

# Emergence of a multidrug-resistant and virulent *Streptococcus pneumoniae* lineage mediates serotype replacement after PCV13: an international whole-genome sequencing study



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## Summary

**Background** Serotype 24F is one of the emerging pneumococcal serotypes after the introduction of pneumococcal conjugate vaccine (PCV). We aimed to identify lineages driving the increase of serotype 24F in France and place these findings into a global context.

**Methods** Whole-genome sequencing was performed on a collection of serotype 24F pneumococci from asymptomatic colonisation (n=229) and invasive disease (n=190) isolates among individuals younger than 18 years in France, from 2003 to 2018. To provide a global context, we included an additional collection of 24F isolates in the Global Pneumococcal Sequencing (GPS) project database for analysis. A Global Pneumococcal Sequence Cluster (GPSC) and a clonal complex (CC) were assigned to each genome. Phylogenetic, evolutionary, and spatiotemporal analysis were conducted using the same 24F collection and supplemented with a global collection of genomes belonging to the lineage of interest from the GPS project database (n=25 590).

**Findings** Serotype 24F was identified in numerous countries mainly due to the clonal spread of three lineages: GPSC10 (CC230), GPSC16 (CC156), and GPSC206 (CC7701). GPSC10 was the only multidrug-resistant lineage. GPSC10 drove the increase in 24F in France and had high invasive disease potential. The international dataset of GPSC10 (n=888) revealed that this lineage expressed 16 other serotypes, with only six included in 13-valent PCV (PCV13). All serotype 24F isolates were clustered in a single clade within the GPSC10 phylogeny and long-range transmissions were detected from Europe to other continents. Spatiotemporal analysis showed GPSC10-24F took 3–5 years to spread across France and a rapid change of serotype composition from PCV13 serotype 19A to 24F during the introduction of PCV13 was observed in neighbouring country Spain.

**Interpretation** Our work reveals that GPSC10 alone is a challenge for serotype-based vaccine strategy. More systematic investigation to identify lineages like GPSC10 will better inform and improve next-generation preventive strategies against pneumococcal diseases.

**Funding** Bill & Melinda Gates Foundation, Wellcome Sanger Institute, and the US Centers for Disease Control and Prevention.

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## Introduction

Pneumococcal conjugate vaccines (PCVs) have been effective at reducing pneumococcal disease worldwide.<sup>1</sup> However, increases in replacement with non-PCV serotypes remain a concern,<sup>2–3</sup> such as the increase of serotype 19A after introduction of PCV7 in the USA in 2000.<sup>4</sup> In France, a big increase in pneumococcal meningitis cases occurred 5 years after the roll out of PCV13, mainly driven by serotype 24F.<sup>2,3</sup> This serotype also mediated serotype replacement in multiple other countries, such as Argentina,<sup>5</sup> Canada,<sup>6</sup> Denmark,<sup>7</sup>

Germany,<sup>8</sup> Israel,<sup>9</sup> Italy,<sup>10</sup> Japan,<sup>11</sup> Lebanon,<sup>12</sup> Norway,<sup>13</sup> Spain,<sup>14,15</sup> and the UK,<sup>16</sup> and was reported to be the predominant serotype causing invasive pneumococcal disease (IPD) in Portugal<sup>17</sup> after PCV13 introduction.

The serotype 24F capsule has a high invasive disease potential and propensity to cause meningitis.<sup>18</sup> In France, the fatality rate for meningitis due to serotype 24F pneumococci was 13%, similar to that caused by pneumococci expressing other serotypes (11%) between 2001 and 2016.<sup>2</sup> In some countries, the increase in serotype 24F was concomitant with increasing prevalence

*Lancet Microbe* 2022; 3: e735–43

Published Online  
August 16, 2022  
[https://doi.org/10.1016/S2666-5247\(22\)00158-6](https://doi.org/10.1016/S2666-5247(22)00158-6)

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## Research in context

### Evidence before this study

We searched PubMed using the terms “*Streptococcus pneumoniae*” AND “24F” OR “CC230” OR “GPSC10” for papers published in English between Jan 1, 2000, and Feb 19, 2021. We searched for population-based studies which reported changes in serotype 24F before and after the introduction of pneumococcal conjugate vaccines (PCV) in the country or region. After reviewing 59 articles, 28 met the inclusion criteria. The effects of 7-valent PCV were measured in six studies and 13-valent PCV (PCV13) in 23 studies. Most studies used samples from children or adults with invasive pneumococcal disease (IPD; n=20), four studies included isolates from both carriage and IPD cases, and four studies analysed isolates from carriage alone. Studies were conducted at the national or regional level, and isolates were typed using Quellung, latex agglutination, or PCR-based methods. Among IPD cases in children, 24F was identified as the predominant serotype post PCV13 in eight studies representing cases from France, Denmark, Spain, Italy, and Japan. For the three studies with serotype stratified by age, the predominance of 24F was only observed in children younger than 5 years. Serotype 24F was the second most common serotype, or the most common together with 12F, in four further studies of IPD cases post PCV13 in Germany, France, and the UK. An increase in 24F post PCV7 was reported among IPD cases from Spain, Italy, and France, carriage and IPD cases in Norway, and carriage in Portugal.

### Added value of this study

We have an enhanced understanding of the multidrug resistant lineage of *Streptococcus pneumoniae* which has driven the

increase in serotype 24F in France after PCV13. Additionally, we utilised a global collection of *S pneumoniae* isolates from 56 countries to contextualise the isolates from France, identifying the predominant lineage (GPSC10) associated with the increase of multidrug resistance in serotype 24F globally. This study is complemented by the knowledge we gained from previous country-specific analyses to demonstrate GPSC10 might pose a global threat after PCV13 due to a high risk of vaccine evasion.

### Implications of all the available evidence

The increase in serotype 24F post PCV13 in France was attributed to the multidrug-resistant lineage GPSC10. Concerningly, GPSC10 has a relatively high invasive disease potential and propensity to cause meningitis, independent of serotype. GPSC10 appears to be highly capable of acquiring DNA that might result in antimicrobial resistance and serotype switches. Analyses of GPSC10 isolates from a global dataset of *S pneumoniae* genomes have identified expression of an additional 16 serotypes of which only six are included in PCV13. Antimicrobial use might have contributed to selection of GPSC10 in France and Spain, decreasing the benefit of PCV for reduction of antimicrobial resistance. GPSC10 has transmitted among European countries, with long-range transmissions to other continents. The evidence suggests that the expansion of GPSC10 might be a challenging problem to address using a serotype-based vaccine strategy.

of penicillin resistance in IPD overall<sup>2,10</sup> and IPD due to non-vaccine serotypes.<sup>5</sup> Despite serotype 24F being an important emerging serotype with high invasiveness and potential for antimicrobial resistance, it is not included in any upcoming PCV formulations (PCV15, PCV20, and PCV24).

Pneumococcal lineages driving the increase in serotype 24F varied between countries. In Argentina,<sup>5</sup> Lebanon,<sup>12</sup> and Spain,<sup>19</sup> the increase was mainly driven by the Global Pneumococcal Sequence Cluster (GPSC) and clonal complex (CC) GPSC10 (CC230), in Denmark<sup>7</sup> by GPSC6 (CC156), and in Japan by GPSC106 (CC2572).<sup>11</sup> In the present study, we used whole-genome sequencing to investigate the pneumococcal lineages driving the increase in serotype 24F after PCV13 introduction in France, then placed this lineage in global context and elucidated its spatiotemporal transmission in France and across the world.

## Methods

### Study design

A representative set of serotype 24F pneumococcal isolates was randomly selected from the datasets collected through a nationwide hospital-based active surveillance for IPD<sup>2,3</sup> and carriage survey<sup>20</sup> across France and were

whole-genome sequenced. The collection included 190 isolates from invasive disease cases and 229 isolates from asymptomatic colonisation among individuals younger than 18 years, from 2003 to 2018 (appendix 1 p 6). The study period spanned the routine use of PCV7 in 2006 and PCV13 in 2010. To identify the pneumococcal lineage driving the increase in serotype 24F, we grouped isolates into GPSCs and CCs and evaluated each lineage's invasive disease potential and propensity to cause meningitis by calculating odds ratio (OR) with reference to carriage.

We then contextualised serotype 24F and the driver lineage (GPSC10) with a global collection of additional genomes from the Global Pneumococcal Sequencing (GPS) Project database (n=25 590 from 57 countries, last accessed on Oct 2, 2021). For comparison, we also sequenced a collection of 91 pneumococcal isolates that were responsible for the increase in serotype 24F in Spain. These isolates were collected from children aged younger than 5 years in the Catalan support laboratory for non-mandatory molecular surveillance of IPD located at Hospital Sant Joan de Déu, Barcelona, from 2009 to 2018.<sup>19</sup> In summary, phylogenetic analysis was carried out on 642 serotype 24F isolates from 29 countries across Africa, Asia, Europe, North America, South America,

and Oceania. An international collection of 888 GPSC10 isolates from 33 countries, regardless of serotype, were also included in phylogenetic, evolutionary, and spatiotemporal analyses.

### Genome sequencing and genomic characterisation

The pneumococcal isolates in this study were whole-genome sequenced using a Illumina HiSeq sequencer (Illumina, San Diego, CA, USA). The sequence reads were subjected to quality control as previously described (appendix 1 pp 4–5).<sup>21</sup> We characterised each genome by assigning GPSC using PopPUNK (version 2.4.0),<sup>21</sup> sequence type using MLSTcheck (version 2.1.1706216),<sup>22</sup> CC using goeBURST (version 1.2.1), and serotype using SeroBA (version 1.0.0),<sup>23</sup> and resistance profile against 17 antimicrobials, including penicillin, chloramphenicol, erythromycin, co-trimoxazole, and tetracycline, using a pipeline developed by the Streptococcus Laboratory at the Centers for Disease Control and Prevention, Atlanta, GA, USA.<sup>24</sup> Multidrug resistance was defined as an isolate resistant to three or more antibiotic classes. In our previous large-scale analysis, we showed high concordance between GPSC and CC, therefore sequence types identified in previous studies that did not have genome data were used to infer GPSC in this study.<sup>21</sup> All sequencing reads were deposited in European Nucleotide Archive (ENA) and the accession numbers and in silico output are included in appendix 2. Additional details on methodology are in appendix 1 (pp 4–5).

### Phylogenetic analysis

We performed phylogenetic analysis on all serotype 24F isolates by constructing a maximum likelihood tree using FastTree (version 2.1.10) with the general time reversible substitution model.<sup>25</sup> Phylogenies were built based on single nucleotide polymorphisms (SNPs) extracted from individual alignment generated by mapping reads to a reference genome of *Streptococcus pneumoniae* ATCC 700669 (National Center for Biotechnology Information [NCBI] accession: FM211187) using SMALT (version 0.74), with default settings and to a reference sequence of serotype 24F capsular encoding region (ie, *cps*, NCBI accession: CR931688) using Burrows Wheeler Aligner (version 0.7.17-r1188).<sup>26</sup> The phylogenetic trees were then overlaid with epidemiological data and in silico output, as described earlier, and visualised in Microreact.

### Evolutionary and spatiotemporal analysis

Sequence reads of GPSC10 (CC230) isolates were mapped to the GPSC10 reference genome Denmark<sup>14-32</sup> (ENA accession: ERS1706837) using Burrows Wheeler Aligner (version 0.7.17-r1188).<sup>26</sup> Recombination was detected and removed using Gubbins (version 2.4.1).<sup>27</sup> A recombination-free phylogeny was produced with RAxML (version 8.2.8)<sup>28</sup> and then visualised in Microreact, together with metadata. Bayesian phylogenetic analysis on a subset of serotype 24F isolates within GPSC10 was

conducted to generate a time-resolved phylogeny and provide estimates of the median effective population size over time with a 95% highest posterior density to detect any exponential increase in population using BEAST Bayesian skyline model (version 2.6.3).<sup>29</sup> The time-resolved phylogeny was visualised in Microreact.

To calculate the time it took for GPSC10-24F to spread across France, we inferred the evolutionary time between pairs of genomes from the time-resolved phylogeny. We used a risk ratio framework to calculate the odds that a pair of genomes from within the same province (0–50 km) compared with pairs isolated from locations between 250 and 350 km apart (the mean distance between different French provinces was 359 km with a range of 54–729 km) had a specified time-to-most-recent-common-ancestor (tMRCA) across rolling 2 year divergence time windows from 2 to 20 years.<sup>30</sup> We restricted the analysis to pairs isolated within the same year to mitigate variable geographic sampling across years. A risk ratio close to 1 indicated an equal chance that a pair of isolates diverged from the same tMRCA within and between provinces. To determine uncertainty, we calculated the risk ratio using 1000 bootstrap iterations. The spatiotemporal analysis was run using R (version 3.6.0).

### Statistical analysis

We grouped the French isolates into four vaccine periods as previously described:<sup>2</sup> (1) targeted PCV7 period (2003–05) in which PCV7 was only reimbursed and recommended for children in a day-care centre with two or more other children, children in families with more than two children, or children breastfed for fewer than 2 months; (2) generalised PCV7 period (2006–10) during which PCV7 was offered to all children younger than 2 years; (3) early PCV13 period (2011–14); and (4) late PCV13 period (2015–18) during which PCV7 was replaced with PCV13, without catch-up. We compared the prevalence of GPSCs between a vaccine period and the previous vaccine period, except for the earliest targeted PCV7 period. To determine if differences in proportions between groups were significant, a two-sided Fisher's exact test was used. Statistical tests were performed in R (version 3.6.0). Multiple testing correction was carried out using the Benjamini-Hochberg false discovery rate of 5%. Two-sided p values of less than 0.05 were considered significant.

### Role of funding source

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

### Results

The 419 French serotype 24F pneumococcal isolates were sequenced and all passed sequencing quality control. The isolates belonged mainly to three lineages: GPSC10 (CC230, 174 [41.5%]), GPSC6 (CC156, 160 [38.2%]), and GPSC16 (CC66, 74 [17.7%]). A small

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See Online for appendix 1

For more on the Global Pneumococcal Sequencing Project see <https://www.pneumogen.net/gps>

See Online for appendix 2

For more on the SMALT software see <http://www.sanger.ac.uk/science/tools/smalt-0>

For the Global 24F phylogeny see [https://microreact.org/project/global\\_24F](https://microreact.org/project/global_24F)

For the Global 24F cps phylogeny see [https://microreact.org/project/global\\_24F\\_cps](https://microreact.org/project/global_24F_cps)

percentage of isolates belonged to other lineages: GPSC44 (CC177, six [1.4%]), GPSC18 (CC15, three [0.7%]), and GPSC5 (CC172, two [0.5%]). Over the study period (2003–18), clonal replacement was observed in overall disease, meningitis, and carriage isolates (figure 1). Overall, the proportion of serotype 24F isolates belonging to GPSC16 decreased significantly from 60.5% in the generalised PCV7 period (2006–10) to 10.6% in the early PCV13 period (2011–14;  $p < 0.0001$ ). By contrast, GPSC6 significantly increased from 9.9% in the generalised PCV7 period to 62.7% in the early PCV13 period (2011–14;  $p < 0.0001$ ). The proportion of 24F isolates that were GPSC6 decreased from 62.7% in the early PCV13 period to 30.2% in the late PCV13 period (2015–18;  $p < 0.0001$ ), and the proportion that belonged to GPSC10 significantly increased from 23.0% in the early PCV13 period to 65.1% in the late PCV13 period ( $p < 0.0001$ ; appendix 1 p 14). In the late PCV13 period, GPSC10 became the predominant lineage among 24F isolates, accounting for 100% of the ten pneumonia cases, 35 (74%) of 47 meningitis cases, four (67%) of six other infections, 11 (50%) of 22 bacteraemias, and 50 (60%) of 84 asymptomatic colonisation. In contrast to the pan-susceptible GPSC16, the replacement lineage GPSC6 was co-trimoxazole-resistant and GPSC10 was multidrug resistant. Stratified by IPD and carriage, we did not observe any significant difference in GPSC, sequence type, and antimicrobial resistance between children younger than 5 years and individuals aged 5–17 years (appendix 1 pp 15–17).

Compared with other serotype 24F lineages, GPSC10 was more frequently detected in overall invasive disease (OR 1.38 [95% CI 0.93–2.04],  $p = 0.11$ ) and meningitis cases (OR 1.57 [95% CI 0.98–2.51],  $p = 0.060$ ) than in asymptomatic colonisation. Although the finding did not reach statistical significance, it suggested that this lineage had relatively high invasive disease potential and propensity to cause meningitis (figure 2; appendix 1 p 18). By contrast, GPSC6 was more frequently identified in carriage than meningitis isolates (OR 0.58 [95% CI 0.35–0.97],  $p = 0.038$ ).

Analyses on an international collection of 642 serotype 24F isolates revealed that this serotype was widely distributed across 29 countries (appendix 1 p 7). These isolates were assigned into 20 GPSCs. GPSC10, GPSC16, GPSC150, and GPSC206 were the most common lineages, accounting for 439 (68%) of 642 of the overall GPS collection and 96 (78%) of 123 of a subcollection that included isolates randomly selected from disease surveillance systems and carriage surveys from 21 countries (appendix 1 p 7). GPSC10, GPSC16, and GPSC206 are globally spreading lineages, and GPSC150 was only detected in west Africa, except for three isolates from Israel. Among these most common lineages, only GPSC10 was multidrug resistant, with most isolates exhibiting resistance to penicillin, co-trimoxazole, erythromycin, and tetracycline (appendix 1 p 8).

Phylogenetic analysis of the serotype 24F *cps* revealed a strong clonal but not geographical structure (appendix 1 p 9). The *cps* gene belonging to the same GPSC was clustered together regardless of the isolates'

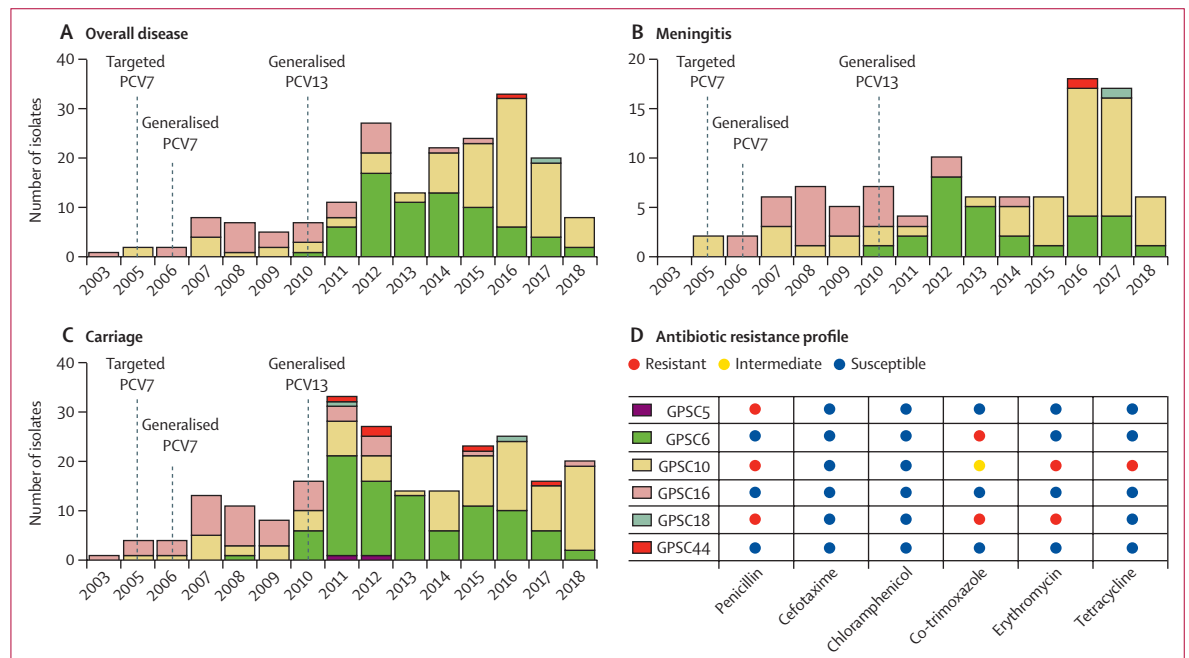


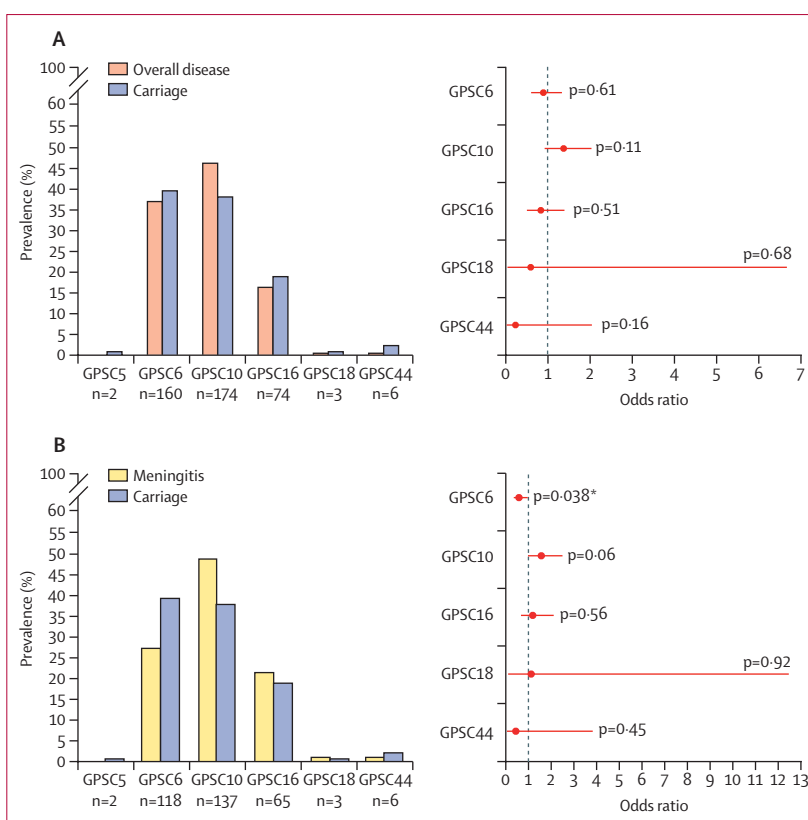
Figure 1: Proportion of serotype 24F pneumococcal lineages from France between 2003 and 2018 (A–C) and predominant antibiotic resistance profiles of each lineage (D) GPSC=global pneumococcal sequence cluster. PCV=pneumococcal conjugate vaccine.



country of origin. This finding indicated the linkage between *cps* and its genetic background, and further confirmed that the emergence of serotype 24F in different geographical regions was due to clonal spread, rather than independent capsular switching. Only a few capsular switching events were observed based on the high similarity in *cps* between lineages. For example, three GPSC18 isolates from France share highly similar *cps* with GPSC10, suggesting a capsular switching between GPSC10 and GPSC18. GPSC18 expressing serotype 24F was not detected elsewhere but in France, according to the GPS project database and PubMLST database (last accessed on Aug 12, 2021). GPSC18-24F was first detected in 2011 in this collection of isolates, a year after the introduction of PCV13 in France, without statistically significant expansion (figure 1).

Of all serotype 24F lineages, GPSC10 was the only one detected with high invasive disease potential and multidrug resistance. GPSC10 was responsible for the increase of serotype 24F in France, and one of the major lineages mediating the global spread of serotype 24F. We further investigated this lineage using an international collection of 888 GPSC10 isolates. This lineage was detected in 33 countries across Africa, Asia, Europe, North America, South America, and Oceania (appendix 1 p 10). GPSC10 expressed 17 different serotypes, including 3, 6A, 6C, 7B, 10A, 11A, 13, 14, 15B, 15C, 17F, 19A, 19F, 23A, 23B, 23F, and 24F. Only six of these serotypes (serotypes 3, 6A, 14, 19A, 19F, and 23F) are covered by the current PCVs (PCV10 and PCV13) approved for use in children. An additional three serotypes (10A, 11A, and 15B) are covered by Pfizer's planned 20-valent vaccine, and four (10A, 11A, 15B, and 17F) by Merck's planned 24-valent vaccine. These two vaccines had not been approved for use in children at the time of writing. At present, no single vaccine is known to cover all 17 serotypes expressed by GPSC10. Concerningly, GPSC10 is among the top five lineages in India,<sup>31</sup> Pakistan, and Nepal (appendix 1 p 12), where pneumococcal disease burden is highest.<sup>1</sup> In these three countries, 15 serotype variants were detected in GPSC10 and only six were included in PCV13, underlining the potential of GPSC10 to cause serotype replacement in the future. Globally, GPSC10 was consistently found to express multiple serotypes and be multidrug resistant.

The global GPSC10 phylogeny showed that all serotype 24F isolates except one were clustered together, regardless of their country of origin (figure 3A). This finding is consistent with the 24F *cps* phylogeny, which indicated that the global dissemination of GPSC10-24F was largely mediated by clonal spread. We analysed an additional collection of 91 GPSC10 isolates from Spain and detected a rapid switch in serotype composition from 19A to 24F, after the implementation of PCV13 in the target group of children (appendix 1 p 11). The 19A and 24F serotype variants from Spain were separated in long branches on the global phylogeny, further indicating that



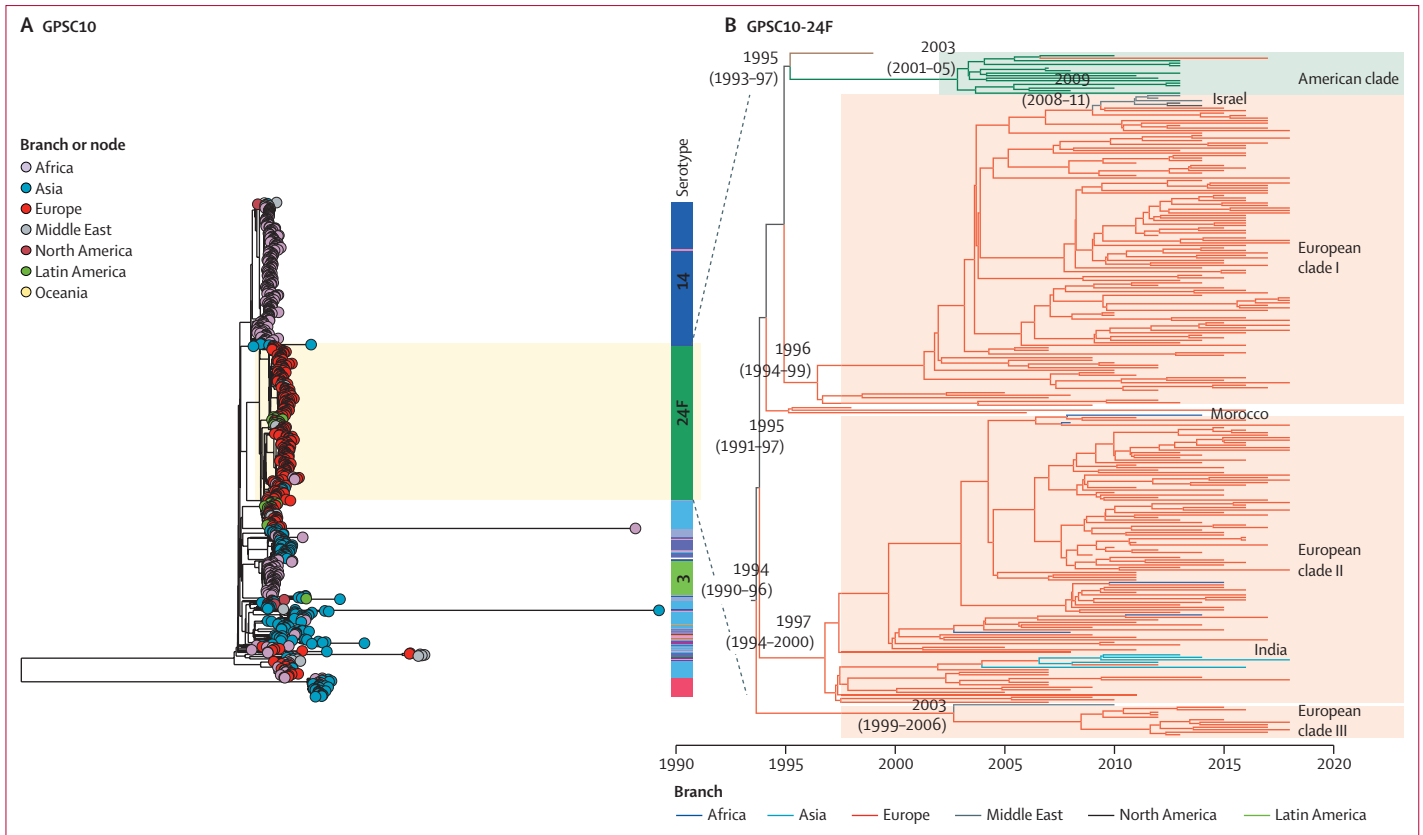
**Figure 2: Prevalence of pneumococcal lineages by clinical manifestations and odds ratio for causing overall invasive diseases (A) and meningitis by reference to carriage (B)**

The odds ratio and 95% CI were calculated using Fisher's Exact test. \*Indicates statistical significance. GPSC=global pneumococcal sequence cluster.

the capsular switch predates the vaccine introduction and coexistence of both GPSC10 serotype variants in Spain.

A time-resolved phylogeny was built on the cluster of 276 GPSC10-24F isolates and revealed four subclusters: EU-clade-I (dominated by ST4253), EU-clade-II (ST230 and ST4677), EU-clade-III (ST230), and American clade (ST230) (figure 3B). EU-clade-I and EU-clade-II were estimated to emerge around the 1990s and then clonally expand. EU-clade-III was relatively small and estimated to emerge around late 1990s to early 2000s, with majority of isolates from France, and one from Qatar. Throughout the study period, EU-clade-I accounted for most (112 [70%] of 160) of the French GPSC10-24F isolates and drove the 24F increase, and most of Spanish isolates (58 [88%] of 66) belonged to EU-clade II (appendix 1 p 12). In the EU-clade-I, long-range transmission was observed from Europe to Israel in the late 2000s. In the EU-clade-II, multiple transmissions from Europe to Morocco were detected, and a single transmission from Europe to India in the early 2000s was followed by a clonal expansion.

We reconstructed the population dynamics over time on EU-clade-I and EU-clade-II using the Bayesian skyline model. The EU-clade-I was predicted to have three exponential increases in effective population size in



**Figure 3: Global phylogeny of GPSC10 (A) and time-resolved phylogeny of a cluster of GPSC10-24F *Streptococcus pneumoniae* (B)**  
 All but one serotype 24F isolates were clustered together, regardless of their country of origin, indicating a clonal spread of GPSC10-24F across the world. The GPSC10 global phylogeny and time-resolved phylogeny can be interactively visualised in Microreact. GPSC=global pneumococcal sequence cluster.

For an interactive visualisation of the GPSC10 global phylogeny see [https://microreact.org/project/global\\_GPSC10/21948517](https://microreact.org/project/global_GPSC10/21948517)

For an interactive visualisation of the GPSC10 time-resolved phylogeny see <https://microreact.org/project/GPSC10-24F/8735223a>

around 1994, 2004, and 2013, and the EU-clade-II had one in around 2010 (figure 4A, C). The most recent increase in EU-clade-I coincided with the observed prevalence increase of GPSC10-24F in both overall disease and carriage isolates from France (figure 4A, B, D). Spatiotemporal analysis of the 174 GPSC10-24F isolates from France indicated that a pair of pneumococcal isolates had a higher risk ratio of being recovered within the same province if they were less than 5 years diverged from their most recent common ancestor (MRCA), compared with pairs that were 5 or more years diverged from their MRCA. Pairs which had diverged 5 or more years ago had risk ratio spanning 1, suggesting that the GPSC10-24F population was homogeneous across French provinces in 3–5 years (appendix 1 pp 13, 18).

### Discussion

Our results showed the emergence of the virulent and multidrug resistant pneumococcal lineage GPSC10 (CC230) that was responsible for the increase of invasive pneumococcal disease in France and the global spread of the invasive serotype 24F, which is not included in the current or upcoming PCVs. Due to its recombinogenic nature,<sup>32</sup> this lineage is capable of simultaneously expressing a wide range of serotypes to facilitate its

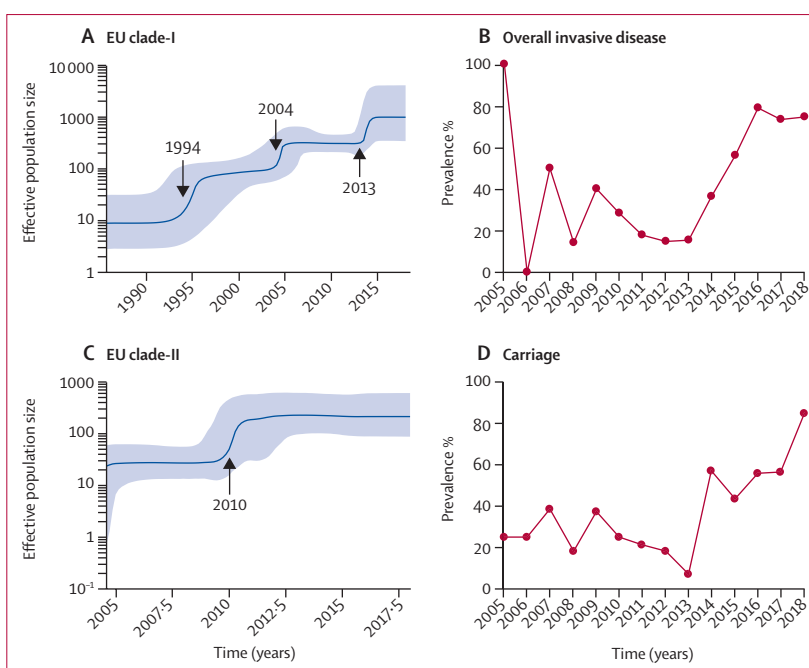
adaptation under the vaccine-selective pressure. Together with its transmissibility, GPSC10 should therefore be regarded as a high-risk lineage that could diminish the benefits of the vaccination programme worldwide over time.

Since the advent of the first PCV in 2000, GPSC10 has mediated serotype replacement in multiple countries. After the introduction of PCV7 and PCV10, 19A became the predominant serotype causing invasive disease in Europe; this increase was partially driven by GPSC10, together with GPSC1 (CC320).<sup>33–38</sup> In France and Spain, the major contributor of serotype 19A was GPSC10 in the post-PCV7 period.<sup>39</sup> The use of PCV13 (which targets 19A) effectively reduced serotype 19A but with a concurrent increase in serotype 24F in Europe.<sup>2,10,17,19,40</sup> Using the Spanish collection, we observed a rapid change in serotype composition within GPSC10 from 19A to 24F after PCV13. A similar serotype change within GPSC10 was also observed in Argentina<sup>3</sup> and Israel<sup>41</sup> after PCV13, demonstrating that the pre-existence of serotype variants enabled GPSC10 to survive and expand under the vaccine-selective pressure.

Serotype 24F pneumococci were infrequent causes of invasive disease in Europe in the 1980s,<sup>42</sup> and the earliest detection of the GPSC10 lineage was an isolate from

Denmark in 1996, which expressed serotype 14 (pubMLST, strain M132-14). GPSC10-24F was first reported from three adult patients in Naples, Italy between 1997 and 1998;<sup>42</sup> these findings coincide with our model prediction of the emergence of this clone in Europe. Since the late 1990s, GPSC10-24F has become more frequently detected in children<sup>2,33</sup> and adults<sup>31,43</sup> from southern Europe. Despite the geographical proximity between France and Spain, the increase in serotype 24F was mainly driven by two different clones, GPSC10 EU-clade-I in France and EU-clade-II in Spain. This finding might suggest trans-border transmission is present, but not as frequently as transmission within a country, resulting in evolution of two clones in parallel.

Although the global spread of serotype 24F is largely due to the clonal spread of three pneumococcal lineages (GPSC10, GPSC16, and GPSC206), the lineage driving the increase in serotype 24F differs between countries. This variation could be partially explained by the differences in antibiotic-selective pressure. For example, the 24F driver lineage in Denmark was GPSC6.<sup>7</sup> Unlike GPSC10, GPSC6 was susceptible to penicillin and erythromycin. This observation coincided with the lower consumption of penicillin and macrolides (a class of antibiotic that includes erythromycin) in Denmark, as compared with other countries such as France,<sup>2,3</sup> Lebanon,<sup>12</sup> and Spain<sup>14,15</sup> where the increase in serotype 24F was mediated by the multidrug-resistant GPSC10 (appendix 1 p 23) lineage. According to ResistanceMap, France, Lebanon, and Spain consumed 1.8–2.2 times more penicillin and 1.2–2.0 times more macrolides than Denmark in 2015. GPSC10 also mediated the increase in serotype 24F in Argentina,<sup>5</sup> where macrolide consumption was 1.2 times higher than in Denmark in 2015, even though penicillin consumption was similar. The high consumption of penicillin or erythromycin, or both, in Argentina, France, Lebanon, and Spain potentially selected for GPSC10, and serotype replacement in low antibiotic consumption settings was mainly observed to be mediated by susceptible lineages.<sup>7,44</sup> Among countries with an increase in serotype 24F, Japan has the highest consumption of erythromycin and the least consumption of penicillin, and the driver lineage in Japan, GPSC106 (CC2572), only exhibits erythromycin resistance.<sup>11</sup> These observations suggest that vaccine-selective and antibiotic-selective pressure are shaping the post-vaccine population structure, and that the reduction in antibiotic resistance achieved by PCVs might gradually diminish as a result.<sup>41</sup> However, resistance alone was not sufficient for clonal expansion, as GPSC10-24F was detected in all six countries but only expanded in settings with high antibiotic use. This finding echoed similar observations in other bacterial species such as *Escherichia coli*.<sup>15</sup> Because acquired antibiotic-resistant genes are part of the accessory genome (genes not present in all isolates of a species), their frequencies are under negative-frequency



**Figure 4:** Bayesian skyline plots of estimated median effective population size of EU-clade-I and EU-clade II of GPSC10-24F *Streptococcus pneumoniae* over time (A, C) and observed prevalence of GPSC10-24F *S pneumoniae* from overall invasive disease and carriage in France over the collection year (B, D)

The figure shows three exponential increases in effective population size in the EU-clade-I and one exponential increase in the EU-clade-II. The most recent increase in EU-clade-I coincided with the observed prevalence increase of GPSC10-24F in both overall disease and carriage isolates in France. GPSC=global pneumococcal sequence cluster.

dependent selection,<sup>46</sup> which might explain the coexistence of susceptible and resistant strains in the pneumococcal population.

The limitations of this study were that the sampling strategy and collection timeframe were not consistent between countries, and the relatively small sample size after stratifications (eg, carriage and IPD). However, the GPS project, which was formulated to create a pre-PCV and post-PCV dataset in each participating country to evaluate the effect of PCVs on the pneumococcal population, not only provided a large global collection of pneumococcal genomes, but also a comprehensive set of epidemiological metadata. This unprecedented database allowed us to complement knowledge we gained from a collection of country-specific analyses to this overarching study, providing an international perspective on GPSC10 and highlighting this lineage as carrying a high future risk for pneumococcal disease requiring targeted prevention. This study also underlines the need for policy makers to evaluate the overall effect of PCVs including changes in IPD incidence and detection of emerging non-vaccine serotypes. Focusing only on vaccine serotypes in estimating effect could be misleading as it would identify a rise in serotype 19A, observed in some settings with PCV10 use, but would not detect the increase in serotype 24F, associated with PCV13 use. Knowledge gained through implementation of an effective and sustainable surveillance system for

For more on ResistanceMap see <https://resistancemap.cddep.org/AntibioticUse.php>

*S pneumoniae* could guide timely policy making including choice of PCV to stabilise the incidence of IPD cases at a low level or even further reduce incidence.

Our work further illustrates the usefulness of bacterial genome sequencing to better understand the pneumococcal lineages behind serotype changes and reveals that GPSC10 alone is a challenge for a serotype-based vaccine strategy. More systematic investigation to identify lineages like GPSC10 will better inform and improve next-generation preventive strategies against pneumococcal disease.

#### The Global Pneumococcal Sequencing Project Consortium

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#### Contributors

SWL, RC, KPK, GR, RFB, LM, SDB, and EV conceptualised the study. SWL performed the data analysis, produced the tables and figures, and wrote the manuscript. KM did the literature review. KM and NK verified the data. SWL, KM, RC, SB, KPK, GR, RFB, LM, SDB, CMA, and EV contributed to data analysis and interpretation. All authors reviewed or edited the manuscript. All authors, including the members of the Global Pneumococcal Sequencing Consortium, contributed to data collection. SWL and SDB had access to the raw data. SWL, SDB, CMA, and EV made the final decision to submit. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Declaration of interests

AvG reports grants from the US Centers for Disease Control and Prevention and Sanofi; travel fees from Pfizer, Sanofi, and Merck; and is the chairperson of South Africa National Advisory Group on Immunisation, outside of the submitted work. AJP reports grants from Gavi, WHO, and AstraZeneca, and is a member of the Joint Committee on Vaccination and Immunisation and WHO Strategic Advisory Group of Experts on Immunization, outside of the submitted work. IN reports grants from BMGF and travel fees from the International Symposium on Pneumococci and Pneumococcal Diseases, outside of the submitted work. JM is an employee of Pharma Vaccines, outside of the submitted work. RD reports grants from Pfizer, MSD, and MedImmune–AstraZeneca, consulting and speaker fees from Pfizer and MSD, outside of the submitted work. SDB reports personal fees from Pfizer and Merck, outside the submitted work. CMA reports grants from Pfizer, outside of the submitted work. EV reports grants from Santé Publique France, Pfizer, and MSD, outside of the submitted work. All other authors declare no competing interests.

#### Data sharing

All sequencing reads were deposited in the European Nucleotide Archive (<https://www.ebi.ac.uk/ena/browser/home>) and the accession numbers are with the metadata and in silico output in appendix 2.

#### Acknowledgments

The study was cofunded by the Bill & Melinda Gates Foundation (grant code OPP1034556) and the Wellcome Sanger Institute (core Wellcome

grants 098051 and 206194). Particular thanks go to all members of the Global Pneumococcal Sequencing Consortium for their contributions of sample collection, processing, and collaborative spirit. We acknowledge Corinne Lévy, Naïm Ouldali, and Stéphane Béchet who manage children data collection and have helped in establishing the study sample for France. We are also grateful to Assiya El Mniai and Cécile Culeux (French National Reference Laboratory for pneumococci) for their technical assistance in preparing DNA for whole genome sequencing. We acknowledge the support from the sequencing facility and the Pathogen Informatics team at the Wellcome Sanger Institute, Hinxton, UK. The findings and conclusions detailed in this manuscript are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

#### References

- 1 Wahl B, O'Brien KL, Greenbaum A, et al. Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. *Lancet Glob Health* 2018; **6**: e744–57.
- 2 Ouldali N, Levy C, Varon E, et al. Incidence of paediatric pneumococcal meningitis and emergence of new serotypes: a time-series analysis of a 16-year French national survey. *Lancet Infect Dis* 2018; **18**: 983–91.
- 3 Ouldali N, Varon E, Levy C, et al. Invasive pneumococcal disease incidence in children and adults in France during the pneumococcal conjugate vaccine era: an interrupted time-series analysis of data from a 17-year national prospective surveillance study. *Lancet Infect Dis* 2021; **21**: 137–47.
- 4 Pai R, Moore MR, Pilišvili T, Gertz RE, Whitney CG, Beall B. Postvaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *J Infect Dis* 2005; **192**: 1988–95.
- 5 Galetti P, Lo SW, Hawkins PA, et al. Population genetic structure, serotype distribution and antibiotic resistance of *Streptococcus pneumoniae* causing invasive disease in children in Argentina. *Microb Genom* 2021; **7**: 000636.
- 6 Adam HJ, Golden AR, Karlowsky JA, et al. Analysis of multidrug resistance in the predominant *Streptococcus pneumoniae* serotypes in Canada: the SAVE study, 2011–15. *J Antimicrob Chemother* 2018; **73** (suppl 7): vii12–19.
- 7 Kavalari ID, Fuursted K, Kroghfelt KA, Slotved H-C. Molecular characterization and epidemiology of *Streptococcus pneumoniae* serotype 24F in Denmark. *Sci Rep* 2019; **9**: 5481.
- 8 van der Linden M, Falkenhörst G, Perniciaro S, Imöhl M. Effects of infant pneumococcal conjugate vaccination on serotype distribution in invasive pneumococcal disease among children and adults in Germany. *PLoS One* 2015; **10**: e0131494.
- 9 Phillips MT, Warren JL, Givon-Lavi N, et al. Evaluating post-vaccine expansion patterns of pneumococcal serotypes. *Vaccine* 2020; **38**: 7756–63.
- 10 Camilli R, D'Ambrosio F, Del Grosso M, et al. Impact of pneumococcal conjugate vaccine (PCV7 and PCV13) on pneumococcal invasive diseases in Italian children and insight into evolution of pneumococcal population structure. *Vaccine* 2017; **35**: 4587–93.
- 11 Ubukata K, Takata M, Morozumi M, et al. Effects of pneumococcal conjugate vaccine on genotypic penicillin resistance and serotype changes, Japan, 2010–2017. *Emerg Infect Dis* 2018; **24**: 2010–20.
- 12 Reslan L, Finianos M, Bitar I, et al. The emergence of invasive *Streptococcus pneumoniae* serotype 24F in Lebanon: complete genome sequencing reveals high virulence and antimicrobial resistance characteristics. *Front Microbiol* 2021; **12**: 637813.
- 13 Siira L, Vestheim DF, Winje BA, Caugant DA, Steens A. Antimicrobial susceptibility and clonality of *Streptococcus pneumoniae* isolates recovered from invasive disease cases during a period with changes in pneumococcal childhood vaccination, Norway, 2004–016. *Vaccine* 2020; **38**: 5454–63.
- 14 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* 2020; **73**: e3778–87.
- 15 Latasa Zamalloa P, Sanz Moreno JC, Ordobás Gavín M, et al. Trends of invasive pneumococcal disease and its serotypes in the Autonomous Community of Madrid. *Enferm Infecc Microbiol Clin (Engl Ed)* 2018; **36**: 612–20.



- 16 Kandasamy R, Voysey M, Collins S, et al. Persistent circulation of vaccine serotypes and serotype replacement after 5 years of infant immunization with 13-valent pneumococcal conjugate vaccine in the United Kingdom. *J Infect Dis* 2020; **221**: 1361–70.
- 17 Silva-Costa C, Brito MJ, Aguiar SI, Lopes JP, Ramirez M, Melo-Cristino J. Dominance of vaccine serotypes in pediatric invasive pneumococcal infections in Portugal (2012–2015). *Sci Rep* 2019; **9**: 6.
- 18 Balsells E, Dagan R, Yildirim I, et al. The relative invasive disease potential of *Streptococcus pneumoniae* among children after PCV introduction: a systematic review and meta-analysis. *J Infect* 2018; **77**: 368–78.
- 19 Ludwig G, Garcia-Garcia S, Lanaspá M, et al. Serotype and clonal distribution dynamics of invasive pneumococcal strains after PCV13 introduction (2011–2016): Surveillance data from 23 sites in Catalonia, Spain. *PLoS One* 2020; **15**: e0228612.
- 20 Ouldali N, Cohen R, Levy C, et al. Pneumococcal susceptibility to antibiotics in carriage: a 17 year time series analysis of the adaptive evolution of non-vaccine emerging serotypes to a new selective pressure environment. *J Antimicrob Chemother* 2019; **74**: 3077–86.
- 21 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.
- 22 Page AJ, Taylor B, Keane JA. Multilocus sequence typing by blast from de novo assemblies against PubMLST. *J Open Source Softw* 2016; **1**: 118.
- 23 Epping L, van Tonder AJ, Gladstone RA, Bentley SD, Page AJ, Keane JA. SeroBA: rapid high-throughput serotyping of *Streptococcus pneumoniae* from whole genome sequence data. *Microb Genom* 2018; **4**: e000186.
- 24 Metcalf BJ, Chochua S, Gertz RE Jr, et al. Using whole genome sequencing to identify resistance determinants and predict antimicrobial resistance phenotypes for year 2015 invasive pneumococcal disease isolates recovered in the United States. *Clin Microbiol Infect* 2016; **22**: 1002.e1–8.
- 25 Price MN, Dehal PS, Arkin AP. FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS One* 2010; **5**: e9490.
- 26 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009; **25**: 1754–60.
- 27 Croucher NJ, Page AJ, Connor TR, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res* 2015; **43**: e15.
- 28 Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 2006; **22**: 2688–90.
- 29 Bouckaert R, Heled J, Kühnert D, et al. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* 2014; **10**: e1003537.
- 30 Salje H, Lessler J, Maljkovic Berry I, et al. Dengue diversity across spatial and temporal scales: local structure and the effect of host population size. *Science* 2017; **355**: 1302–06.
- 31 Horácio AN, Silva-Costa C, Diamantino-Miranda J, Lopes JP, Ramirez M, Melo-Cristino J. Population Structure of *Streptococcus pneumoniae* causing invasive disease in adults in Portugal before PCV13 availability for adults: 2008–2011. *PLoS One* 2016; **11**: e0153602.
- 32 Lo SW, Gladstone RA, van Tonder AJ, et al. A mosaic tetracycline resistance gene tet(S/M) detected in an MDR pneumococcal CC230 lineage that underwent capsular switching in South Africa. *J Antimicrob Chemother* 2020; **75**: 512–20.
- 33 Simões AS, Pereira L, Nunes S, Brito-Avó A, de Lencastre H, Sá-Leão R. Clonal evolution leading to maintenance of antibiotic resistance rates among colonizing Pneumococci in the PCV7 era in Portugal. *J Clin Microbiol* 2011; **49**: 2810–17.
- 34 Gherardi G, D'Ambrosio F, Visaggio D, Dicuonzo G, Del Grosso M, Pantosti A. Serotype and clonal evolution of penicillin-nonsusceptible invasive *Streptococcus pneumoniae* in the 7-valent pneumococcal conjugate vaccine era in Italy. *Antimicrob Agents Chemother* 2012; **56**: 4965–68.
- 35 Setchanova LP, Alexandrova A, Dacheva D, Mitov I, Kaneva R, Mitev V. Dominance of multidrug-resistant Denmark(14)-32 (ST230) clone among *Streptococcus pneumoniae* serotype 19A isolates causing pneumococcal disease in Bulgaria from 1992 to 2013. *Microb Drug Resist* 2015; **21**: 35–42.
- 36 Ardanuy C, Marimón JM, Calatayud L, et al. Epidemiology of invasive pneumococcal disease in older people in Spain (2007–2009): implications for future vaccination strategies. *PLoS One* 2012; **7**: e43619.
- 37 Mayanskiy N, Savinova T, Alyabieva N, et al. Antimicrobial resistance, penicillin-binding protein sequences, and pilus islet carriage in relation to clonal evolution of *Streptococcus pneumoniae* serotype 19A in Russia, 2002–2013. *Epidemiol Infect* 2017; **145**: 1708–19.
- 38 Hurmic O, Grall N, Al Nakib M, et al. Evidence of a clonal expansion of *Streptococcus pneumoniae* serotype 19A in adults as in children assessed by the DiversiLab® system. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 2067–73.
- 39 Muñoz-Almagro C, Esteve C, de Sevilla MF, Selva L, Gene A, Pallares R. Emergence of invasive pneumococcal disease caused by multidrug-resistant serotype 19A among children in Barcelona. *J Infect* 2009; **59**: 75–82.
- 40 Balsells E, Guillot L, Nair H, Kyaw MH. Serotype distribution of *Streptococcus pneumoniae* causing invasive disease in children in the post-PCV era: a systematic review and meta-analysis. *PLoS One* 2017; **12**: e0177113.
- 41 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**: 759–69.
- 42 Pantosti A, Gherardi G, Conte M, Faella F, Dicuonzo G, Beall B. A novel, multiple drug-resistant, serotype 24F strain of *Streptococcus pneumoniae* that caused meningitis in patients in Naples, Italy. *Clin Infect Dis* 2002; **35**: 205–08.
- 43 González-Díaz A, Càmará J, Ercibengoa M, et al. Emerging non-13-valent pneumococcal conjugate vaccine (PCV13) serotypes causing adult invasive pneumococcal disease in the late-PCV13 period in Spain. *Clin Microbiol Infect* 2020; **26**: 753–59.
- 44 Vestrheim DF, Steinbakk M, Aaberge IS, Caugant DA. Postvaccination increase in serotype 19A pneumococcal disease in Norway is driven by expansion of penicillin-susceptible strains of the ST199 complex. *Clin Vaccine Immunol* 2012; **19**: 443–45.
- 45 Gladstone RA, McNally A, Pöntinen AK, et al. Emergence and dissemination of antimicrobial resistance in *Escherichia coli* causing bloodstream infections in Norway in 2002–17: a nationwide, longitudinal, microbial population genomic study. *Lancet Microbe* 2021; **2**: e331–41.
- 46 Corander J, Fraser C, Gutmann MU, et al. Frequency-dependent selection in vaccine-associated pneumococcal population dynamics. *Nat Ecol Evol* 2017; **1**: 1950–60.