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Evolutionary dynamics and global spread of macrolide-resistant *Bordetella pertussis* during the post-pandemic pertussis resurgence

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SUMMARY

Objectives: A global resurgence of pertussis has followed the COVID-19 pandemic, yet the underlying drivers remain unclear. We investigated the contribution of Bordetella pertussis genomic evolution to the post-pandemic resurgence.
Methods: We analyzed 8117 B. pertussis genomes from 35 countries, including 249 newly sequenced isolates collected from six regions in China. Temporal dynamics of genotypes, antigenic profiles, and macrolide resistance were examined before, during, and after the pandemic.
Results: Shared post-pandemic evolutionary patterns were identified across global B. pertussis populations. These included the emergence of macrolide-resistant strains in France, the United States, and Australia, and their dominance in eastern China, driven by the expansion of the MR-MT28 sublineage (ptxP3/fim3–1/prn150 or PRN-deficient). Parallel antigenic shifts were observed, with declining PRN-deficient strains accompanied by increasing prn2 allele frequencies in multiple regions. Region-specific dynamics were also evident. In China, the post-pandemic resurgence was characterized by near-complete replacement of circulating strains by the MR-MT28 sublineage. In contrast, the resurgence in Europe and the United States

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largely reflected the persistence and expansion of locally established lineages. Expanded sampling further revealed that MR-MT28 sublineage is no longer genetically homogeneous, having diversified into multiple phylogenetic groups, with most isolates detected outside China (72%, 8/11) being PRN-deficient.
Conclusions: This unified global genomic analysis demonstrates both shared and region-specific post-pandemic shifts in B. pertussis populations. The dominance of the MR-MT28 sublineage in China, together with its international dissemination and ongoing diversification, represents a growing public health concern and underscores the need for coordinated global genomic surveillance.

Introduction

Pertussis, a highly contagious respiratory disease primarily caused by *Bordetella pertussis*, declined globally following the introduction of whole-cell and acellular vaccines.[1,2] Despite sustained high vaccination coverage, many countries have observed a resurgence over the past two decades, with a marked increase post-COVID-19 pandemic.[3–5]

According to the World Health Organization (WHO),[6] following a sharp decline during the COVID-19 pandemic (January 2020–May 2023, when WHO declared the end of the COVID-19 pandemic), global pertussis cases rose substantially. In the post-pandemic period (after May 2023), more than 800,000 cases were reported worldwide in 2024, over four times higher than the pre-pandemic average. In Asia, China and South Korea reported more than 10-fold and 20-fold increases, respectively.[7–9] Several European countries, including Denmark, France, Czechia, and Finland, recorded historically high incidence.[10–16] Similar surges were observed in Australia[17] and the United States.[18]

Multiple factors may underlie this resurgence, including the relaxation of COVID-19 pandemic-related non-pharmaceutical interventions such as mask wearing and social distancing, waning vaccine-induced immunity, and evolution of *B. pertussis*.[2–5,7,8] Over recent decades, *B. pertussis* has undergone notable genetic shifts, including the emergence and prevalence of strains with *ptxP3* alleles potentially associated with increased virulence,[19] and pertactin (PRN)-deficient variants, which may evade vaccine-induced immunity.[20] In parallel, the emergence of macrolide-resistant *B. pertussis* (MRBP), particularly its predominance in China, has raised additional concern regarding potential transmission and treatment failures.[21–24] Of particular concern is the *ptxP3* MR-MT28 sublineage, which has been proposed as a major driver of China's post-pandemic resurgence due to its combined features of vaccine escape and antimicrobial resistance.[23–25] Notably, this genotype has recently been detected in multiple regions outside China, including France,[12,13] Finland,[16] and Australia.[17]

While the post-pandemic resurgence of pertussis in China appears associated with the expansion of the MR-MT28 sublineage,[23,25] the contribution of *B. pertussis* genomic evolution to post-pandemic resurgence at the global level, particularly outside China, has not been systematically examined. In this study, we investigated the genomic dynamics of *B. pertussis* in the context of the global post-pandemic resurgence by analyzing 8117 genomes from 35 countries, including 249 newly sequenced isolates collected from six regions in China. Beyond confirming previously reported regional trends, our integrated framework enables direct cross-regional comparisons and reveals both shared and region-specific evolutionary patterns. We also substantially expanded the genomic representation of the MR-MT28 sublineage, providing a more resolved view of its diversification and international dissemination.

Methods

Pertussis case data

Pertussis case numbers and incidence data were obtained from the WHO website[6] to provide epidemiological context and to enable qualitative comparison with temporal changes in *B. pertussis* genotype dynamics. For countries or regions with incomplete WHO data, supplementary case counts were searched and sourced from national public health agencies, including the US Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC). Specifically, the 2024 case count for the United States (35,435 cases) was manually updated from the US CDC,[26] and the 2024 count for France (36,938 cases) was based on published statistics from recent European studies.[27]

Genome collection and sequencing

We analyzed a total of 8430 non-duplicate *B. pertussis* genomes with unique Biosample accession numbers, comprising 8181 publicly available genomes and 249 newly sequenced isolates. Public genomes were retrieved from the NCBI Sequence Read Archive (SRA) and GenBank, with isolation year and country extracted from Biosample metadata.

The 249 newly sequenced *B. pertussis* isolates were collected from tertiary hospitals and local CDCs across six regions in China. As this study focused on the post-pandemic period, all archived isolates obtained from clinically diagnosed or PCR-confirmed pertussis cases during 2024 (January–December) at each participating center were included: Shanghai (n = 42), Zhejiang (n = 165), Anhui (n = 7), Jiangsu (n = 3), Sichuan (n = 19), and Guangdong (n = 13). Among 245 isolates with available patient metadata, 216 (88.2%) were from children aged > 1 year (Table S1).

Whole-genome sequencing for newly sequenced isolates was performed on the Illumina NovaSeq platform. Newly generated sequencing data are available in the NCBI Genbank under accession number PRJNA1288341. Raw sequencing reads were quality-trimmed using Trimmomatic v0.39.[28] Genome assembly was conducted using the shovill v1.1.0 pipeline (https://github.com/tseemann/shovill). Assembly quality was assessed using CheckM v1.1.3.[29] Only high-quality genomes (completeness > 95%, contamination < 5%) were retained in the following analysis.

After genome quality control, a total of 8117 high-quality genomes were used in the analysis, including isolates collected between 1900 and 2024 from 35 countries across six continents: Europe (16 countries), Asia,[6] North America,[5] Africa,[4] Oceania,[2] and South America.[2] The five main contributing countries were China (n = 1645), Australia (n = 445), France (n = 1308), Belgium (n = 429), and the United States (n = 2798) (Fig. S1).

Phylogenetic and temporal analysis

Core-genome single-nucleotide polymorphisms (SNPs) were identified using Snippy v4.6.0 (https://github.com/tseemann/snippy), based on raw reads or assemblies where reads were unavailable, with *B. pertussis* Tohama I (NC_002929.2) as the reference. SNPs within repetitive regions were identified using Tandem Repeats Finder (TRF) v4.07b and self-aligning by BLASTN, as previously described.[30] Maximum-likelihood phylogenies were constructed based on non-repetitive core-SNPs using FastTree v2.1.10[31] (all strains, Figs. 1B, S2) or RAxML-NG v1.0.1[32] (MR-MT28 sublineage) with the General Time Reversible substitution model with a

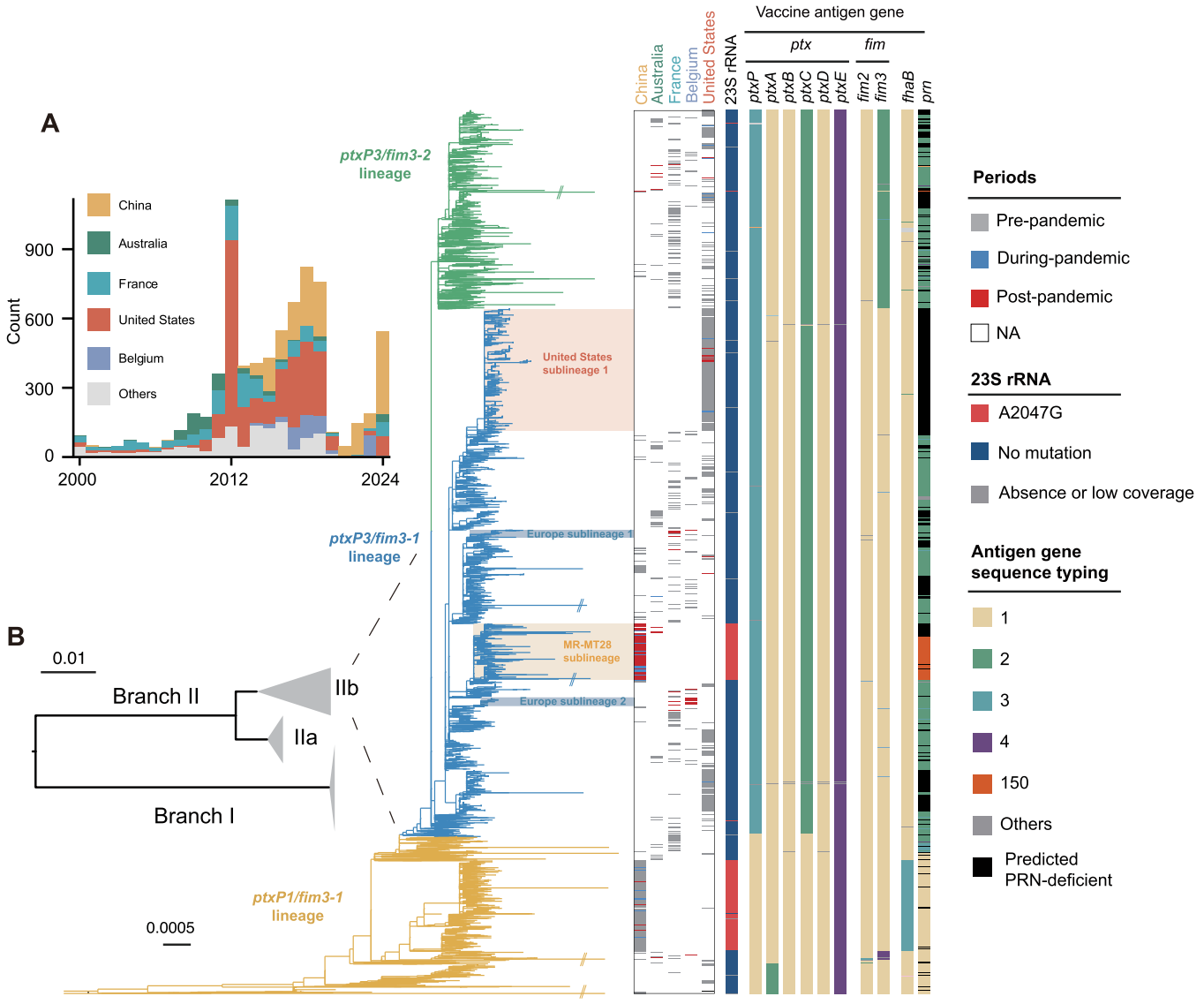

Fig. 1. Global population structure and resistance and antigenic profiles of *B. pertussis*. (A) Annual numbers of high-quality genomes from China, Australia, France, Belgium, and the United States. (B) Maximum-likelihood phylogenetic tree of global isolates, annotated with geographic origin, macrolide resistance status, and vaccine antigen profiles. Branches marked with "//" indicate artificially shortened branches corresponding to putative mutator strains with exceptionally long branch lengths (Fig. S3). Genomes lacking isolation date information are shown in white. Gray shading in the resistance column indicates strains for which 23S rRNA alleles could not be reliably determined because of incomplete or missing sequence data.

Gamma distribution (GTR+Γ). Putative mutator strains indicated by abnormal tree branches were artificially shortened to improve visual clarity (Fig. S3 and Table S1).

Strains were assigned to branches and lineages based on the phylogenetic framework proposed by Bart et al.[33] Specifically, *B. pertussis* strains were first divided into two deep branches (I and II), with branch II further subdivided into IIa and IIb. As most isolates belong to branch IIb, this branch was further resolved into lineages defined primarily by antigen genotypes (*ptxP* and *fim3*) (Fig. S2).

Within these established branches and lineages, we further defined sublineages as monophyletic phylogenetic clades. Using this criterion, we defined four major sublineages within the focal lineage, predominantly associated with strains from the United States (United States sublineage 1), Europe (Europe sublineage 1 and Europe sublineage 2), and China (MR-MT28 sublineage), respectively. The China-associated sublineage is dominated by *ptxP3* macrolide-resistant strains corresponding to the MR-MT28 clone originally defined in our previous work,[22,23,25] based on the predominance of MLVA type 28. To maintain consistency with existing literature, we retain this nomenclature and refer to this clade as the MR-MT28 sublineage in this study, noting that not all constituent strains necessarily exhibit MLVA type 28. In addition, we observed genetic diversification within the MR-MT28 sublineage and therefore further defined phylogenetic groups (PGs) within this sublineage. Strain assignments to branches, lineages, sublineages, and phylogenetic groups are provided in Table S1.

Time-resolved phylogenies and effective population size dynamics were inferred using BEAST v1.10.4.[34] TempEst was used to check for the time signal (Fig. S4). We applied the GTR+Γ substitution model and used the isolation date (years) for tip date calibration. Six combinations of molecular clocks (strict and uncorrelated relaxed clock) and coalescent tree models (constant size and Bayesian Skyline and Skygrid) were tested, and the relaxed clock with Skygrid model was the best fit based on path sampling and stepping-stone sampling analysis. We ran ten independent Markov chain Monte Carlo chains for 30 million steps, sampling every 3000 steps. The results of different runs were combined to generate the maximum clade credibility trees using TreeAnnotator v1.10.4. Effective population size dynamics were inferred using Tracer v1.7.1.[35]

Vaccine antigen and macrolide resistance profiles

Vaccine antigen alleles were assigned by comparison with the curated *Bordetella* allele database on BIGSdb-Pasteur,[36] using BLASTn searches against assembled genomes (Table S1). So far, the only mechanism associated with macrolide resistance has been the point mutation of A2047G in the 23S rRNA gene of *B. pertussis*. Accordingly, strains carrying the A2047G mutation (allele 13) were classified as macrolide resistant.

Strains were classified as predicted PRN-deficient if they carried incomplete or disrupted *prn* coding sequences or promoters, determined by BLASTn or TBLASTn coverage < 99%, according to the criteria defined by Lefrancq et al.[37] Mechanisms underlying PRN deficiency were examined and classified as IS481 insertion (the predominant mechanism within the MR-MT28 sublineage) or as "other" mechanisms, including point mutations and alternative disruptions, following the definitions of Lefrancq et al.[37]

Results

Global genomic diversity and population structure of B. pertussis

We analyzed 8117 high-quality genomes, including 249 newly sequenced isolates collected between April and October in 2024 from six regions in China. These genomes spanned 35 countries across six continents (Fig. S1). Five countries (China, USA, Australia, Belgium, and France) from four continents had more than ten genomes from both pre-pandemic and post-pandemic periods, enabling temporal and geographical comparisons (Fig. 1A).

A maximum-likelihood phylogenetic tree based on 17,181 core-genome SNPs was constructed to characterize the global population structure of *B. pertussis* (Figs. 1B, S3). Strains were assigned to phylogenetic branches and lineages following previously reported frameworks.[33] Consistent with earlier studies,[33] 99.6% (8087/8117) of isolates clustered within branch IIb, whereas a small fraction (0.3%, 30/8117) formed distinct clades corresponding to branch I and IIa.

To achieve higher-resolution classification within branch IIb, we further subdivided this branch into three major lineages based on *ptxP* and *fim3* genotypes: *ptxP1/fim3–1* (17%, 1364/8087), *ptxP3/fim3–1* (60%, 4812/8087), *ptxP3/fim3–2* (22%, 1816/8087), with the remaining 1% (95/8087) assigned to minor clades not fitting these combinations. The difference between *ptxP1* and *ptxP3* is a point mutation of C to A at position −65 in the promoter of the pertussis toxin gene, and the difference between *fim3–1* and *fim3–2* alleles is a point mutation of C260A, causing an amino acid change (Ala87Glu).[19,38]

The MRBP-associated A2047G mutation in the 23S rRNA gene was detected in 59% (811/1364) of *ptxP1/fim3–1*, 11% (519/4812) of *ptxP3/fim3–1,* and 0.3% (5/1816) of *ptxP3/fim3–2* strains. As nearly all *ptxP3/fim3–1* MRBP strains formed a single monophyletic clade corresponding to the MR-MT28 strains previously reported predominantly in China,[25,39] we refer to this clade as the MR-MT28 sublineage, following established usage (Fig. 1B).

In total, 513 MR-MT28 sublineage strains were identified, carrying either a p*txP3/fim3–1/prn150* profile (74%, 382/513) or a *ptxP3/fim3–1* genotype with predicted PRN deficiency (25%, 129/513). Among the 249 newly sequenced Chinese isolates, 217 (87.1%) belonged to the MR-MT28 sublineage, while the remaining isolates were primarily assigned to the *ptxP1/fim3–1* lineage (31/249, 12.4%). Notably, two strains classified within the MR-MT28 sublineage lacked the A2047G mutation in the 23S rRNA gene, which were collected in Shanghai, China, in 2019 and 2022, respectively, indicating potential heterogeneity in resistance-associated mutations during the evolution of this sublineage.

Lineage dynamics and variation in vaccine antigen and antibiotic resistance profiles

We focused on five countries (China, USA, Australia, Belgium, and France) spanning four continents to examine post-pandemic shifts in genomic dynamics (Fig. 2A) in the context of increased pertussis incidence. Across regions, we observed both shared and region-specific changes in lineage composition, vaccine antigen variants, and macrolide resistance among circulating *B. pertussis* populations.

In China, a total of 1645 genomes were analyzed. Using this substantially expanded dataset, our results confirm previously reported post-pandemic expansion of *ptxP3/fim3–1* lineage, together with a marked rise in MRBP frequency and pronounced changes in antigen profiles, primarily driven by rapid expansion of the MR-MT28 sublineage during and after the COVID-19 pandemic.[22,23,25,39] Consequently, MR-MT28 sublineage accounted for 99% of circulating strains in the post-pandemic period (Figs. 2A, 3), replacing the pre-pandemic predominance of *ptxP1/fim3–1* MRBP isolates (96%, 714/747) with *ptxP3/fim3–1* isolates (98%, 386/390). This lineage replacement was accompanied by a marked reduction in genetic diversity (median pairwise SNP distance: 8 post-pandemic vs 31 pre-pandemic, Fig. 2B).

Similarly, in Australia (n = 445), substantial post-pandemic shifts were observed in lineage distribution and antigen gene composition, accompanied by an increase in MRBP prevalence (Fig. 2A), consistent with previous reports.[17] Based on our genomic analysis, 9 of 40 post-pandemic isolates (23%) were MRBP, indicating a notably higher frequency than previously reported outside China,[24] although the sample size remains limited. This rise was also associated with the introduction of the MR-MT28 sublineage.

In contrast to China, changes in lineage composition and antigen gene profiles in Australia were largely driven by the rise of *ptxP3/fim3–2* lineage and *fim3–2* and *prn2* alleles, along with a decreased frequency of predicted PRN-deficient strains (Fig. 2A). Moreover, post-pandemic strains in Australia remained genetically diverse, with *ptxP3/fim3–2* and *ptxP3/fim3–1* lineages present at 45% (18/40) and 53% (21/40), respectively. Consistent with this pattern, genetic diversity remained stable in the post-pandemic period (median pairwise SNP distance: 24 post-pandemic vs 19 pre-pandemic, Fig. 2B). Phylogenetic analysis showed that close relatives of most post-pandemic strains were already circulating before the pandemic, except for the MR-MT28 sublineage, suggesting that the resurgence in Australia might be driven by the expansion of locally pre-existing lineages (Figs. 1B, 2A).

In Europe (n = 2595), represented by France (n = 1308) and Belgium (n = 429), noticeable post-pandemic changes were observed in the vaccine antigen gene *prn*. As in Australia, the frequency of the *prn2* allele increased post-pandemic, while the frequency of predicted PRN-deficient strains decreased (Fig. 2A). Within this dataset, MR-MT28 sublineage was detected in France, consistent with a recent report identifying 14 MR-MT28 sublineage strains.[13]

Post-pandemic strains in Europe mainly (65%) belong to two sublineages (Europe sublineage 1 and 2) (Fig. 1B). Both sublineages were already present pre-pandemic, indicating continued transmission of locally endemic strains. However, patterns varied by country: post-pandemic strains in Belgium showed significantly reduced diversity (median pairwise SNP distance: 5 post-pandemic vs 16 pre-pandemic, Fig. 2B), with 70% (64/91) belonging to Europe sublineage 2, whereas no comparable reduction in genetic diversity was observed in France.

In the United States (n = 2798), lineage composition and genetic diversity remained relatively stable (median pairwise SNP distance: 19 post-pandemic vs 23 pre-pandemic, Fig. 2). We identified North America sublineage 1, which predominated pre-pandemic, and remained dominant post-pandemic (67%, 69/103), continuing to drive endemic circulation (Fig. 1B). One MR-MT28 sublineage strain was identified in the United States in 2024.

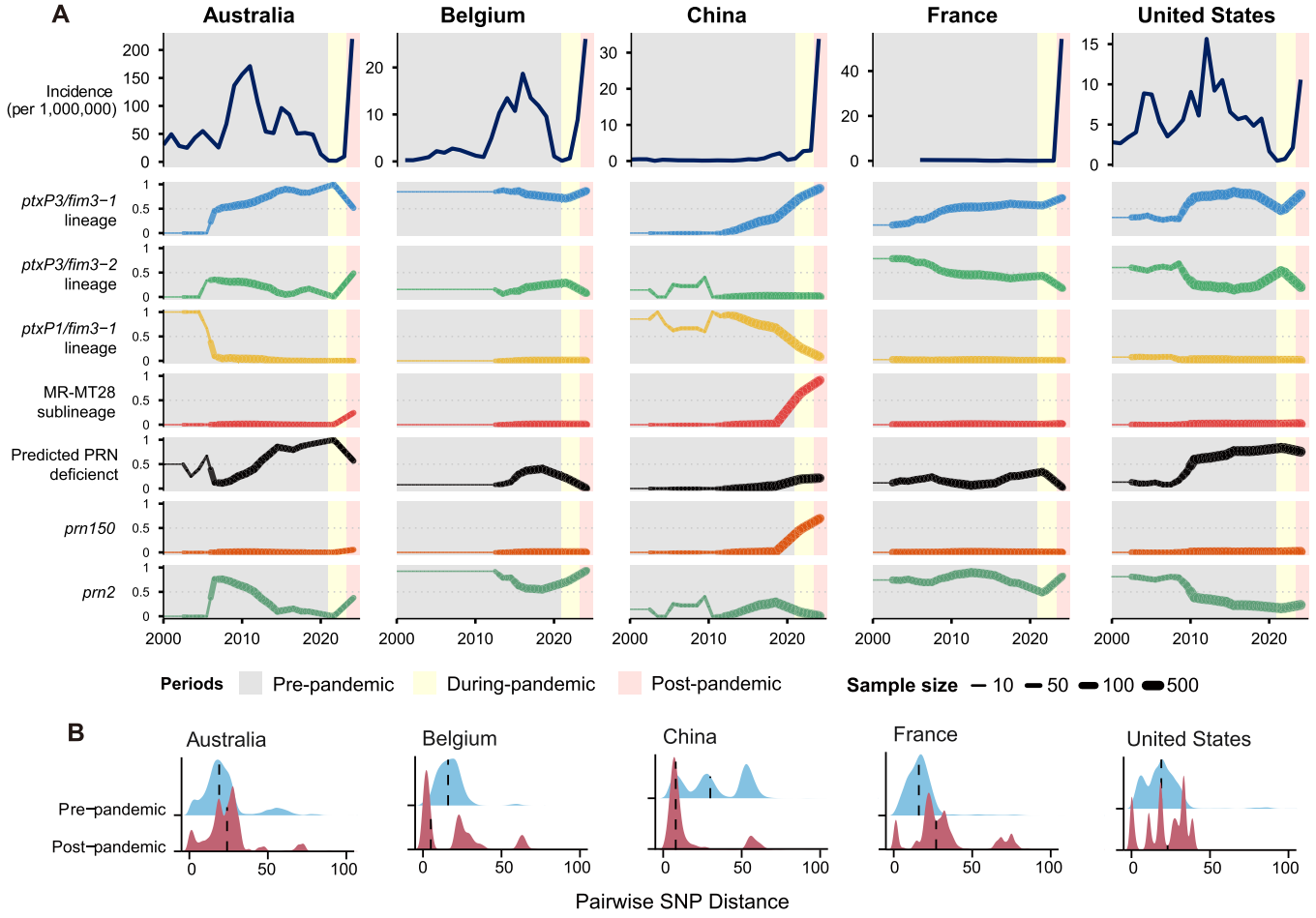

Fig. 2. Temporal dynamics of pertussis incidence and *B. pertussis* population and genetic diversity across five countries. (A) Annual pertussis incidence rates (per 1,000,000 population, top panel) and proportions of specific lineages, sublineages, and antigenic profiles (other panels). Pertussis incidence rates were derived from WHO data and supplemented with national public health surveillance reports.[27] Trend lines are weighted by sample size. Background colors indicate different time periods: pre-pandemic (2000–2020), pandemic period (January 2020–May 2023), and post-pandemic (after May 2023). (B) Genetic diversity represented by pairwise core-genome SNP distances among pre-pandemic and post-pandemic strains from five countries. The x-axis denotes pairwise SNP distance, and the y-axis denotes the density of pairwise comparisons.

Evolutionary dynamics and global spread of the MR-MT28 sublineage

The MR-MT28 sublineage has been previously reported independently in several countries using geographically isolated datasets.[12,13,16,17,40] Here, by assembling a unified global genomic dataset of 513 MR-MT28 sublineage genomes from four countries, including 217 newly sequenced isolates from six regions in China, we confirm its global occurrence (Fig. 3A, B) and enable a systematic analysis of its origin, diversification, and international spread within a single framework.

Phylogenetic analysis confirmed that the MR-MT28 sublineage evolved from the MS-MT28 strains, which were primarily identified in Europe and North America pre-pandemic (Fig. 3C, D). Molecular clock analysis estimated the emergence of MR-MT28 sublineage around 2016 (95% CI: 2014–2018), most likely originating in China, where it now predominates (Fig. 3D). As previously described,[25] we confirmed that MR-MT28 sublineage is defined by two characteristic mutations relative to MS-MT28: the macrolide resistance–associated A2047G mutation in the 23S rRNA gene, and a synonymous G1959A substitution in *BP0685* (dehydrogenase/oxidase). In addition, we observed that the antigen allele *prn150*, which is highly prevalent within MR-MT28, was also present in a single MS-MT28 strain from Shanghai, suggesting that this allele likely arose prior to, or during, the early emergence of the MR-MT28 sublineage.

Leveraging the newly generated Chinese dataset, we show that MR-MT28 sublineage expanded rapidly and extensively across China in the post-pandemic period, becoming dominant particularly in eastern China, while remaining at lower frequencies in western regions (Fig. 3B). Outside China, MR-MT28 sublineage was detected in 2024 in three countries (France, Australia and United States), and were phylogenetically interspersed among Chinese strains rather than forming a single external cluster (Fig. 3C, D), indicating multiple independent international introductions. Consistent with its rapid expansion in China and subsequent geographic dissemination, Bayesian skyline analysis revealed a sharp post-pandemic increase in the effective population size of the MR-MT28 sublineage, exceeding a 1000-fold rise between 2021 and 2024 (Fig. 3E).

This expanded dataset further shows that the MR-MT28 sublineage has undergone genetic diversification and is no longer homogeneous, with two distinct phylogenetic groups (PG1 and PG2) identified (Fig. 3C, D). PG1, estimated to have emerged around 2019, is characterized by predicted PRN deficiency, a nonsynonymous mutation in BP3197 (G287T; Arg96Leu; NAD(P)-dependent oxidoreductase), and, in a subset of strains, an additional mutation in BP0026 (G370T; Ala124Ser; thiolase family protein). Notably, most MR-MT28 isolates detected outside China, including 7 of 9 from Australia and the single isolate from France, belonged to PG1. Given previous evidence that PRN deficiency is associated with increased fitness in populations with long-standing acellular pertussis vaccination programs,[20,37,41] this enrichment may be relevant to the international dissemination of this phylogenetic group.

PG2 was defined by a distinct set of mutations, including *BP2642A* (C664T; Pro222Ser; ABC transporter ATP-binding protein), *BP3467* (G1621A; Asp541Asn; AsmA family protein), and a

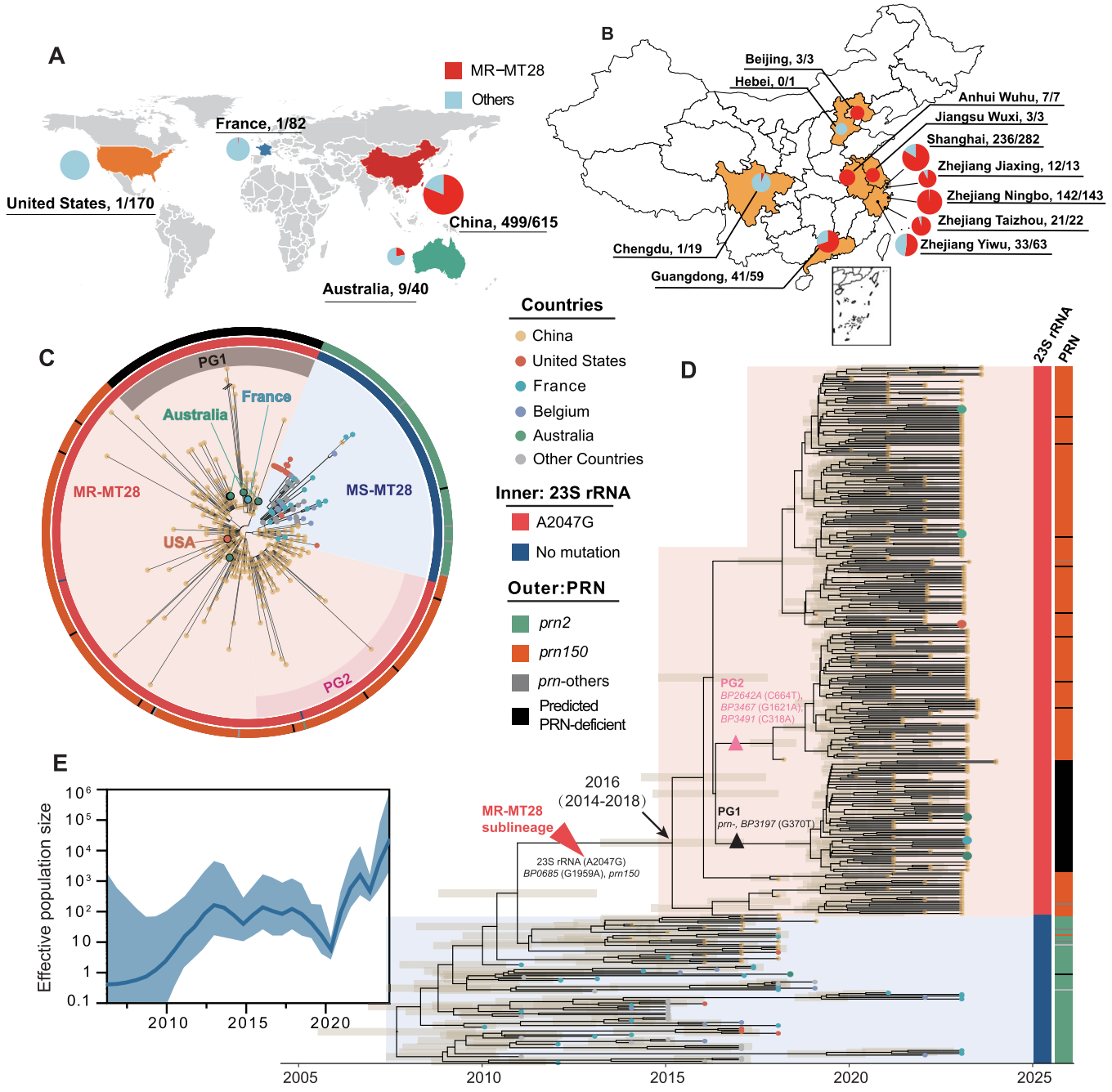

Fig. 3. Evolutionary dynamics and global dissemination of the MR-MT28 sublineage. (A, B) Geographic distribution of MR-MT28 sublineage during and after the pandemic (post-2020), shown at the global scale and within China. Pie charts indicate the regional proportions of the MR-MT28 sublineage. (C) Maximum-likelihood phylogeny of the MR-MT28 sublineage and phylogenetically closely related macrolide-susceptible strains (MS-MT28 sublineage). Tip colors denote the country of origin. Rings indicate 23S rRNA (inner) and *prn* allele (outer) profiles. (D) Time-calibrated phylogeny of the MR-MT28 sublineage and closely related MS-MT28 strains, annotated with 23S rRNA status (inner) and *prn* allele (outer) profiles. Key time points of inferred genetic events, and 95% confidence intervals are indicated by arrows, triangles, and shaded orange rectangles, respectively. (E) Temporal changes in the effective population size of the MR-MT28 sublineage inferred using Bayesian Skygrid analysis.

synonymous substitution in *BP3491* (C318A; NAD(P)/FAD-dependent oxidoreductase). Although these mutations do not currently have known functional implications, it is notable that both phylogenetic groups independently accumulated changes in genes encoding NAD (P)-dependent oxidoreductases (*BP3197* in PG1 and *BP3491* in PG2), representing potential targets for future functional investigation.

Discussion

Using a large-scale, unified global genomic dataset, we investigated changes in *B. pertussis* population dynamics in the post-pandemic period. Unlike previous studies that examined individual countries in isolation,[12,16,17,42] our integrated framework enables regional trends to be evaluated consistently while also allowing direct cross-regional comparisons. In addition, by newly sequencing isolates from six regions of China, we substantially expanded the genomic representation of the MR-MT28 sublineage, providing a more resolved view of its internal diversification and international dissemination.

Across countries, several shared post-pandemic patterns emerged. First, the prevalence of *ptxP3*-associated MRBP increased markedly in both China and Australia,[17,39] largely driven by the

expansion of the MR-MT28 sublineage. Second, the *ptxP3/fim3–1* lineage, which already predominated before the pandemic, further increased in frequency in China, Europe, and the United States. Third, PRN-deficient strains declined in Europe, Australia, and the United States, accompanied by a concurrent rise in the *prn2* allele.

The decline of PRN-deficient strains in Europe is consistent with recent surveillance data showing a marked resurgence of PRN-positive isolates in 2024, with frequencies increasing from 48.7% to 98.5% in France and from 75.8% to 99.5% in Finland.[12,16] This pattern likely reflects changes in vaccine-mediated selection. In several European countries, including France and Denmark, the recent adoption of PRN-free acellular pertussis (aP) vaccines may have reduced immune pressure against PRN expression, thereby diminishing the selective advantage of PRN deficiency and facilitating the re-emergence of PRN-positive strains.[12,43,44] Additionally, the migration of PRN-positive strains into Europe, followed by spread in the absence of competing PRN-deficient strains, represents an alternative possibility.

In contrast, PRN-deficient strains increased in China after the pandemic, despite being rarely reported previously. This trend is less likely to be driven by vaccine-mediated selection, as aP vaccines used in China generally do not include PRN,[45,46] and PRN deficiency would therefore not confer a direct immunological advantage. Instead, PRN-deficient isolates in China were almost exclusively associated with the MR-MT28 sublineage, which is characterized by macrolide resistance. Given the extensive use of antibiotics in China,[24] we propose that the rise in PRN-deficient strains may reflect a hitchhiking effect accompanying the rapid expansion of this antibiotic-resistant lineage.

Beyond these contrasting PRN dynamics, additional region-specific patterns were evident. In China, the post-pandemic resurgence was dominated by the near-complete replacement of circulating strains by the recently emerged MR-MT28 sublineage, indicating a prominent role for *B. pertussis* evolution in the post-pandemic resurgence.[25] In contrast, resurgence in Europe and the United States was characterized by the persistence and expansion of locally established lineages, suggesting that factors such as waning vaccine-induced immunity and social or behavioral changes, rather than recent bacterial evolution, may have played a larger role in these regions. Moreover, in Australia, the *fim3–2* allele increased uniquely in the post-pandemic period. This allele has previously been associated with enhanced biofilm formation[47] and showed a global rise beginning in the mid-1990s, coinciding with the transition from whole-cell to aP vaccines.[33] Notably, its global frequency has declined since 2020 for reasons that remain unclear. Together, these findings highlight the role of region-specific selective pressures and local evolutionary trajectories in shaping post-pandemic *B. pertussis* resurgence.

By analyzing MR-MT28 sublineage genomes from multiple continents within a unified phylogenomic framework, we confirm its international occurrence[13,17,42] and provide new insight into its diversity and global spread. We showed that it has widely spread in China in the post-pandemic period, becoming dominant particularly in eastern China. Moreover, the MR-MT28 sublineage detected outside China did not form a single phylogenetic cluster, supporting multiple independent introductions. Beyond establishing its global presence, our expanded dataset shows that the MR-MT28 sublineage is no longer genetically homogeneous. We identify two distinct phylogenetic groups (PG1 and PG2), indicating ongoing diversification within this recently emerged sublineage. PG1, characterized by PRN deficiency and over-represented among non-Chinese isolates, may harbor traits that facilitate persistence or dissemination in settings with long-standing use of acellular pertussis vaccines containing PRN.[37] Although the functional consequences of the mutations distinguishing these groups remain uncertain, their emergence underscores the evolutionary plasticity of the MR-MT28 sublineage and highlights priorities for future experimental investigation.

The global spread of MR-MT28 sublineage contrasts sharply with that of earlier macrolide-resistant *B. pertussis* lineages. Previous *ptxP1*-associated MRBP strains remained largely confined to China for more than a decade,[21–24] whereas MR-MT28 sublineage has now been detected across multiple continents. Its rapid expansion within China is likely driven by strong antibiotic selection pressure. In contrast, outside China, where macrolide use is more tightly regulated, the MR-MT28 sublineage may not gain a sustained selective advantage, and its detection is more likely to reflect sporadic introductions, such as by travelers, rather than widespread local expansion.

Our genomic analysis confirmed a notable post-pandemic rise in MRBP prevalence in Australia.[17] This expansion was further supported by targeted culture-independent sequencing screening, which estimated MRBP prevalence of 4.4%.[17] In addition to the MR-MT28 sublineage strains identified in this study from France, a recent French study showed that overall MRBP accounted for 2.8% (17/593) of the tests (culture and qPCR) in 2024.[13] Phylogenetic analysis revealed that the 14 MRBP belong, together with isolates from China, to a cluster of 349 MRBP isolates. All 14 MRBP carried *ptxP3*, and 13 of them were PRN-deficient.[12,13] MRBP strains have recently been identified in other European countries, such as Finland.[16] Thus, the geographic range of MR-MT28 sublineage described here only represents a minimum estimate.

The repeated international detection of MR-MT28 sublineage, together with its globally prevalent *ptxP3* genetic background and prior reports linking this lineage to extensive transmission in vaccinated populations, indicating enhanced immune evasion,[25] underscores the need for strengthened international genomic surveillance, particularly for PRN-deficient MR-MT28 strains.

This study has several limitations. First, publicly available genomes are unevenly distributed across regions, and post-pandemic data remain limited or unavailable for some countries, introducing unavoidable sampling biases in regional comparisons. Second, vaccination programs, vaccine formulations, and the implementation of non-pharmaceutical interventions varied across countries, leading to heterogeneous selective pressures that complicate direct cross-country inference. Finally, assembly-based detection of macrolide resistance reflects a consensus sequence; it typically identifies the A2047G mutation only when it is present in at least two of the three 23S rRNA gene copies. As a result, mutations confined to a single gene copy, although potentially sufficient to confer macrolide resistance, may be missed.

In summary, this study provides a unified global view of *B. pertussis* population dynamics in the post-pandemic era. Beyond confirming previously reported regional trends, our integrated framework enables direct cross-regional comparisons and reveals both shared and region-specific evolutionary patterns. By newly sequencing isolates from six regions of China, we substantially expanded the genomic representation of the MR-MT28 sublineage and demonstrated its rapid post-pandemic expansion and dominance in eastern China. We further show that MR-MT28 is no longer genetically homogeneous, having diversified into distinct phylogenetic groups, and that its international presence reflects multiple independent introductions. Together, these findings highlight the need for coordinated global genomic surveillance to monitor the ongoing evolution and spread of the MR-MT28 sublineage.

CRediT authorship contribution statement

P.F. and Y.C. contributed to the conception of this project. Z.R., Y.Z., P.F., Z.K., Y.Y. B.W., D.B., S.G., C.N., X.S., S.W., and S.H. were responsible for the collection of strains and metadata. H.Z., C.Y., H.L., N.W., J.W., F.J., and S.T. performed bioinformatics analysis of whole genome sequence data. Y.C., P.F. and Q.H. prepared the manuscript. All authors contributed to the interpretation of results and critical review of the manuscript.

Data availability

Accession numbers for the publicly available genome sequences and associated metadata can be found in supplementary materials. Newly sequenced genomes in this study are available from the National Centre for Biotechnology Information database under BioProject PRJNA1288341.

Declaration of Competing Interest

We declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2026.106718.

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