly replaced by PCV13 in 2010. Although PCV13 was mainly administered for private use, it had a good acceptance and it was in 2016 when this vaccine was included in the pediatric calendar of the public health system with high coverage rates. In adults pneumococcal vaccine coverage rates are unavailable publicly although in 2018 it was 22% for Spanish regions using PCV13 and 26% for Spanish regions using PPV23.[5] A marked reduction in the incidence of IPD by PCV13 serotypes has recently been reported in Spain not only in children but also in adults due to herd effects by the pediatric vaccination.[5] Another important benefit after the use of PCVs is their contribution reducing the burden of antimicrobial resistance by controlling serotypes with reduced susceptibility.[6] However, the increase of non-PCV13 serotypes in the last years, mainly in adults, might jeopardize the effectiveness of this vaccine.[5,7]

The rise of serotypes associated with antimicrobial resistance has been constant with declines after the introduction of PCVs and increases of non-PCV serotypes after their implementation in the pediatric population.[8] In addition, the emergence of multidrug resistance (MDR) isolates of serotype 19A isolates was reported shortly after the introduction of PCV7 worldwide.[8] This is consistent with a recent report exploring the global antimicrobial resistance rates in *S. pneumoniae* worldwide showing that susceptibility rates have decreased throughout the years in particular regions.[9] Hence, we have performed a national longitudinal study to characterize the evolution of antibiotic susceptibility during the last 16 years (period 2004-2020) with a special focus on third oral cephalosporins as they are widely used antibiotics in Spain to treat non-hospitalized pneumonia cases. In addition, another major goal of the study was to evaluate the contribution of PCV7, PCV13 and the recent pandemic SARS-CoV-2 to the emergence of non-vaccine serotypes associated with antibiotic resistance. In this sense, recent studies suggest that *S. pneumoniae* may interact with SARS-CoV-2.[10,11] Vaccination with PCV13 has been associated with a reduced risk of COVID-19 diagnosis, hospitalization and mortality in patients infected by SARS-CoV-2.[10] Moreover, pneumococcal carriage has been linked with impaired anti-SARS-CoV-2 immune responses, affecting mucosal IgA levels among individuals with mild or asymptomatic infection and cellular memory responses in infected patients.[11] Hence, vaccination with PCVs that reduces the carrier state may preserve the immune response against SARS-CoV-2 and might be the reason for the lower risk of COVID-19.[10 11]

Methods

Study design

In this study, we have characterized 3017 non-susceptible clinical isolates to penicillin received at the Spanish Pneumococcal Reference Laboratory (SPRL) during the period 2004-2020. These isolates were from adult population hospitalized with IPD or with non-bacteraemic pneumococcal pneumonia. We did not include strains from adults with meningitis. We also analyzed the impact of PCVs in the epidemiology of *S. pneumoniae* strains with reduced susceptibility to penicillin assessing at different periods. A comparison of 2019 (pre-COVID-19) and 2020 (COVID-19) years was performed to analyze the impact of SARS-CoV-2 in the antimicrobial susceptibility of *S. pneumoniae* **(Figure S1 and Table S1).**

We included around 500 clinical isolates (non-susceptible to penicillin; minimum inhibitory concentration MIC ≥0·12 µg/ml) from the years 2004 (early PCV7), 2008 (late PCV7), 2012 (early PCV13), 2016 (middle PCV13), 2019 (late PCV13 and pre-COVID-19), and 2020 (COVID-19) **(Table S1).** These strains were selected using our strain collection at the SPRL from hospitals distributed throughout the entire country. To avoid possible bias, we performed a random selection from our database to assure a generic distribution around the country.

Characterization of pneumococcal serotypes and antibiotic susceptibility

Serotyping was performed by Quellung reaction, dot blot assay using specific antisera, and/or by PCR-sequencing.[5,12] For antimicrobial susceptibility, we analyzed different β-lactam antibiotics including penicillin, amoxicillin, cefotaxime, cefditoren, cefixime, and cefpodoxime. In addition, we analyzed other, antibiotic groups such as erythromycin and levofloxacin as representatives for macrolides and fluoroquinolones respectively. PCV7 vaccine contains serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. PCV13 vaccine contains PCV7 plus 1, 3 and 6A. PCV15 vaccine contains PCV13 serotypes plus 22F and 33F whereas PCV20 contains PCV15 serotypes plus 8, 10A, 11A, 12F and 15A.

Antibiotic susceptibility was evaluated by the test diffusion method and the MICs were determined by the agar dilution technique in accordance with the EUCAST criteria, following their breakpoints recommendations for data interpretation.[8] For those antibiotics without a defined breakpoint by EUCAST or CLSI such as cefixime and cefditoren, we used the same breakpoints as cefotaxime **(Table S2).**

Statistical analysis

Statistical analysis was performed by using a two-tailed Student's *t*-test (for two group's comparisons), whereas analysis of variance (ANOVA) followed by a Dunnett's post hoc test was chosen for multiple comparisons. The impact of vaccination against resistant serotypes was calculated by comparing the rates during the different periods and calculating the incidence rate ratio (IRR) with 95% confidence interval (CI) using Poisson regression models. The impact of SARS-CoV-2 in the rise of pneumococcal resistant strains was measured using a Fisher´s exact test. GraphPad InStat version 8.0 (GraphPad Software, San Diego, CA) was used for statistical analysis. Differences were considered statistically significant with $P < 0.05$ (*) and highly significant with $P < 0.01$ (**) and $P < 0.001$ (***).

Results

To characterize the evolution during 2004-2020 of the clinical isolates depending on their susceptibility pattern, we categorized the strains following EUCAST criteria as fully susceptible (S)**,** susceptible with increased exposure (I), and resistant (R) **(Figure 1 and S2)**. In the case of cefotaxime, more than 40% of strains were susceptible with increased exposure followed by cefixime, amoxicillin, cefditoren, cefpodoxime and erythromycin **(Figure 1A)**. Among resistant strains, cefixime was the antibiotic associated with a higher proportion of resistant strains (>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%), followed by cefotaxime (<5%), penicillin (<6·5%) and levofloxacin (<7%) **(Figure 1B)**. Interestingly, a decrease in the proportion of resistant strains was observed after the introduction of PCVs (late PCV7 and early/middle-PCV13 periods) confirming that these vaccines have been effective to control the emergence of resistant strains. However, a moderate increasing trend to certain antibiotics was observed in the late-PCV13 period **(Figure 1B).** Comparison of 2019 (Pre-COVID-19) with 2020 (COVID-19) periods, also showed a rise (P <0·05) in the proportion of resistant strains to different antibiotics such as penicillin (3% vs 6%), amoxicillin (33% vs 36%), cefixime (68% vs 72%), cefpodoxime (50% vs 56%), and erythromycin (55% vs 59%) with no differences in the case of cefditoren and levofloxacin **(Figure 1B)**. For cefotaxime, which is widely used in hospitals as a parenteral antibiotic against respiratory and systemic infection, we also found an increase in the proportion of strains with reduced susceptibility (42% vs 48%) during the first SARS-CoV-2 pandemic year **(Figure 1A)**. Cefditoren was the antibiotic showing the highest proportion of susceptible strains (>81%) followed by cefotaxime (>45%) and erythromycin (>37%) **(Figure S2)**. In contrast, cefixime followed by cefpodoxime had the lowest proportion of susceptible strains **(Figure S2)**.

The contribution of pneumococcal vaccination using PCV7 until 2009 followed by PCV13 since 2010 to the national epidemiology of pneumococcal strains with reduced susceptibility to penicillin (PEN) and erythromycin (ERY) strains (I/R) was evaluated **(Figures 2 and 3)**. Pneumococcal serotypes I/R included in PCV7 and PCV13 decreased in the middle and late periods after the introduction of these vaccines. For PCV7 (IRR, 0·31; CI 0·26-0·38 for PEN-I/R and IRR, 0·35; CI 0·27-0·46 for ERY-I/R). For PCV13 (IRR, 0·37; CI 0·32-0·43 for PEN-I/R and IRR, 0·38; CI 0·31-0·47 for ERY I/R). Serotype 14 accounted for the highest proportion of non-susceptible strains with a constant and steady trend in the last years, especially for penicillin **(Figures 2 and 3)**. A reduction of PCV13 strains (I/R) from 88% in 2004 for both antibiotics to 40-46% in the year 2020 to penicillin and erythromycin respectively was obtained after the introduction of these PCVs confirming the importance of these vaccines against antibiotic resistance **(Figures 2 and 3).** In the case of erythromycin, as we selected PEN-I/R strains, a limitation of our study is that we did not measure the impact of PCVs against PEN-S strains that are ERY-R. In addition, an increase of 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 allowed the control of serotype 19A strains with reduced susceptibility to penicillin and erythromycin although, in the last years, a situation of stability is observed for both antibiotics **(Figures 2 and 3)**. Interestingly, we found an increase of non-PCV13 strains (I/R) since the introduction of both PCVs, ranging from 12% in 2004 to 54-60% in the year 2020 for erythromycin and penicillin respectively **(Figures 2 and 3).** In terms of non-PCV13 serotypes, we have observed an increase of

strains PEN I/R associated with serotype 11A and an increase of serotype 24F strains with non-susceptibility to PEN and ERY **(Figures 2 and 3).** In the case of penicillin resistance, serotype 11A followed by serotype 24F, are currently the two most frequent causes of pneumococcal disease caused by non-susceptible strains, accounting for 30% of all cases associated with reduced susceptibility to this antibiotic. For simultaneous resistance to penicillin and erythromycin, epidemiological data from the last years confirmed that serotype 24F was responsible for 24% of all cases with a secondary role for serotype 11A as only 5% of cases by this serotype had resistance to both antibiotics.

To evaluate the impact of PCVs and SARS-CoV-2 in the MIC values to β-lactams, we explored the evolution of MIC_{50} **(Table 1)** and MIC_{90} **(Table 1)**, analyzing the three most prevalent PCV13 serotypes (19A, 14 and 19F) and non-PCV13 serotypes (11A, 24F and 23B) associated to reduced susceptibility (I/R). Among third-generation oral cephalosporins, cefixime had the highest $MIC_{50/90}$ values irrespective of the serotype, followed by cefpodoxime, whereas cefditoren was the most active cephalosporin showing the lowest $MIC_{50/90,}$ being even lower than cefotaxime, which is one of the most widely used parenteral cephalosporins against IPD **(Table 1).** Overall, these $MIC_{50\text{-}90}$ results indicate that cefotaxime and cefditoren to a higher extent were the β-lactam antibiotics with the highest activity against the most frequent (I/R) serotypes. Hence, our results showing that cefditoren achieved the lowest MIC levels along the period 2004-2020 were statistically significant in comparison to each β-lactam antibiotic including cefotaxime (P <0·001, two-tailed Student *t*-test) and even when multiple comparisons were performed with oral cephalosporins such as cefixime and cefpodoxime (P <0·01, one-way ANOVA followed by a Dunnett´s post hoc test). In addition, PCV13 serotypes (19A, 14 and 19F) and serotype 11A as non-PCV13 serotype had higher $MIC_{50/90}$ values to all these cephalosporins compared to serotypes 24F and 23B.

For penicillin and amoxicillin, the three most frequent PCV13 serotypes (19A, 14 and 19F) had higher $MIC_{50/90}$ levels 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 $MIC_{50/90}$ values since 2008 being even higher than the three PCV13 serotypes studied **(Table 1).** In terms of antibiotic resistance and SARS-CoV-2, we found an increase of MIC_{90} to penicillin for serotype 11A that changes the interpretation from susceptible with increased exposure (I) to resistant (R). Hence, the MIC_{90} value for serotype 11A increased from 2 µg/ml in the period 2016-2019 to 4 µg/ml in 2020 **(Table 1).**

In this study, we explored the fraction 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 (years 2008 to 2012), the majority of pneumococcal cases associated with reduced susceptibility were caused by PCV13 serotypes **(Figure 4).** Our results show that PCV20 would increase up to 30% the potential coverage of cases by strains with reduced susceptibility to β-lactams in comparison to PCV13 or PCV15 **(Figure 4)**. Overall, the use of PPV23 despite containing three additional serotypes than PCV20 offered similar protection against resistant strains **(Figure 4).**

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

Discussion

Antibiotic treatment with β-lactam antibiotics including the use of 3rd generation cephalosporins is one the first options to manage pneumococcal infections.[13,14] A major threat in public health is the rise of resistant strains that can increase the mortality rates by limiting the efficacy of the antibiotic treatment.[15] The use of PCVs in children and adults has been demonstrated as an effective intervention to control the burden of invasive and non-invasive disease and a great measure to reduce the impact of antimicrobial resistance.[16,17]

In this study, we have analyzed the evolution of antimicrobial resistance in *S. pneumoniae* in penicillin non-susceptible strains 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 (PBPs).[18] Our results showed that the cephalosporin with the highest activity in terms of $MIC_{50/90}$ was cefditoren, showing the greater proportion of susceptible strains (>80%) during the last 16 years. These results are in agreement with previous reports confirming a marked activity of this cephalosporin against penicillin-resistant pneumococcal strains due to its high affinity to PBP2x.[19,20] Due to its high antimicrobial activity, the proportion of resistant strains to cefditoren in our study was dramatically low (<0·4%), despite the long-term use of this oral antibiotic in Spain since 2004.[21] These results are substantially different from those obtained with the other oral cephalosporins tested (cefixime and cefpodoxime) with more than 68% and 50% of resistant strains respectively. In addition, cefditoren followed by cefotaxime were the cephalosporins with higher activity during the study period. This is important against respiratory infections because cefditoren has a similar bacterial spectrum to cefotaxime or ceftriaxone, and can be used as oral treatment against non-hospitalized CABP or after intravenous treatment with parenteral cephalosporins.[21-23] Additional benefits to de-escalate the treatment using cefditoren is because the intrinsic activity of cefditoren is higher and it may help to reduce the hospitalization duration, preventing therefore, the risk of acquiring hospital-associated infections by MDR strains.[24] Levofloxacin was one of the antibiotics with the lowest proportion of resistant strains. This is in agreement with a recent surveillance study comparing different countries being levofloxacin one the most active agents against MDR pneumococcal strains.[6]

Our data demonstrate the effectiveness of the different PCVs to control the dissemination of pneumococcal resistant strains, confirming that the implementation of these vaccines in national immunization calendars is a cost-effective measure against the antibiotic resistance problem.[16] In the pre-PCV period, the majority of cases were caused by serotypes included in the vaccines and were associated with MDR resistance.[8,15,25] Our results, confirmed this phenomenon but also demonstrate a clear benefit in reducing vaccine-serotypes after the use of PCV7 and PCV13, although for serotype 19A, a situation of stability is observed in the last years. This is intriguing because PCV13 was included in the national immunization pediatric calendar with high coverage rates since 2016 and therefore, a more profound impact would have been expected, suggesting that this plateau is maybe the maximum benefit that can be achieved after several years of use. However, the emergence of non-vaccine serotypes harboring antibiotic resistance in our

study, confirms that this is a global threat as many other countries have reported similar replacement in pneumococcal serotypes and lineages.[5,25-27]

The emergence of penicillin-resistant strains of serotype 11A is worrisome from the 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 diverting very efficiently the host immune response.[15] Hence, the profound potential of this serotype to produce infection might explain why serotype 11A was the second serotype with the highest fatality rate in a recent lethality study.[28] Another non-PCV13 serotype that is emerging in the last years is serotype 24F. This serotype is also alarming because displays resistance to penicillin and erythromycin and its prevalence in the pediatric and adult population is increasing in different countries.[5]

As limitations for our study, we selected around 500 PEN-I/R strains in each year and not all the pneumococcal PEN/IR strains and therefore, our results may underestimate the potential impact of PCVs reducing the burden of disease caused by resistant serotypes. We did not include pediatric strains and although the majority of serotypes affecting children are similar than those in adults[5], our results might not be applied for children.

During the first year of the SARS-CoV-2 pandemic, the generic use of antibiotics to avoid co-infections with bacterial pathogens might explain the increased proportion of pneumococcal resistant strains to different antimicrobial drugs.[29] This is consistent with a recent clinical trial advising against the routine use of azithromycin for people with suspected COVID-19 in the community because it may exacerbate the antimicrobial resistance problem.[30] The increased resistance to penicillin for serotype 11A in Spain during the SARS-CoV-2 pandemic is worrisome and deserves further attention because it changes the consideration from being a serotype with reduced susceptibility to resistant according to the MIC_{90} values between 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 impact of PCV15 compared to 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 non-susceptible strains to cefotaxime.

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

Overall, our results confirm the potential of cefditoren as one of the best options for oral administration based on its high antimicrobial activity and alarm the increase of non-PCV13 serotypes, especially serotype 11A that can be prevented by the use of PCV20.

Contributors

JY is the scientific leader of the national reference laboratory and was responsible for the management of the epidemiological surveillance data. JY wrote the first draft of the paper.

ML, JS, BLR, IDR, CPG, DL, MG, PC, FGC, MD and JY provided technical support for the study. MG, PC, MD, and JY contributed to the study conception, design, data analysis and interpretation. All authors contributed to the review of the different drafts, and approved all versions of the manuscript.

Declaration of interests

JY has received grants from MSD-USA (MISP Call), and Pfizer that are not related to this work. JY has participated in advisory boards organized by MSD, and Pfizer. MG and PC are members of the Medical Department of Meiji Pharma Spain. The other authors declare no competing interests.

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Figure legends

Figure 1: Evolution of the different strains based on their susceptibility pattern to different antibiotics during the period 2004-2020.
The strains were categorized as susceptible with increased exposure (A) and resistant (B). CFX (cefixime), CPD (cefpodoxime), CDN (cefditoren), PEN (penicillin), AMX (amoxicillin), CTX (cefotaxime), ERY (erythromycin), and LVX (levofloxacin).

Figure 2: Evolution of pneumococcal serotypes with reduced susceptibility to penicillin (MIC ≥0·12 µg/ml) during the period 2004-2020.
Number of IPD cases by PCV7 serotypes (red bars), by additional serotypes included in PCV13 (green bars), and by Non-PCV13 serotypes (blue bars). The values on top of the bars on the charts indicate the number and proportion of pneumococcal PEN I/R cases caused by serotypes covered by PCV7, PCV13 or not included in PCV13.

Figure 3: Evolution of pneumococcal serotypes with reduced susceptibility to erythromycin (MIC ≥0·5 µg/ml) among PEN-I/R strains during the period 2004-2020.
Number of IPD cases by PCV7 serotypes (red bars), by additional serotypes included in PCV13 (green bars), and by Non-PCV13 serotypes (blue bars). The values on top of the bars on the charts indicate the number and proportion of pneumococcal PEN I/R and ERY-I/R cases caused by serotypes covered by PCV7, PCV13 or not included in PCV13.

Figure 4: Fraction of pneumococcal disease caused by strains with reduced susceptibility to different antibiotics that is potentially covered by different PCVs and PPV23 during the period 2004-2020.
Proportion of pneumococcal cases caused by serotypes included in PCV13 (red bars), PCV15 (green bars), PCV20 (blue bars) or PPV23 (black bars). Penicillin (A), Erythromycin (B), Cefotaxime (C), Cefixime (D), Cefpodoxime (E), and Cefditoren (F).

References

1. Aliberti S, Dela Cruz CS, Amati F, Sotgiu G, Restrepo MI. Community-acquired pneumonia. *Lancet* 2021; **398**(10303): 906-19.
2. Collaborators GLRI. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis* 2018. *18* (11):1191-210.
3. Bonten MJ, Huijts SM, Bolkenbaas M, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *N Engl J Med* 2015; **372**(12): 1114-25.
4. Torres A, Menendez R, Espana PP, et al. The evolution and distribution of pneumococcal serotypes in adults hospitalized with community-acquired pneumonia in Spain using a serotype-specific urinary antigen detection test: The CAPA Study, 2011-2018. *Clin Infect Dis* 2021; **73**(6): 1075-85.
5. de Miguel S, Domenech M, González-Camacho F, et al. Nationwide trends of invasive pneumococcal disease in Spain from 2009 through 2019 in children and adults during the pneumococcal conjugate vaccine era. *Clin Infect Dis* 2021. 73(11):e3778-e3787.
6. Sader HS, Mendes RE, Le J, Denys G, Flamm RK, Jones RN. Antimicrobial susceptibility of *Streptococcus pneumoniae* from North America, Europe, Latin America, and the Asia-Pacific Region: results from 20 years of the SENTRY antimicrobial surveillance program (1997-2016). *Open Forum Infect Dis* 2019; **6**(Suppl 1): S14-S23.
7. Sempere J, de Miguel S, González-Camacho F, Yuste J, Domenech M. Clinical relevance and molecular pathogenesis of the emerging serotypes 22F and 33F of *Streptococcus pneumoniae* in Spain. *Front Microbiol* 2020; **11**(309).
8. Fenoll A, Granizo JJ, Giménez MJ, Yuste J, Aguilar L. Secular trends (1990-2013) in serotypes and associated non-susceptibility of *S. pneumoniae* isolates causing invasive disease in the pre-/post-era of pneumococcal conjugate vaccines in Spanish regions without universal paediatric pneumococcal vaccination. *Vaccine* 2015; **33**(42): 5691-9.
9. Rosen JB, Thomas AR, Lexau CA, et al. Geographic variation in invasive pneumococcal disease following pneumococcal conjugate vaccine introduction in the United States. *Clin Infect Dis* 2011; **53**(2): 137-43.
10. Lewnard JA, Bruxvoort KJ, Fischer H, et al. Prevention of COVID-19 among older adults receiving pneumococcal conjugate vaccine suggests interactions between Streptococcus pneumoniae and SARS-CoV-2 in the respiratory tract. *J Infect Dis* 2021.
11. Mitsi E, Reine J, Urban BC, et al. *Streptococcus pneumoniae* colonization associates with impaired adaptive immune responses against SARS-CoV-2. *J Clin Invest* 2022; **132**(7).
12. Bornstein DL, Schiffman G, Bernheimer HP, Austrian R. Capsulation of pneumococcus with soluble C-like (Cs) polysaccharide. I. Biological and genetic properties of Cs pneumococcal strains. *J Exp Med* 1968; **128**(6): 1385-400.
13. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007; **44 Suppl 2**: S27-72.
14. Tomczyk S, Jain S, Bramley AM, et al. Antibiotic prescribing for adults hospitalized in the etiology of pneumonia in the community study. *Open Forum Infect Dis* 2017; **4**(2): ofx088.
15. Aguinagalde L, Corsini B, Domenech A, et al. Emergence of amoxicillin-resistant variants of spain^{9v}-ST156 pneumococci expressing serotype 11A correlates with their ability to evade the host immune response. *PLoS One* 2015; **10**(9): e0137565.
16. Atkins KE, Flasche S. Vaccination to reduce antimicrobial resistance. *Lancet Glob Health* 2018; **6**(3): e252.

17. Andrejko K, Ratnasiri B, Hausdorff WP, Laxminarayan R, Lewnard JA. Antimicrobial resistance in paediatric *Streptococcus pneumoniae* isolates amid global implementation of pneumococcal conjugate vaccines: a systematic review and meta-regression analysis. *Lancet Microbe* 2021; **2**(9): e450-e60.
18. Nagai K, Davies TA, Jacobs MR, Appelbaum PC. Effects of amino acid alterations in penicillin-binding proteins (PBPs) 1a, 2b, and 2x on PBP affinities of penicillin, ampicillin, amoxicillin, cefditoren, cefuroxime, cefprozil, and cefaclor in 18 clinical isolates of penicillin-susceptible, -intermediate, and -resistant pneumococci. *Antimicrob Agents Chemother* 2002; **46**(5): 1273-80.
19. Yamada M, Watanabe T, Miyara T, et al. Crystal structure of cefditoren complexed with Streptococcus pneumoniae penicillin-binding protein 2X: structural basis for its high antimicrobial activity. *Antimicrob Agents Chemother* 2007; **51**(11): 3902-7.
20. Yang Q, Xu Y, Chen M, et al. In vitro activity of cefditoren and other comparators against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* causing community-acquired respiratory tract infections in China. *Diagn Microbiol Infect Dis* 2012; **73**(2): 187-91.
21. Granizo JJ, Giménez MJ, Barberan J, Coronel P, Gimeno M, Aguilar L. The efficacy of cefditoren pivoxil in the treatment of lower respiratory tract infections, with a focus on the per-pathogen bacteriologic response in infections caused by *Streptococcus pneumoniae* and *Haemophilus influenzae*: a pooled analysis of seven clinical trials. *Clin Ther* 2006; **28**(12): 2061-9.
22. Di Marco F, Braido F, Santus P, Scichilone N, Blasi F. The role of cefditoren in the treatment of lower community-acquired respiratory tract infections (LRTIs): from bacterial eradication to reduced lung inflammation and epithelial damage. *Eur Rev Med Pharmacol Sci* 2014; **18**(3): 321-32.
23. Monmaturapoj T, Montakantikul P, Mootsikapun P, Tragulpiankit P. A prospective, randomized, double dummy, placebo-controlled trial of oral cefditoren pivoxil 400mg once daily as switch therapy after intravenous ceftriaxone in the treatment of acute pyelonephritis. *Int J Infect Dis* 2012; **16**(12): e843-9.
24. Blasi F, Concia E, Del Prato B, et al. The most appropriate therapeutic strategy for acute lower respiratory tract infections: a Delphi-based approach. *J Chemother* 2017; **29**(5): 274-86.
25. Cassiolato AP, Almeida SCG, Andrade AL, Minamisava R, Brandileone MCC. Expansion of the multidrug-resistant clonal complex 320 among invasive *Streptococcus pneumoniae* serotype 19A after the introduction of a ten-valent pneumococcal conjugate vaccine in Brazil. *PLoS One* 2018; **13**(11): e0208211.
26. Lo SW, Gladstone RA, van Tonder AJ, et al. Pneumococcal lineages associated with serotype replacement and antibiotic resistance in childhood invasive pneumococcal disease in the post-PCV13 era: an international whole-genome sequencing study. *Lancet Infect Dis* 2019; **19**(7): 759-69.
27. Gladstone RA, Lo SW, Lees JA, et al. International genomic definition of pneumococcal lineages, to contextualise disease, antibiotic resistance and vaccine impact. *EBioMedicine* 2019; **43**: 338-46.
28. De Miguel S, Latasa P, Yuste J, et al. Age-dependent serotype-associated case-fatality rate in invasive pneumococcal disease in the autonomous community of madrid between 2007 and 2020. *Microorganisms* 2021; **9**(11): 2286.
29. Langford BJ, So M, Raybardhan S, et al. Antibiotic prescribing in patients with COVID-19: rapid review and meta-analysis. *Clin Microbiol Infect* 2021; **27**(4): 520-31.

30. Group PTC. Azithromycin for community treatment of suspected COVID-19 in people at increased risk of an adverse clinical course in the UK (PRINCIPLE): a randomised, controlled, open-label, adaptive platform trial. *Lancet* 2021; **397**(10279): 1063-74.

Figure

Figure 1

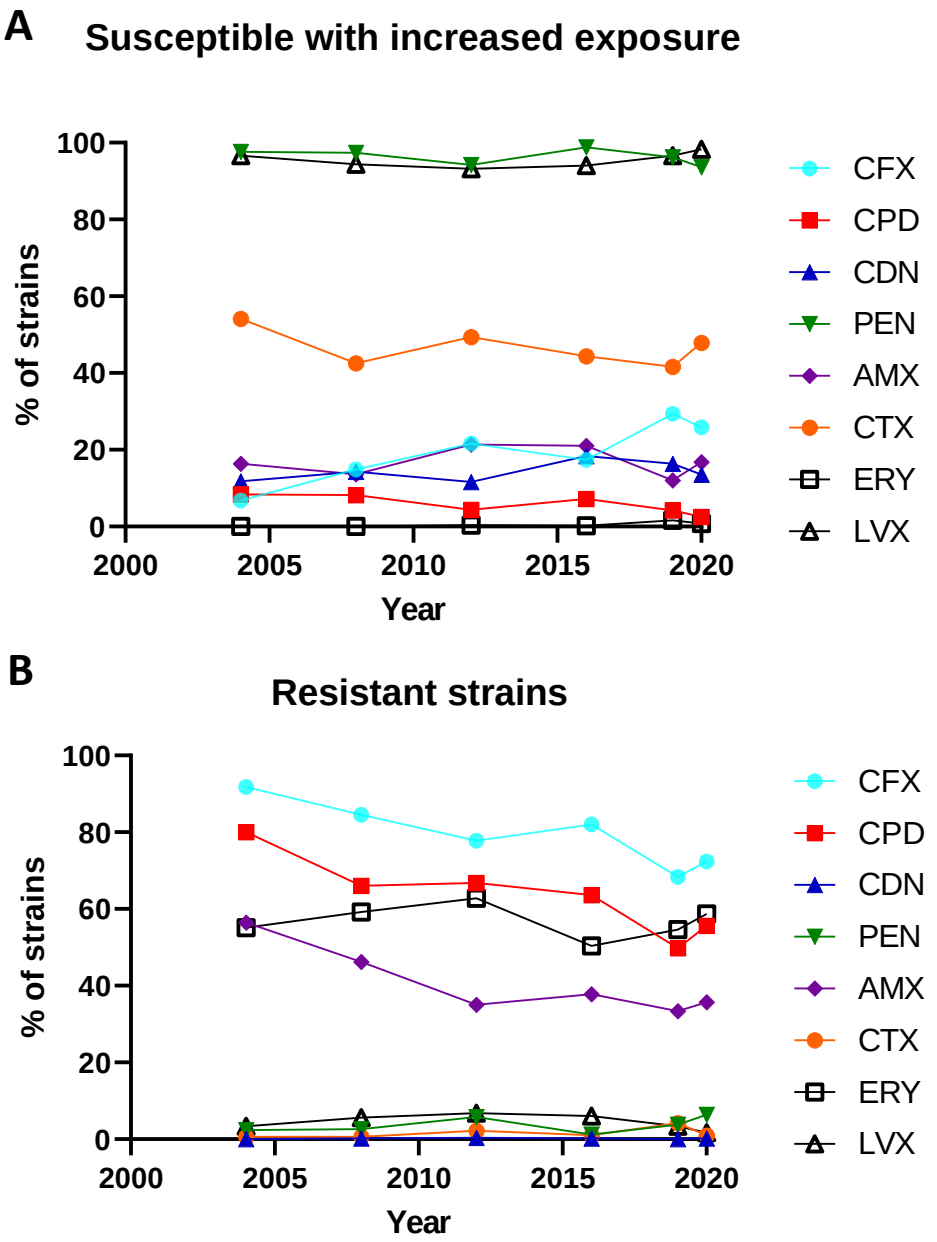


Figure 2

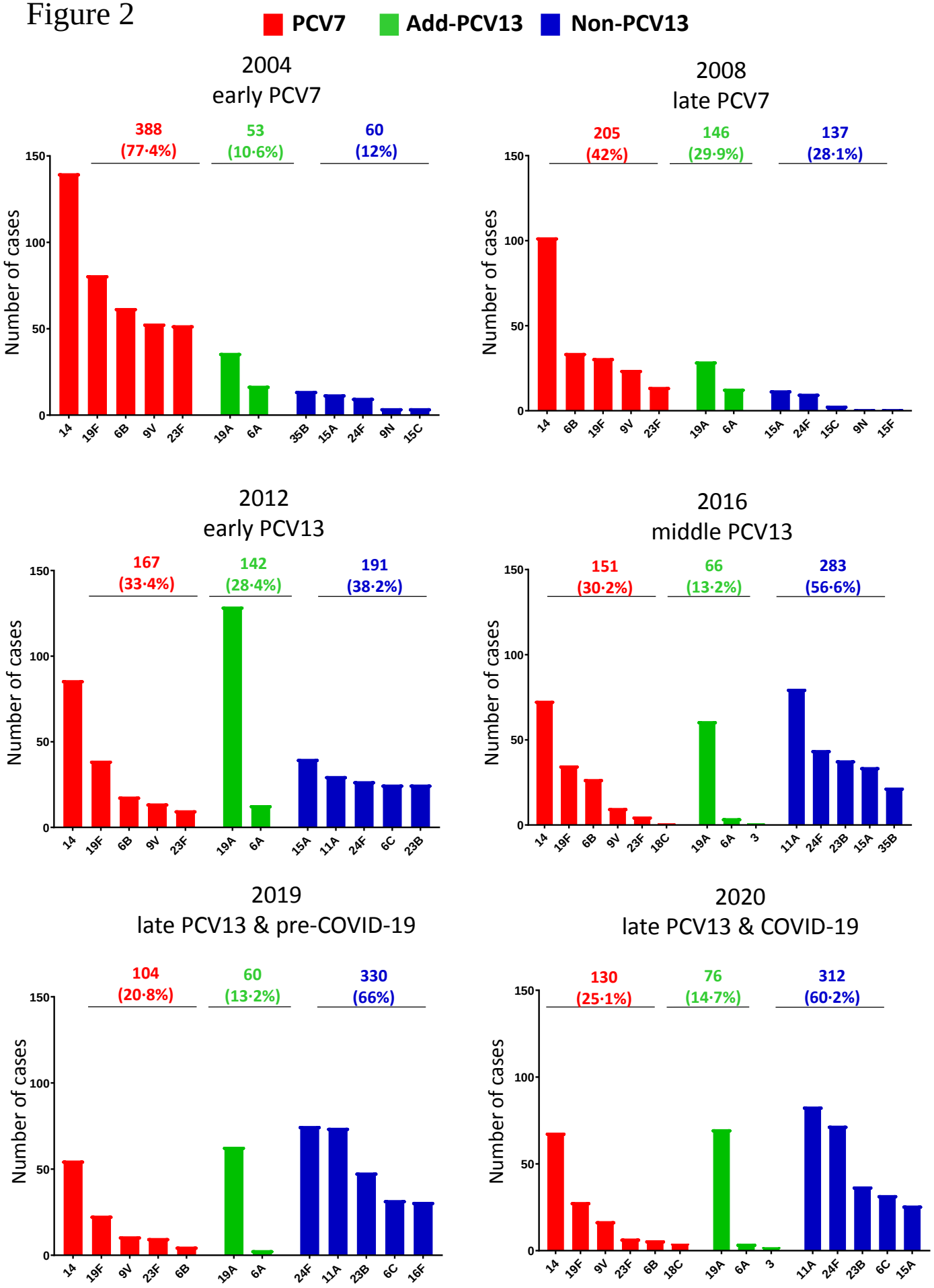

PCV7
Add-PCV13
Non-PCV13
2004
early PCV7
388
(77·4%)
53
(10·6%)
60
(12%)
Number of cases
150
100
50
0
14
19F
6B
9V
23F
19A
6A
35B
15A
24F
9N
15C
2008
late PCV7
205
(42%)
146
(29·9%)
137
(28·1%)
14
6B
19F
9V
23F
19A
6A
15A
24F
15C
9N
15F
2012
early PCV13
167
(33·4%)
142
(28·4%)
191
(38·2%)
14
19F
6B
9V
23F
19A
6A
15A
11A
24F
6C
23B
2016
middle PCV13
151
(30·2%)
66
(13·2%)
283
(56·6%)
14
19F
6B
9V
23F
18C
19A
6A
3
11A
24F
23B
15A
35B
2019
late PCV13 & pre-COVID-19
104
(20·8%)
60
(13·2%)
330
(66%)
14
19F
9V
23F
6B
19A
6A
24F
11A
23B
6C
16F
2020
late PCV13 & COVID-19
130
(25·1%)
76
(14·7%)
312
(60·2%)
14
19F
9V
23F
6B
18C
19A
6A
3
11A
24F
23B
6C
15A

Figure 3

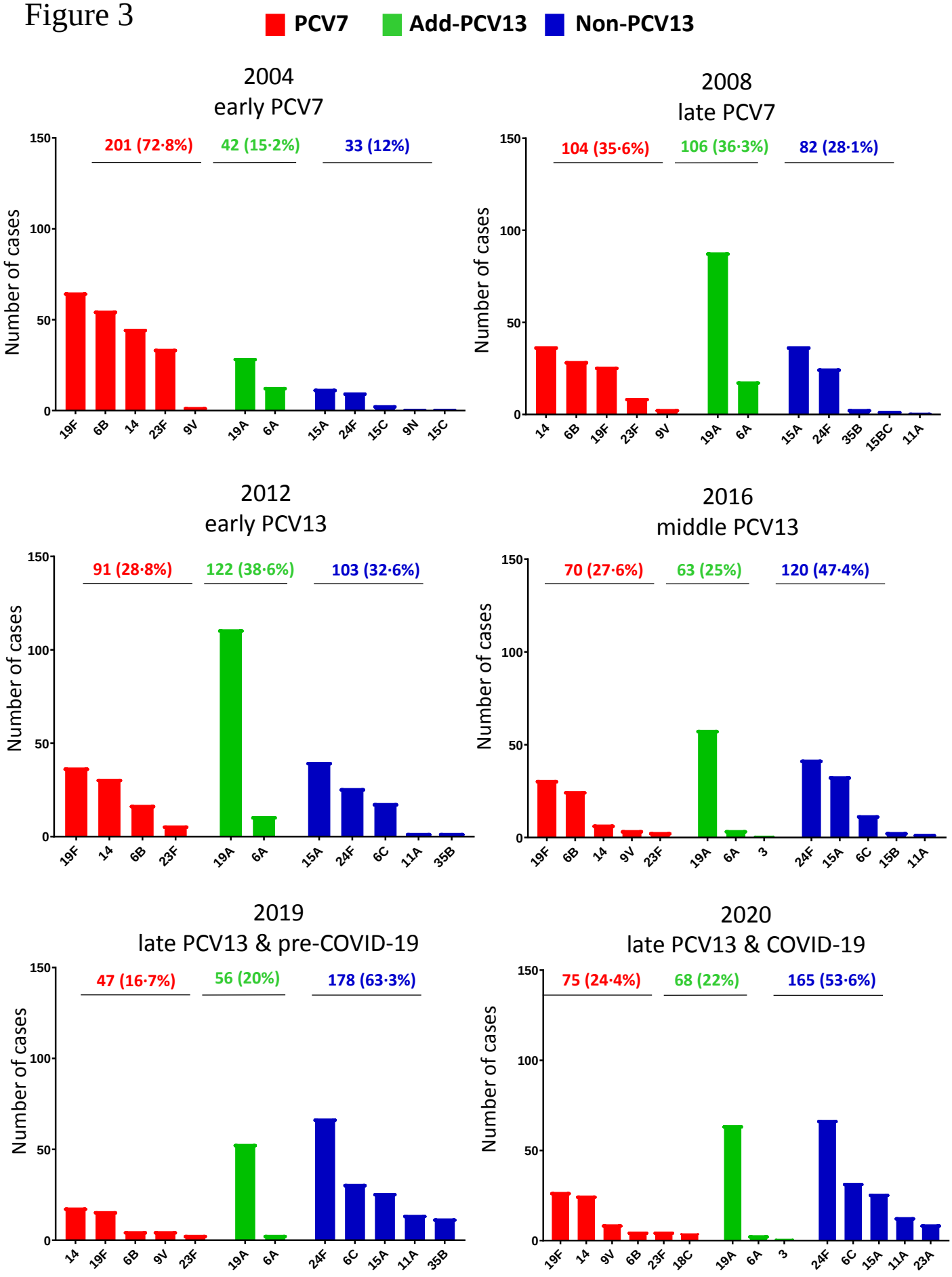
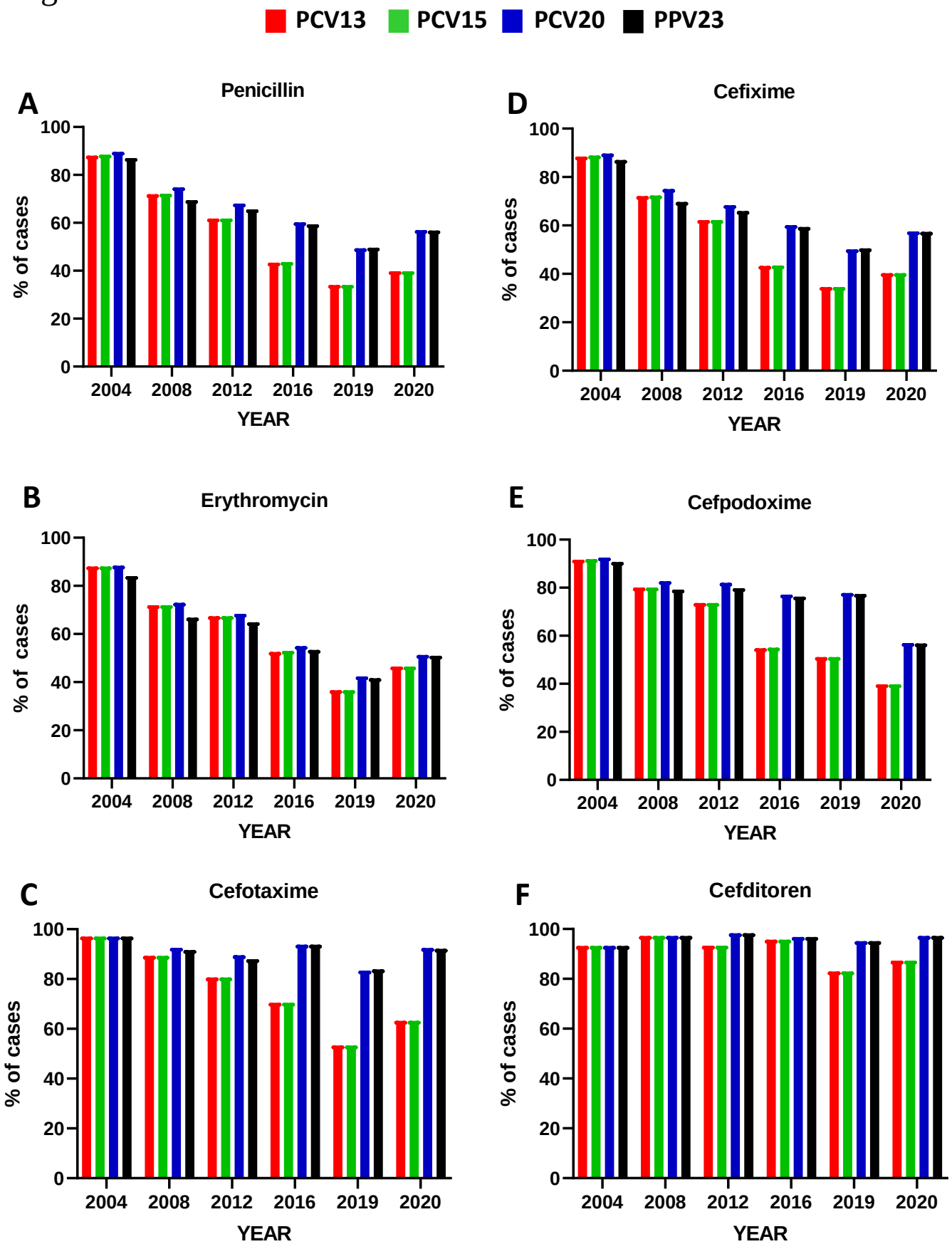

PCV7
Add-PCV13
Non-PCV13
2004
early PCV7
201 (72·8%)
42 (15·2%)
33 (12%)
Number of cases
150
100
50
0
19F
6B
14
23F
9V
19A
6A
15A
24F
15C
9N
15C
2008
late PCV7
104 (35·6%)
106 (36·3%)
82 (28·1%)
14
6B
19F
23F
9V
19A
6A
15A
24F
35B
15BC
11A
2012
early PCV13
91 (28·8%)
122 (38·6%)
103 (32·6%)
19F
14
6B
23F
19A
6A
15A
24F
6C
11A
35B
2016
middle PCV13
70 (27·6%)
63 (25%)
120 (47·4%)
19F
6B
14
9V
23F
19A
6A
3
24F
15A
6C
15B
11A
2019
late PCV13 & pre-COVID-19
47 (16·7%)
56 (20%)
178 (63·3%)
14
19F
6B
9V
23F
19A
6A
24F
6C
15A
11A
35B
2020
late PCV13 & COVID-19
75 (24·4%)
68 (22%)
165 (53·6%)
19F
14
9V
6B
23F
18C
19A
6A
3
24F
6C
15A
11A
23A

Figure 4


PCV13
PCV15
PCV20
PPV23
A
Penicillin
% of cases
YEAR
B
Erythromycin
% of cases
YEAR
C
Cefotaxime
% of cases
YEAR
D
Cefixime
% of cases
YEAR
E
Cefpodoxime
% of cases
YEAR
F
Cefditoren
% of cases
YEAR
0
20
40
60
80
100
2004
2008
2012
2016
2019
2020

Table

	MIC$_{50/90}$ (mg/L)					
	2004	**2008**	**2012**	**2016**	**2019**	**2020**
Serotype 19A						
Cefixime	16/16	16/16	16/16	16/16	16/16	16/16
Cefpodoxime	2/2	2/4	2/4	4/4	4/4	2/4
Cefditoren	0·5/0·5	0·5/1	0·5/1	1/1	1/1	0·5/1
Penicillin	0·5/1	1/2	2/4	1/2	2/2	2/2
Amoxicillin	0·5/2	0·5/4	2/4	2/4	4/4	2/4
Cefotaxime	0·5/1	0·5/1	1/2	1/2	2/4	2/2
Serotype 14						
Cefixime	16/16	16/16	16/16	16/16	16/16	16/16
Cefpodoxime	2/4	2/4	2/4	2/4	2/4	2/4
Cefditoren	0·5/1	0·5/1	0·5/1	1/1	1/2	0·5/1
Penicillin	1/2	1/2	1/2	1/2	2/4	2/2
Amoxicillin	2/8	2/8	1/4	1/4	1/8	1/2
Cefotaxime	1/2	1/2	1/2	1/2	2/4	1/2
Serotype 19F						
Cefixime	8/16	8/16	16/16	16/16	8/16	16/16
Cefpodoxime	1/2	1/2	2/4	2/4	1/2	2/4
Cefditoren	0·25/0·5	0·25/0·5	0·5/0·5	0·5/1	0·25/0·5	0·5/1
Penicillin	0·5/1	0·5/2	1/2	1/2	0·5/2	1/2
Amoxicillin	1/2	1/4	1/2	1/4	0·5/2	1/4
Cefotaxime	0·25/1	0·25/1	1/1	1/2	0·5/2	1/2
Serotype 11A						
Cefixime	8/8	16/16	16/16	16/16	16/16	16/16
Cefpodoxime	0·5/0·5	2/2	2/2	2/2	2/2	2/2
Cefditoren	0·25/0·5	0·5/0·5	0·5/0·5	0·5/0·5	0·5/1	0·5/0·5
Penicillin	0·25/0·25	2/2	2/4	2/2	2/2	2/4
Amoxicillin	0·5/0·5	4/8	4/8	4/4	4/8	4/8
Cefotaxime	0·25/0·25	1/1	1/2	1/1	1/2	1/2
Serotype 24F						
Cefixime	4/4	4/4	4/4	4/4	4/4	4/4
Cefpodoxime	0·25/0·5	0·25/0·25	0·25/0·25	0·25/0·5	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	0·5/0·5	0·5/1	0·5/1	0·5/0·5	0·5/1	0·5/1
Amoxicillin	0·12/0·25	0·12/0·5	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	0·25/0·5	0·25/0·25	0·25/0·5	0·25/0·25
Serotype 23B						
Cefixime	4/4	2/2	2/4	2/2	2/4	2/2
Cefpodoxime	0·25/0·25	0·12/0·12	0·12/1	0·12/0·25	0·12/1	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	0·25/0·5	0·25/0·5
Amoxicillin	0·12/0·12	0·12/0·25	0·06/0·25	0·06/0·06	0·06/0·5	0·06/0·12
Cefotaxime	0·12/0·12	0·06/0·25	0·12/0·25	0·12/0·25	0·12/0·5	0·12/0·25

Table 1: MIC$_{50/90}$ values (mg/L) for the three most prevalent PCV13 and non-PCV13 serotypes against different β-lactam antibiotics during the period 2004-2020.