Genomics 117 (2025) 111132

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Research Paper

# Geogenomic mapping of drug-resistant *Mycobacterium tuberculosis* from Ireland and overseas

Cian Ennis [a,b], Gaetan Thilliez [a,b], Ronan F. O'Toole [a,b,*]

[a] School of Biotechnology, Faculty of Science and Health, Dublin City University, Dublin D09 K20V, Ireland
[b] Life Sciences Institute, Dublin City University, Dublin D09 NR58, Ireland

ARTICLE INFO

*Keywords:*
*Mycobacterium tuberculosis*
Drug-susceptible tuberculosis (DS-TB)
Drug-resistant tuberculosis (DR-TB)
Rifampicin-resistant tuberculosis (RR-TB)
Multi-drug resistant tuberculosis (MDR-TB)
Extensively-drug resistant tuberculosis (XDR-TB)
Whole genome analysis

ABSTRACT

In this study, we performed an in-depth comparison of genome-sequenced *Mycobacterium tuberculosis* isolates from Ireland with isolates from other countries. The sequenced isolates from Ireland mostly belonged to Lineage 4 (64.15 %) with Lineages 2 (17.27 %), 1 (13.21 %), 3 (5.22 %), and 5 (0.15 %) also represented. Of these, Lineages 2 (47.57 %) and 4 (34.95 %) accounted for the majority of the isolates that were resistant to at least rifampicin. By performing hierarchical clustering of the genomes, we determined that many drug-resistant (DR) strains of Lineage 2 collected in Ireland belonged to larger international clusters of the bacterium that were dominant in countries that included Estonia, Georgia, Ukraine, and Moldova. Lineage 4 DR-TB strains isolated in Ireland were also commonly part of large international clusters but the major countries differed *i.e.* Eswatini, Germany, United Kingdom, and Mozambique. Based on single nucleotide polymorphism (SNP) analysis, there was no evidence found of widespread onward transmission of DR-TB isolates in Ireland. This indicates that a key source of DR-TB in Ireland is translocation of *M. tuberculosis* from countries where specific genetic clusters of drug-resistant strains are prevalent. This study has implications for interpreting future trends in TB drug resistance. As an open economy with extensive international travel connections, Ireland is sensitive to the emergence of resistant isolates of *M. tuberculosis* elsewhere. In addition to caution being applied with respect to TB presenting in individuals from high multi-drug resistant (MDR) TB burden countries, vigilance is also needed for TB in persons from countries where large phylogenetic clusters of DR-TB occur.

## 1. Introduction

Antimicrobial resistance (AMR) is a leading cause of death with an estimated 1.25 million (95 % uncertainty interval: 0.91–1.71) mortalities attributed to bacterial AMR in 2019 internationally [1]. *Mycobacterium tuberculosis* is one of the major pathogens that contributes to the burden of AMR disease with approximately 170,000 tuberculosis (TB) deaths associated with AMR in 2019 [1].

Resistance in *M. tuberculosis* to key first- and second-line drugs results in lower treatment success rates according to the World Health Organization (WHO) [2]. For each step in the increased drug resistance profile of an *M. tuberculosis* isolate, the odds of a successful treatment outcome have been found to decline by an adjusted odds ratio of 0.62-fold (95 % confidence interval (CI): 0.56–0.69), independently of other factors [3]. The WHO defines multi-drug resistant (MDR) TB as "TB disease caused by a strain of *M. tuberculosis* complex that is resistant to rifampicin and isoniazid" [4]. Since January 2021, it has defined extensive-drug resistant (XDR) TB as "TB caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) strains that fulfil the definition of MDR/RR-TB and which are also resistant to any fluoroquinolone and at least one additional Group A drug" consisting of either bedaquiline or linezolid (or both) [5]. In addition, it defines pre-XDR-TB as "TB caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) strains that fulfil the definition of MDR/RR-TB and which are also resistant to any fluoroquinolone" [5].

The WHO reported 10.8 million new incidences of TB in 2023 of which approximately 400,000 cases were rifampicin-resistant (RR) or MDR TB [6]. Mathematical modelling has predicted that MDR-TB, as a proportion of incident TB cases will increase in high MDR-TB burden countries, for example to 32.5 % (95 % prediction interval 27.0–35.8) in Russia, and 12.4 % in India (9.4–16.2), and that XDR-TB will rise as a percentage of incident MDR-TB cases in the countries analysed [7].

The above findings indicate that a growing proportion of TB cases in high MDR-TB burden countries will no longer be susceptible to TB drugs

* Corresponding author.
*E-mail address:* ronan.otuathail@gmail.com (R.F. O'Toole).

https://doi.org/10.1016/j.ygeno.2025.111132
Received 17 May 2025; Received in revised form 24 September 2025; Accepted 4 October 2025
Available online 8 October 2025

[8]. This may have implications for low TB burden countries such as Ireland. Over the past 25 years in Ireland, there has been a steady increase in the proportion of TB cases that occur in overseas-born persons from 8.3 % of TB cases in Ireland in 1998 [9] to 72 % of cases in 2024 [10]. Although, the incidence rate of TB in Irish-born persons remains low (1.7/100,000), it is markedly higher in persons born outside Ireland (20.3/100,000) [10]. We previously determined that migration from high burden countries (HBC) for TB can exert a measurable effect on the foreign-born TB notification rate, particularly for countries with a high proportion of overseas-born TB cases [11]. A Europe-wide study reported that the leading countries of origin for overseas-born TB cases in Ireland in 2020 were India, the Philippines, and Pakistan which are all classified as HBCs for TB and MDR-TB [12,13]. The incidence rates of MDR/RR-TB per 100,000 are significantly greater in the Philippines (25 (8.4–42)), India (7.4 (5.7–9.1)), and Pakistan (6.1 (4–8.3)) compared to Ireland (0.11 (0.02–0.2)) [6]. Should multi-drug resistance continue to increase in HBCs for MDR-TB, as predicted by mathematical modelling, Ireland and other low TB burden countries may be at risk of seeing a greater proportion of their TB cases present as MDR.

*M. tuberculosis* consists of a number of genetic lineages which differ in their geographical distribution and burden [14]. For example, Euro-American Lineage 4 is dominant in Western Europe, the Americas and also much of Africa whereas East Asian Lineage 2 is a major lineage in Eastern Asia as well as Eastern Europe [15]. Conversely, Lineages 7 to 10 are relatively rare and are therefore, considered to be of limited public health relevance [15]. Lineage 2 in particular is believed to have an association with MDR and XDR in TB [16–19]. To the best of our knowledge, only one prior study has been published on the lineage distribution of *M. tuberculosis* in Ireland. This analysis was conducted using MIRU-VNTR (mycobacterial-interspersed-repetitive-units variable-number tandem-repeat) typing rather than genomic analyses. It reported dominance by Lineage 4 among total TB isolates in Ireland [20].

To date, few studies have assessed international transmission links to drug-resistant TB isolates in Ireland using genomics. In this study, we collated a large set of genome sequences from RR/MDR-TB and XDR-TB isolates collected around the world and analysed their phylogenetic relatedness and geographical origins. These analyses provided insights into the possible international movement of different strains of drug-resistant *M. tuberculosis* from Lineages 2 and 4 that have presented in Ireland. Furthermore, marked differences were observed between lineages and sub-lineages of *M. tuberculosis* in terms of their geographical relationship with strains isolated in Ireland. The implications of our findings are discussed.

## 2. Materials and methods

### 2.1. Genome sequences

This study compiled a collection of raw sequence reads that were obtained from publicly available projects listed in Supplementary Table S1. Sequence read quality was assessed using Fastp v0.23.4 and reports were compiled using MultiQC v1.26 [21,22]. Sequences with less than 10-fold read depth after filtering were excluded from further analysis. The collection year data for the Ireland isolates were obtained from the National Center for Biotechnology Information (NCBI) and the European Nucleotide Archive (ENA) (Table S1). Hierarchical clustering information was obtained from EnteroBase (https://enterobase.warwick.ac.uk/) [23–25].

### 2.2. Lineage and resistance determination

The lineage and resistance profile of the isolates was determined using the TB-profiler database v6.2.1 (https://tbdr.lshtm.ac.uk/) on raw reads that passed quality control. The WHO-endorsed definitions of MDR-TB, pre-XDR-TB, and XDR-TB were used in this study [4,5]. Eight isolates with resistance calls that were inconsistent with evolutionary patterns were reviewed. Upon inspection, these isolates all possessed a TB-profiler soft-fail quality metric in the *rpoB* gene (Ser450Leu) resulting in one 'other' resistance and seven isoniazid monoresistance calls. As these isolates were all phylogenetically clustered within clades containing only MDR- and Pre-XDR-TB isolates, their respective *rpoB* genes were manually checked for known rifampicin-resistance mutations and their resistance profile adjusted where appropriate.

### 2.3. Variant calling and core genome alignment

Snippy v4.6.0 (https://github.com/tseemann/snippy) was used to identify SNPs and indels in each of the isolate genomes. The .fastq sequence reads were aligned to the reference genome of *M. tuberculosis* strain H37Rv (NC_000962.3) for Lineage 4 genomes and to the reference genome of *M. tuberculosis* strain CCDC5180 (NC_017522.1) for Lineage 2 genomes to achieve lineage-specific alignments. Regions of difference, including deletions up to 28kbp in size, have been reported between individual lineages of *M. tuberculosis* [26]. This reduces the propensity of a reference genome from one lineage to display the diversity present in another lineage. CCDC5180 is a fully-sequenced isolate of *M. tuberculosis* Lineage 2 [27] and has been used previously as a reference genome in other work on the phylogeny of Lineage 2 isolates [28]. After variant extraction, the Snippy-core function was applied to merge the .vcf outputs for each of the isolate genomes into a core alignment .aln file. A cut-off of 85 % coverage with respect to the reference genome was used resulting in a mean coverage of 97.51 % for Lineage 2 and 96.71 % for Lineage 4 genomes. The pseudoalignment for each isolate was processed using Gubbins (https://github.com/nickjcroucher/gubbins) to detect and exclude sequence variations located inside regions of recombination [29]. Core-SNP-filter v0.2.0 was used to filter SNPs that were present in 95 % of the isolates. A threshold of $\leq$5 single nucleotide polymorphisms (SNPs) between *M. tuberculosis* isolates has previously been proposed as an indicator of recent TB transmission involving patients belonging to a genomic cluster, while >12 SNP differences between isolates has been considered as evidence against recent transmission [30–32]. Therefore, an upper threshold of 12 SNP differences was used to identify genomes that are potentially closely related.

### 2.4. Phylogenetic analysis

RAxML Next Generation (RAxML-NG) v1.2.2-master (https://github.com/amkozlov/raxml-ng) [33] was utilized to construct the maximum likelihood phylogenetic trees used in the analysis. The general time-reversible (GTR) model with discrete gamma model of rate heterogeneity (GTR + Gamma) was applied with 1000 bootstrap replicates. Phylogenetic trees generated by RAxML-NG were manually rooted in RStudio with a reference genome of *Mycobacterium canettii* (SRR29477258), a closely related species to *M. tuberculosis* [34] which has been used previously as an outgroup in phylogenetic studies of the *M. tuberculosis* complex [35]. FigTree was used to visualise phylogenetic trees (http://tree.bio.ed.ac.uk/software/figtree/).

### 2.5. Hierarchical clustering of cgMLST data

Core genome multi-locus sequence typing (cgMLST) data from EnteroBase (https://enterobase.warwick.ac.uk/) was used to perform hierarchical clustering (hierCC) of genomes within 12 alleles of one another (HC12) [23]. Country and year of collection data where available were extracted from EnteroBase for isolates belonging to specific HC12 clusters. HierCC enabled identification of genome-sequenced international isolates that were phylogenetically related to the *M. tuberculosis* complex isolates from Ireland.

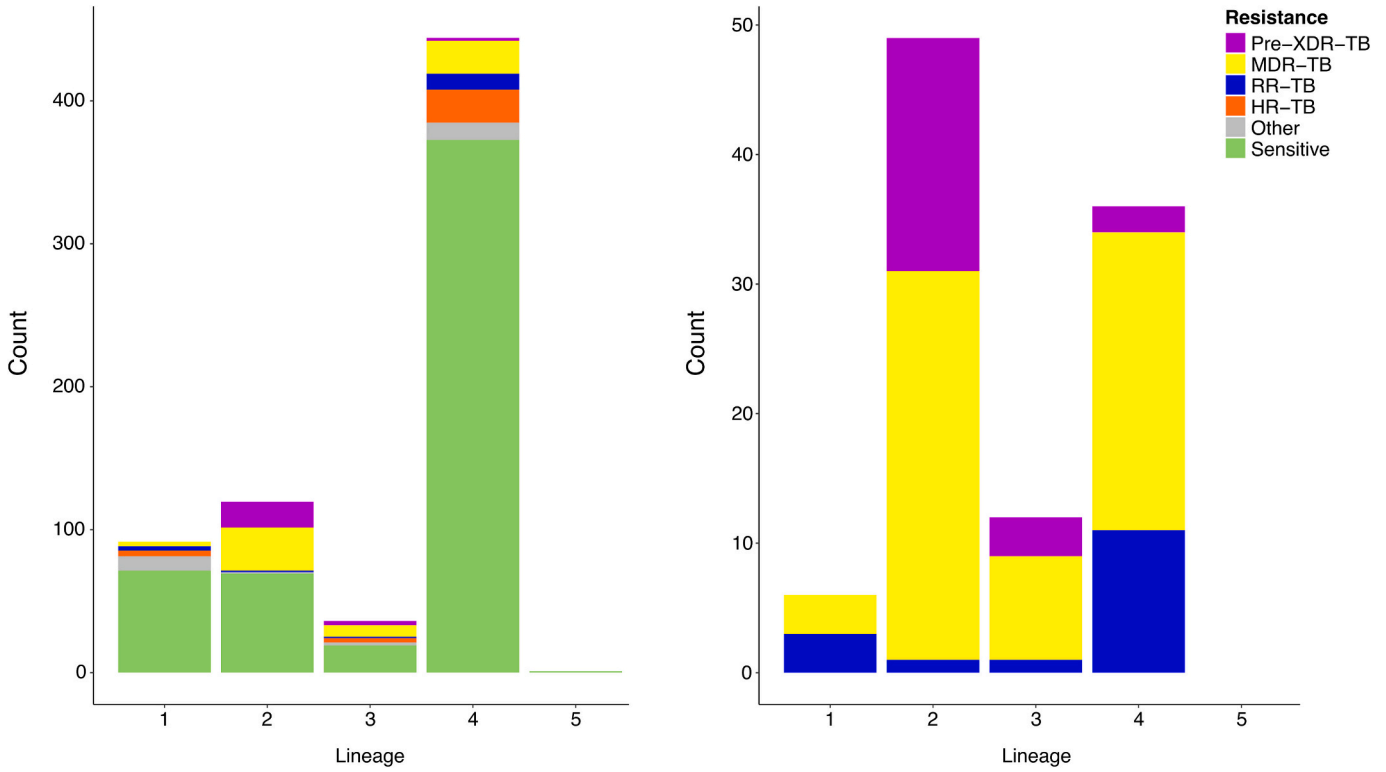


**Fig. 1.** Distribution of genome-sequenced *Mycobacterium tuberculosis* complex (MTBC) isolates from Ireland with respect to genetic lineage. A, lineage distribution for all MTBC genomes. B, lineage distribution for drug-resistant MTBC genomes. Gagneux lineage numbers are indicated. HR-TB, isoniazid-resistant tuberculosis. RR-TB, rifampicin-resistant tuberculosis. MDR-TB, multi-drug resistant tuberculosis. Pre-XDR-TB, pre-extensively drug-resistant tuberculosis.

**Table 1**
Distribution of genetic lineages and drug resistance types among genome sequenced isolates of *Mycobacterium tuberculosis* complex collected between 1994 and 2020 in Ireland.

| | DS-TB | HR-TB | RR-TB | MDR-TB | Pre-XDR-TB | Resistance other | Total (genetic lineage) |
|---|---|---|---|---|---|---|---|
| Lineage 1 | 71 (**13.37 %** \| *78.02 %*) | 4 (**13.33 %** \| *4.40 %*) | 3 (**18.75 %** \| *3.30 %*) | 3 (**4.69 %** \| *3.30 %*) | 0 (**0.00 %** \| *0.00 %*) | 10 (**40.00 %** \| *10.99 %*) | *91 (13.21 %)* |
| Lineage 2 | 69 (**12.99 %** \| *57.98 %*) | 0 (**0.00 %** \| *0.00 %*) | 1 (**6.25 %** \| *0.84 %*) | 30 (**46.88 %** \| *25.21 %*) | 18 (**78.26 %** \| *15.13 %*) | 1 (**4.00 %** \| *0.84 %*) | *119 (17.27 %)* |
| Lineage 3 | 19 (**3.58 %** \| *52.78 %*) | 3 (**10.00 %** \| *8.33 %*) | 1 (**6.25 %** \| *2.78 %*) | 8 (**12.50 %** \| *22.22 %*) | 3 (**13.04 %** \| *8.33 %*) | 2 (**8.00 %** \| *5.56 %*) | *36 (5.22 %)* |
| Lineage 4 | 371 (**69.87 %** \| *83.94 %*) | 23 (**76.67 %** \| *5.20 %*) | 11 (**68.75 %** \| *2.49 %*) | 23 (**35.94 %** \| *5.20 %*) | 2 (**8.70 %** \| *0.45 %*) | 12 (**48.00 %** \| *2.71 %*) | *442 (64.15 %)* |
| Lineage 5 | 1 (**0.19 %** \| *100 %*) | 0 (**0.00 %** \| *0.00 %*) | 0 (**0.00 %** \| *0.00 %*) | 0 (**0.00 %** \| *0.00 %*) | 0 (**0.00 %** \| *0.00 %*) | 0 (**0.00 %** \| *0.00 %*) | *1 (0.15 %)* |
| Total (drug resistance type) | **531** | **30** | **16** | **64** | **23** | **25** | 689 |

The table reports counts of isolates (together with % drug resistance type by genetic lineage in bold | and % genetic lineage by drug resistance type in italices) *e.g.* **13.37 %** of drug susceptible isolates are from Lineage 1 while *78.02 %* of Lineage 1 isolates are drug sensitive, $n = 71$. DS-TB, drug-susceptible tuberculosis. HR-TB, isoniazid-resistant tuberculosis. RR-TB, rifampicin-resistant tuberculosis. MDR-TB, multi-drug resistant tuberculosis. Pre-XDR-TB, pre-extensively drug-resistant tuberculosis. Resistance that does not fall under the above resistance designations is listed under 'Resistance other'.

### *2.6. Data visualisation and statistical analysis*

To combine the RAxML-NG generated phylogenetic trees with heatmaps, the libraries ggplot2, ggtree, and pheatmap were used with the R coding suite. The Inkscape 1.2.2 graphics tool (https://inkscape.org/release/inkscape-1.2.2/) was used for the formatting of heatmap outputs from RStudio. Statistical analysis was conducted in RStudio.

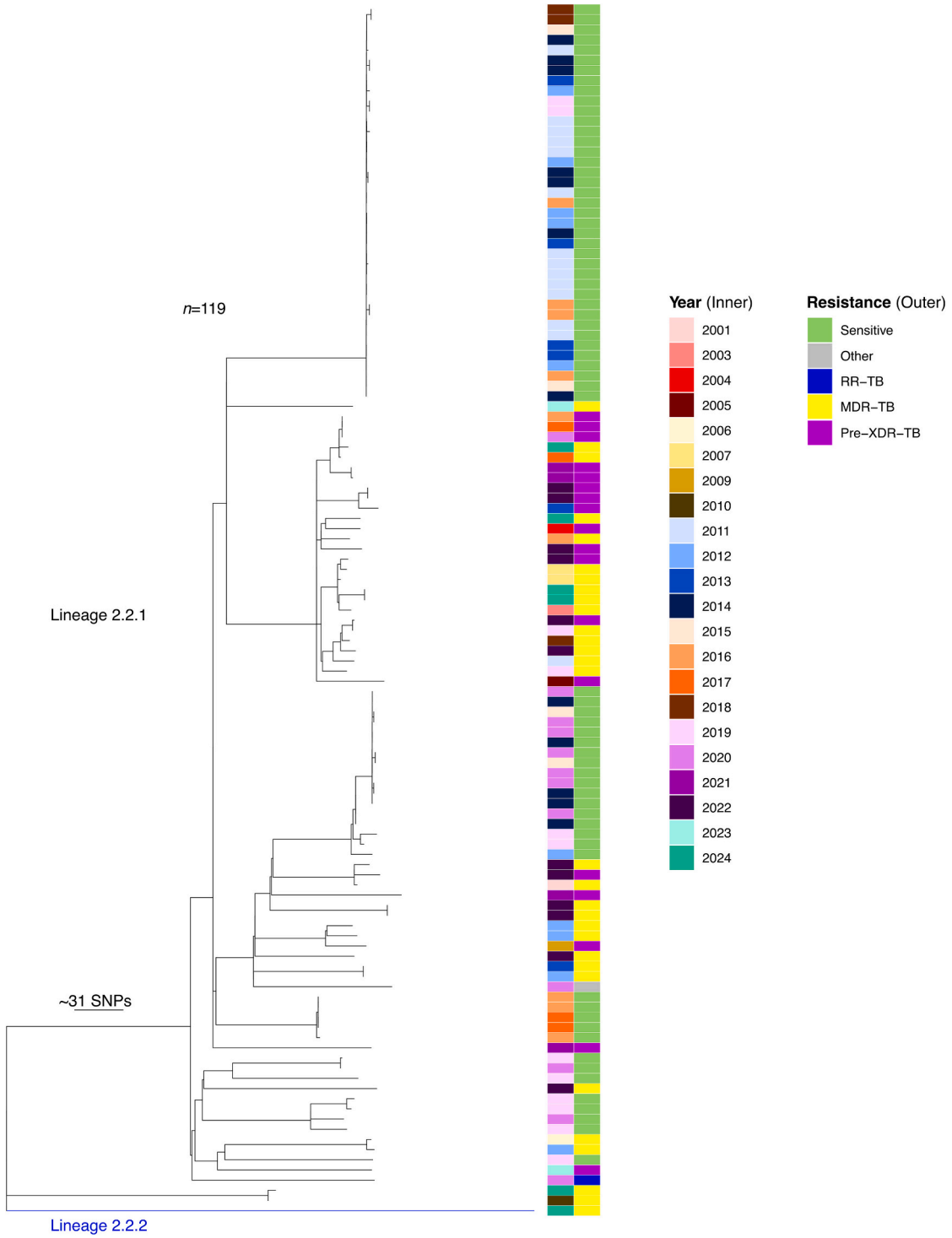


**Fig. 2.** Maximum-likelihood phylogenetic tree based on alignment of whole genome sequences of Lineage 2 *Mycobacterium tuberculosis* complex isolates collected in Ireland. Year of collection is indicated by the inner column. Drug resistance status is depicted by the outer column. RR-TB, rifampicin-resistant tuberculosis. MDR-TB, multi-drug resistant tuberculosis. Pre-XDR-TB, pre-extensively drug-resistant tuberculosis.

## 3. Results

### *3.1. Lineage distribution of sequenced M. tuberculosis genomes from Ireland*

This study examined 689 sequenced *M. tuberculosis* isolates collected in Ireland from the years 1998 to 2024 (Table S1). In terms of phylogenetic lineages, the majority of the sequenced *M. tuberculosis* isolates, 64.15 % ($n = 442$) belonged to Lineage 4 with 17.27 % ($n = 119$), 13.21 % ($n = 91$), 5.22 % ($n = 36$) and 0.15 % ($n = 1$) from Lineages 2, 1, 3, and 5 respectively (Fig. 1, Table 1). Regarding isolates that were at least resistant to rifampicin *i.e.* RR-TB, MDR-TB, pre-XDR-TB and XDR-TB, 47.57 % ($n = 49$) were from Lineage 2, with 34.95 % ($n = 36$), 11.65 % ($n = 12$) and 5.83 % ($n = 6$) from Lineages 4, 3 and 1, respectively (Fig. 1, Table 1).

Statistical tests were applied to assess for an association between

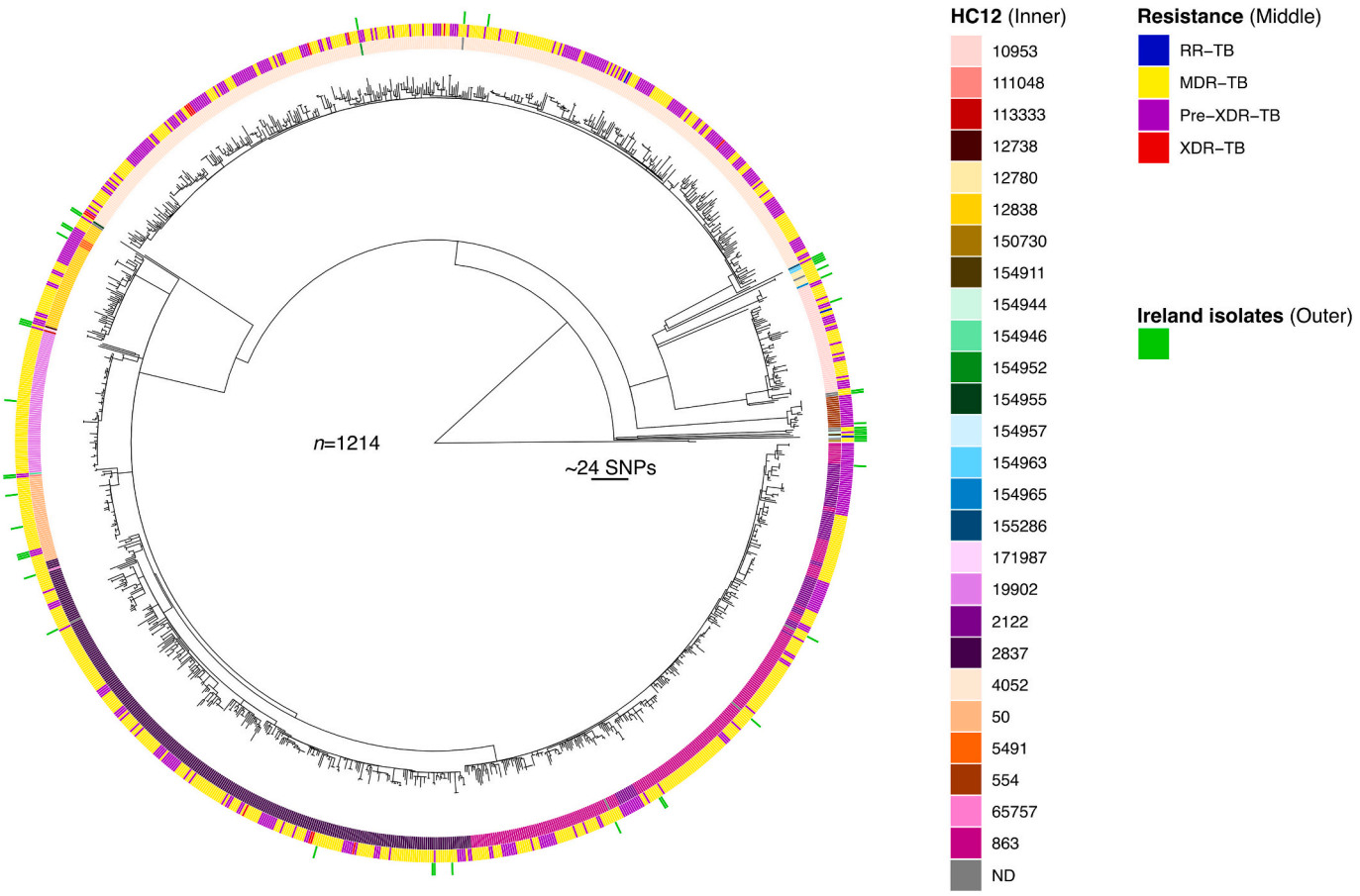


**Fig. 3.** Maximum-likelihood phylogenetic tree of Lineage 2 *Mycobacterium tuberculosis* complex isolates from Ireland combined with drug-resistant international genomes belonging to common hierarchical clusters of genomes within 12 allelic differences (HC12). All genomes within common HC12 groups belonged to sub-lineage 2.2.1 of Lineage 2 and are represented by the inner layer. The middle layer represents the drug resistance status. A total of $n = 1214$ Lineage 2.2.1 genomes were analysed including 48 isolates from Ireland, shown in the outermost layer, that were resistant to at least rifampicin. HR-TB, isoniazid-resistant tuberculosis. RR-TB, rifampicin-resistant tuberculosis. MDR-TB, multi-drug resistant tuberculosis. Pre-XDR-TB, pre-extensively drug-resistant tuberculosis. XDR-TB, extensively-drug resistant tuberculosis.

*M. tuberculosis* lineage and resistance status. The chi-squared and *p*-value test statistics were 153.93 and $2.2 \times 10^{-16}$ respectively ($n = 688$, df = 15), indicating that a significant association existed between lineage and resistance status. Chi-squared standardised residuals ($\chi^2$-SR) were determined for each genetic lineage with respect to drug resistance type. Lineage 4 had a $\chi^2$-SR of 5.77 for DS-TB, and of −4.96 and −5.65 for MDR-TB and pre-XDR-TB respectively (Table S2). This indicates a significant over-representation among drug-susceptible isolates and an under-representation among MDR-TB and pre-XDR-TB isolates for Lineage 4 in this study. A converse trend was seen for Lineage 2 which was under-represented among DS-TB isolates ($\chi^2$-SR of −5.43) but significantly over-represented in MDR-TB (6.57) and pre-XDR-TB (7.86) cases (Table S2).

### 3.2. Phylogenetic analysis of lineage 2 isolates

Lineage 2 was focussed on initially as it was the most dominant lineage among drug-resistant TB cases in Ireland. Lineage 2 isolates belonged to sub-lineage 2.2.1 ($n = 48$) except for one isolate which belonged to sub-lineage 2.2.2 ($n = 1$) (Fig. 2). The analysis did not provide evidence of a switch from a susceptible to a drug-resistant genotype within the same phylogenetic clade or HC12 group. To determine whether the resistant isolates were part of larger transmission networks circulating internationally, the approach was extended to encompass isolates within identical HC12 clusters. The 48 Lineage 2.2.1 *M. tuberculosis* isolates that were collected in Ireland and were resistant to at least rifampicin were analysed using hierarchical clustering. Of these, 12 isolates were not on EnteroBase and therefore possessed no HC12 data. Of the remaining 36 isolates, 20 isolates were in HC12 clusters that contained resistant international isolates, 14 were single isolates in unique HC12 groups not closely related to international or other isolates in Ireland, and 2 isolates were within a HC12 cluster found exclusively in Ireland. From this analysis, most of the Lineage 2.2.1 isolates from Ireland were found to be part of larger international HC12 clusters of genomes (Fig. 3). For example, HC12 number 4052 consisted of 404 genomes with just 1 from Ireland.

### 3.3. Geographical analysis of lineage 2 isolates

Country of collection data were then applied to members of each of the HC12 clusters and the number of SNP differences across the whole genome in relation to isolates from Ireland was calculated. Closely-related Lineage 2 isolates were dominated by isolates from Estonia ($n = 128$), Georgia ($n = 108$), Ukraine ($n = 108$), Moldova ($n = 82$), Germany ($n = 54$), and Lithuania ($n = 28$). Country of collection data were plotted *versus* SNP distance to the closest Irish isolate (Fig. 4). Isolates from Estonia belonged to two HC12 clusters, 863 ($n = 120$) and 2122 ($n = 8$) and ranged from 2 or more SNP differences away from isolates collected in Ireland. Some Lineage 2 isolates from Angola ($n = 1$), Azerbaijan ($n = 1$), Germany ($n = 2$), Latvia ($n = 1$), Lithuania ($n = 2$), Russia ($n = 2$) and the United Kingdom ($n = 3$) exhibited 0 SNP differences to isolates from Ireland (Fig. 4).

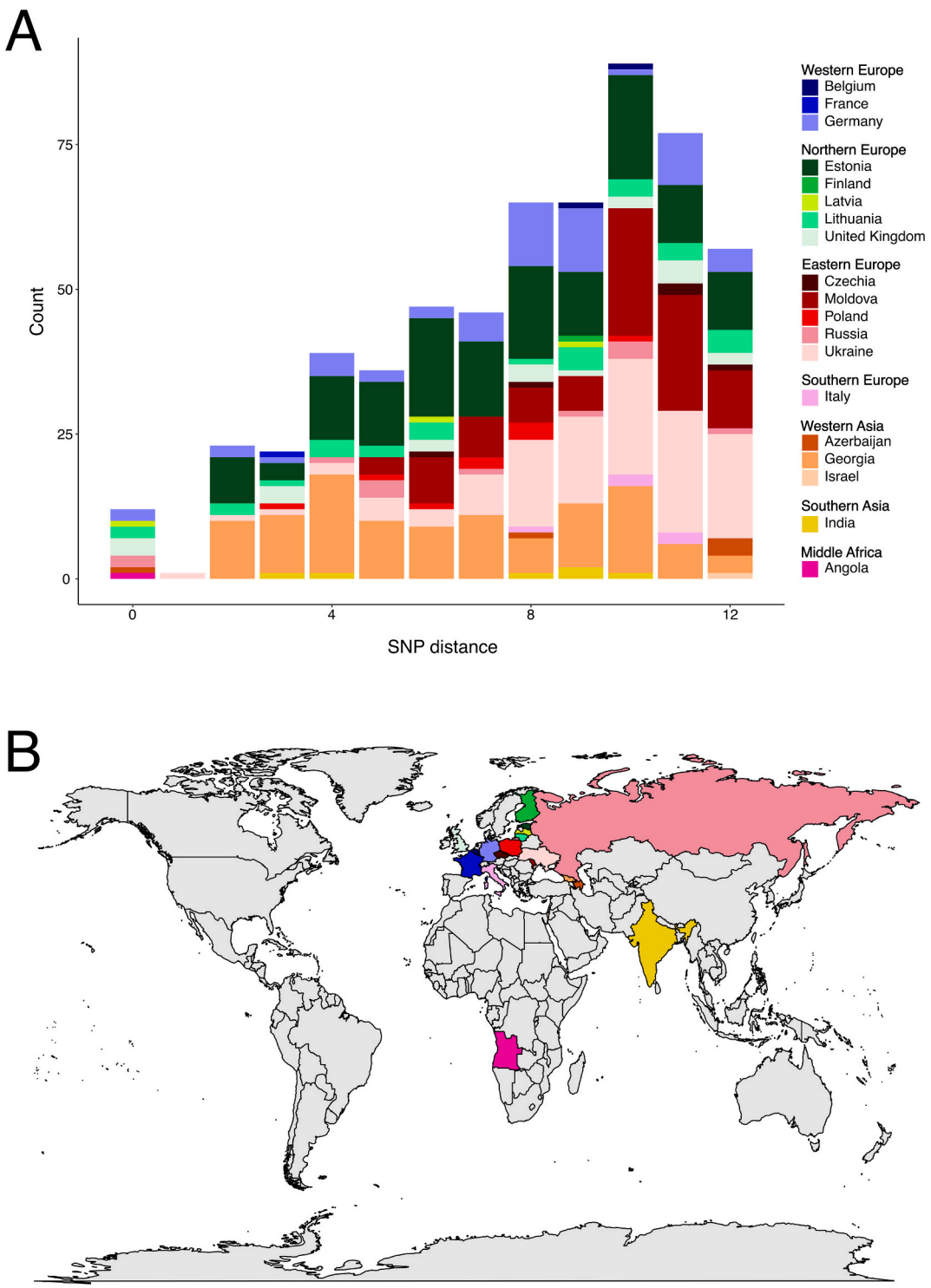


**Fig. 4.** Geographical distribution of international *Mycobacterium tuberculosis* complex isolates belonging to Lineage 2 that are within 12 SNP differences to isolates collected in Ireland. A, range of SNP differences from 0 to 12 by region and country of isolation. B, global map illustrating distribution of countries with related Lineage 2 isolates within 12 SNP differences to Ireland isolates.

### *3.4. Characterisation of lineage 4 isolates*

As Lineage 4 was the dominant lineage among all sequenced *M. tuberculosis* complex genomes from Ireland, we extended the analysis in order to compare Lineage 4 to findings with Lineage 2. There were 36 Lineage 4 *M. tuberculosis* isolates collected in Ireland that were resistant to at least rifampicin. Of these isolates, 26 were within international HC12 clusters, 9 were in unique HC12 groups not closely related to international or other isolates in Ireland, and a single isolate was not on EnteroBase and did not possess HC12 information. There were also 23 isoniazid-resistant tuberculosis (HR-TB) isolates from Ireland belonging to Lineage 4 of which 4 isolates were not represented on Enterobase. Overall, Lineage 4 exhibited greater diversity among TB cases in Ireland with a larger number of sub-lineages represented (Fig. 5) than was seen for Lineage 2 (Fig. 2). The major sub-lineages were 4.1 and 4.3 with sub-lineages 4.2, 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9 also represented. Many of the

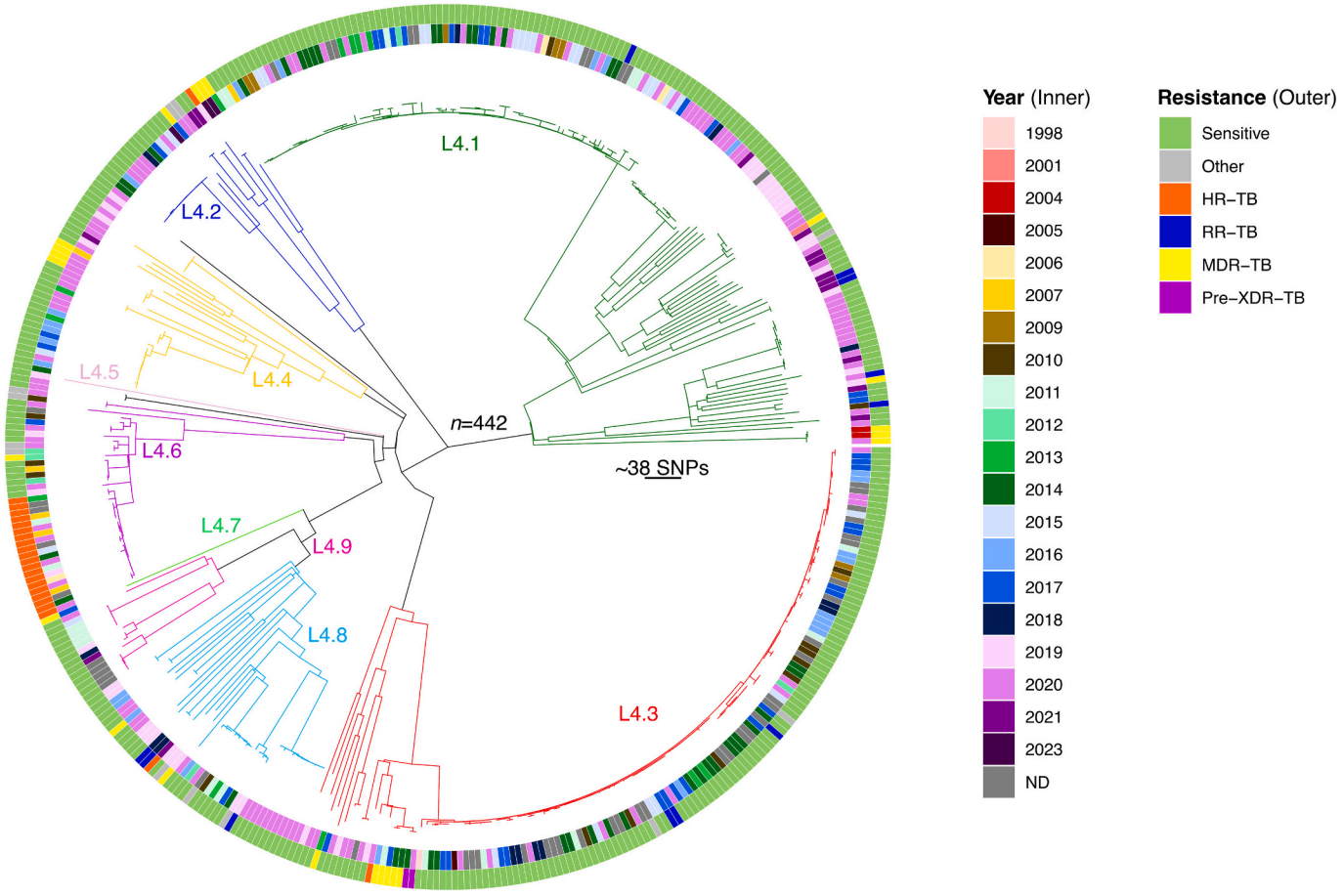


**Fig. 5.** Maximum-likelihood phylogenetic tree based on alignment of whole genome sequences of Lineage 4 *Mycobacterium tuberculosis* complex isolates collected in Ireland. Year of collection is indicated in the inner layer. The drug resistance status for each isolate is shown in the outer layer. HR-TB, isoniazid-resistant tuberculosis. RR-TB, rifampicin-resistant tuberculosis. MDR-TB, multi-drug resistant tuberculosis. Pre-XDR-TB, pre-extensively drug-resistant tuberculosis. Each of the sub-lineages is represented by a different branch colour.

Lineage 4 isolates from Ireland were part of larger international HC12 clusters of genomes (Fig. 6). For example, HC12 group 3759 consisted of 127 genomes with 4 from Ireland. When country of collection data were applied to members of each of the HC12 clusters, closely-related Lineage 4 isolates were dominated by isolates from Eswatini ($n = 53$), Germany ($n = 27$ including 2 HR-TB isolates), United Kingdom ($n = 17$ including 15 HR-TB isolates), Mozambique ($n = 13$), Sweden ($n = 12$), and Latvia ($n = 10$) (Fig. 7). Isolates from Eswatini ranged from 1 or more SNP differences away from isolates collected in Ireland (Fig. 7) and belonged to one HC12 cluster, 3759 (Fig. 8). Some Lineage 4 isolates from Germany ($n = 1$), Ivory Coast ($n = 1$), Latvia ($n = 3$), Moldova ($n = 1$), Nigeria ($n = 1$), Somalia ($n = 1$), and the United Kingdom ($n = 1$) exhibited 0 SNP differences to isolates from Ireland (Fig. 7). Mapping the countries which contributed *M. tuberculosis* complex isolates to HC12 groups shared with Ireland revealed a different pattern when comparing Lineage 2 (Fig. 4) and Lineage 4 (Fig. 7). Country of origin metadata were not available for 16.90 % ($n = 197$) of Lineage 2 and 42.19 % ($n = 305$) of Lineage 4 international genomes from HC12 clusters shared with Ireland and it was therefore, necessary to exclude these genomes from the geographical analysis.

## 4. Discussion

In this study, we report that close to 50 % of genome sequenced isolates of rifampicin- or higher-resistant *M. tuberculosis*, collected between 1998 and 2024 in Ireland, belonged to Lineage 2 (Fig. 1, Table 1). Lineage 2 was significantly over-represented among cases of MDR-TB (46.88 %, $\chi^2$-SR = 6.57) and pre-XDR-TB (78.26 %, $\chi^2$-SR = 7.867). In contrast, Lineage 4 was over-represented among DS-TB cases (69.87 %, $\chi^2$-SR = 5.7783). Lineages 1 and 3, which were minor lineages among DS-TB, were also minor lineages among RR-/MDR-TB (Fig. 1, Table 1).

To investigate potential geographical sources or linkages with cases of MDR-TB in Ireland, we commenced our analysis with Lineage 2. All except one of the Lineage 2 *M. tuberculosis* isolates from Ireland belonged to sub-lineage 2.2.1. There was no apparent relationship between individual clades of sub-lineage 2.2.1 and any one calendar year (Fig. 2). Some clades appeared to be dominated by DS-TB isolates while others contained a range of drug-resistance phenotypes. There was little evidence of widespread national transmission of RR-, MDR- or pre-XDR-TB isolates within Ireland as evidenced by deep rooting of the branches containing DR-TB isolates (Fig. 2). This is in agreement with an earlier study which did not find “conclusive evidence of MDR/XDR-TB transmission within Ireland” [36].

To refine our analysis further, we grouped the *M. tuberculosis* Lineage 2 genomes into hierarchical clusters (HC) containing isolates with up to 12 allelic differences to one another. International isolates of Lineage 2 that belonged to the same HC12 groups were incorporated into the analysis and a total of 26 HC12 groups were detected that contained Irish Lineage 2 isolates and closely-related isolates from other countries (Fig. 3). The common HC12 groups consisted of resistant isolates ranging from isoniazid-resistant tuberculosis (HR-TB) through to XDR-TB but lacked any DS-TB isolates. Pairwise SNP analyses were performed and genomes of international isolates were grouped according to SNP distance from an Irish Lineage 2 isolate up to a maximum of 12

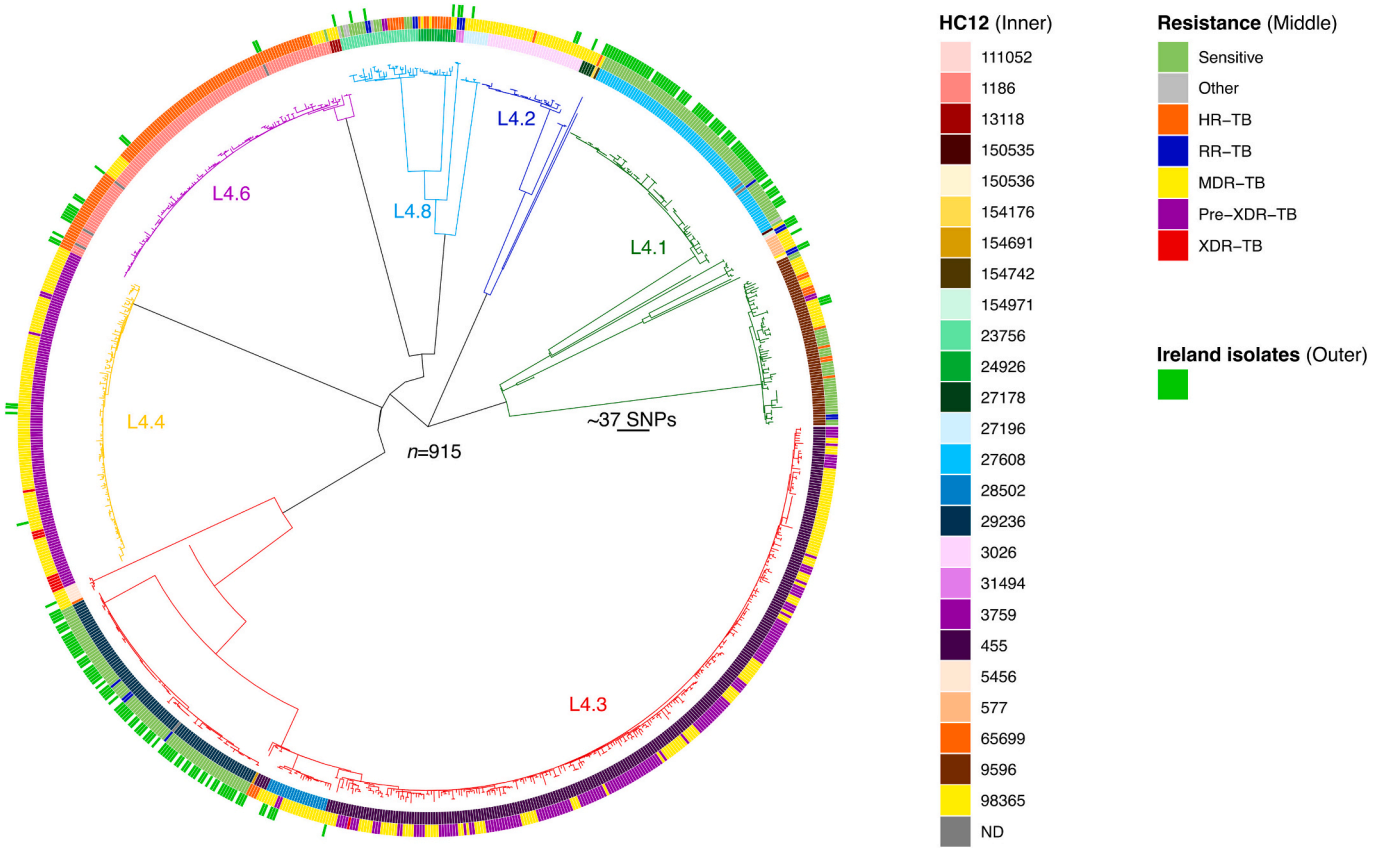


**Fig. 6.** Maximum-likelihood phylogenetic tree of Lineage 4 *Mycobacterium tuberculosis* complex isolates from Ireland combined with drug-resistant international genomes belonging to common hierarchical clusters of genomes within 12 allelic differences (HC12). The HC12 cluster information for each isolate is shown in the inner layer. Sub-lineages of Lineage 4 are indicated by branch colour. Drug resistance status for each isolate is shown in the middle layer. A total of $n = 915$ Lineage 4 genomes were analysed including 36 isolates from Ireland that were at least resistant to rifampicin. Sensitive and resistant isolates collected in Ireland are depicted in the outermost layer. HR-TB, isoniazid-resistant tuberculosis. RR-TB, rifampicin-resistant tuberculosis. MDR-TB, multi-drug resistant tuberculosis. Pre-XDR-TB, pre-extensively drug-resistant tuberculosis. XDR-TB, extensively-drug resistant tuberculosis.

SNPs. From this analysis, it was apparent that isolates within 12 SNPs of Irish isolates had been detected in other European countries, in Western and Southern Asia, and in Middle Africa (Fig. 3, Fig. 4).

Many of the Lineage 2 isolates from Ireland appeared to be part of larger international HC12 clusters of genomes (Fig. 3). For example, HC12 number 4052 consisted of 404 genomes with 160 from Ukraine but just 1 from Ireland. Lineage 2 isolates were detected from Estonia ($n = 128$) that ranged from 2 or more SNP differences away from Irish Lineage 2 isolates (Fig. 4). The Estonia isolates belonged to two HC12 groups, 863 ($n = 120$) and 2122 ($n = 8$) (Fig. 8), which were also found in Ireland but at lower numbers *i.e.* 863 ($n = 2$) and 2122 ($n = 1$). In addition, some Lineage 2 isolates from Angola, Azerbaijan, Latvia (all $n = 1$), Germany, Lithuania, Russia (all $n = 2$), and the United Kingdom ($n = 3$) exhibited 0 SNP differences to isolates from Ireland (Fig. 4), which could indicate membership of common transmission networks based on established SNP thresholds for *M. tuberculosis* genomic relatedness [30–32]. The above data are indicative of the translocation of DR-TB isolates of *M. tuberculosis* from large international clusters to Ireland.

When comparing the geographical distribution of isolates that were within 12 SNPs of Irish isolates, a very different pattern emerged for Lineage 4 with respect to Lineage 2. In the latter case, closely-related Lineage 4 isolates were detected in Western, Eastern and Southern Africa as well as in South America (Fig. 7). Highest numbers of related Lineage 4 isolates were from Eswatini ($n = 53$), Germany ($n = 27$), United Kingdom ($n = 17$), and Mozambique ($n = 13$). Some isolates from Germany, Ivory Coast, Moldova, Nigeria, Somalia, United Kingdom (all $n = 1$), and Latvia ($n = 3$), exhibited 0 SNP differences to isolates from Ireland (Fig. 7). HC12 cluster, 3759, which was detected in four isolates in Ireland, was found in larger numbers of drug-resistant isolates from Eswatini ($n = 53$), Mozambique ($n = 13$) and South Africa ($n = 8$) (Fig. 8). Based on known SNP threshold for genomic relatedness [29–31], these data suggest that many of the DR-TB isolates of Lineage 4 collected in Ireland originated from overseas but from different countries with respect to Lineage 2.

There are a number of implications from this study. Firstly, Lineage 2 of *M. tuberculosis* is strongly represented among RR-/MDR-TB/pre-XDR-TB cases in Ireland. Nearly half of TB cases in Ireland that are caused by Lineage 2 strains are at least resistant to rifampicin. This means that any increase in Lineage 2 as a proportion of TB cases would be expected to be accompanied by a corresponding increase in the proportion of cases that are RR-/MDR-TB/pre-XDR-TB in Ireland. Secondly, an examination of phylogenetically-related isolates from other jurisdictions revealed that many of the Lineage 2 RR-/MDR-TB/pre-XDR-TB isolates belong to larger international clusters that appear to be concentrated in other countries such as Estonia, Georgia, Ukraine, and Moldova. This shows that while Ireland may not be the original source of several of the Lineage 2 strains, RR-/MDR-TB/pre-XDR-TB cases associated with this lineage still present in Ireland. Establishing how these strains reach Ireland is outside the scope of this study, but previously-established factors in the international translocation of *M. tuberculosis* in other countries include travel and population movement such as migration, particularly from HBCs for TB [37–39].

TB cases that present in individuals from countries with large clusters of RR-/MDR-TB/pre-XDR-TB strains may need close scrutiny and contact tracing for early detection of drug-resistant isolates and prevention of further spread. Comparison with Lineage 4 showed that although

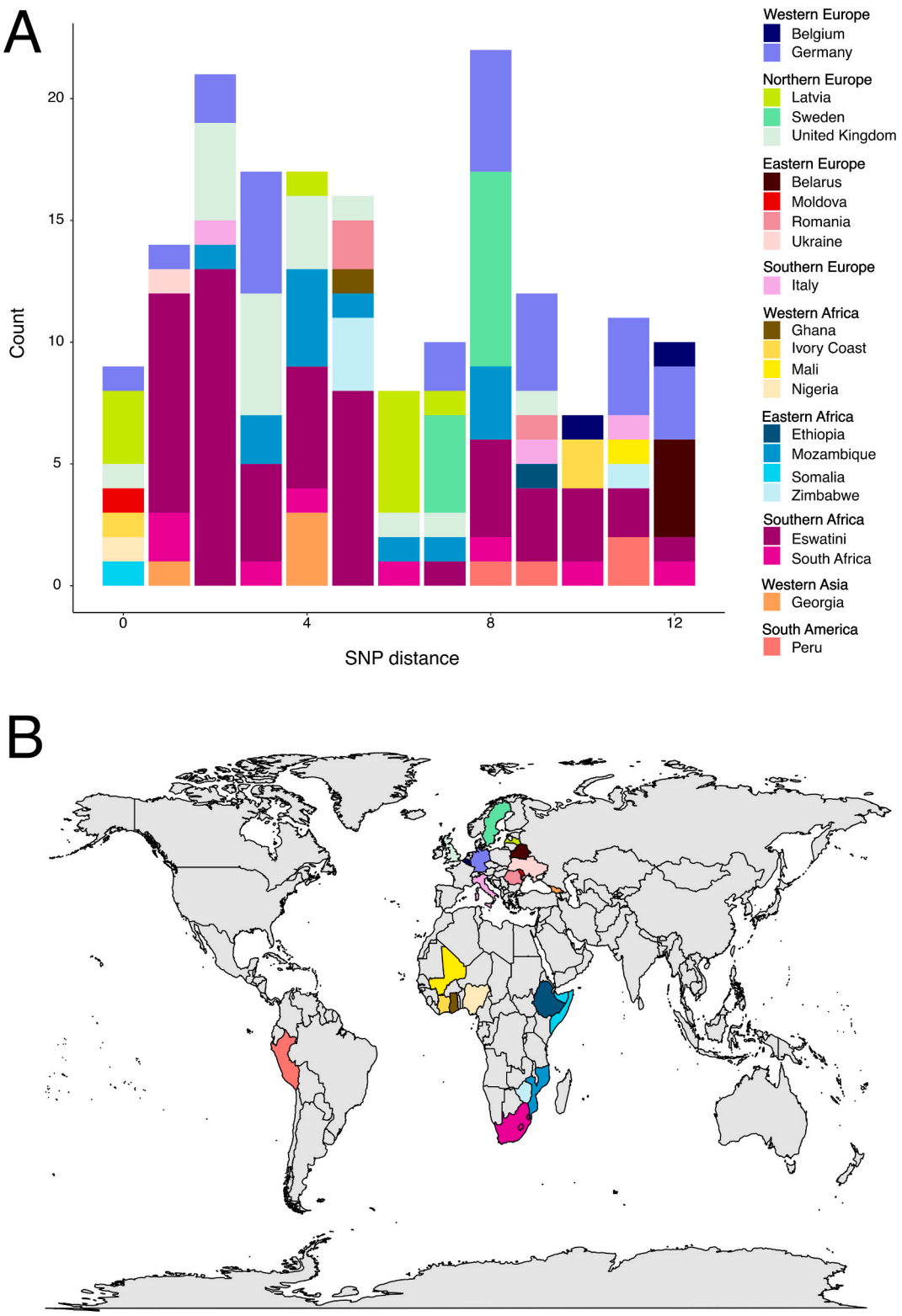


**Fig. 7.** Geographical distribution of international *Mycobacterium tuberculosis* complex isolates belonging to Lineage 4 that are within 12 SNP differences to isolates collected in Ireland. A, range of SNP differences from 0 to 12 by region and country of isolation. B, global map illustrating distribution of countries with related Lineage 4 isolates within 12 SNP differences to Ireland isolates.

fewer of the Lineage 4 isolates were drug resistant, its dominance among total TB cases means that Lineage 4 is also a major contributor to RR-/MDR-TB/pre-XDR-TB in Ireland. As with Lineage 2, many of the drug-resistant isolates from Lineage 4 were part of large international clusters. However, the potential source countries were different with Lineage 4 RR-/MDR-TB/pre-XDR-TB cases in Ireland dominated by isolates belonging to clusters centred in countries that include Eswatini, Germany, the United Kingdom, and Mozambique. Again, vigilance is needed when TB cases are identified in individuals from countries known to contain large clusters of RR-/MDR-TB/pre-XDR-TB or XDR-

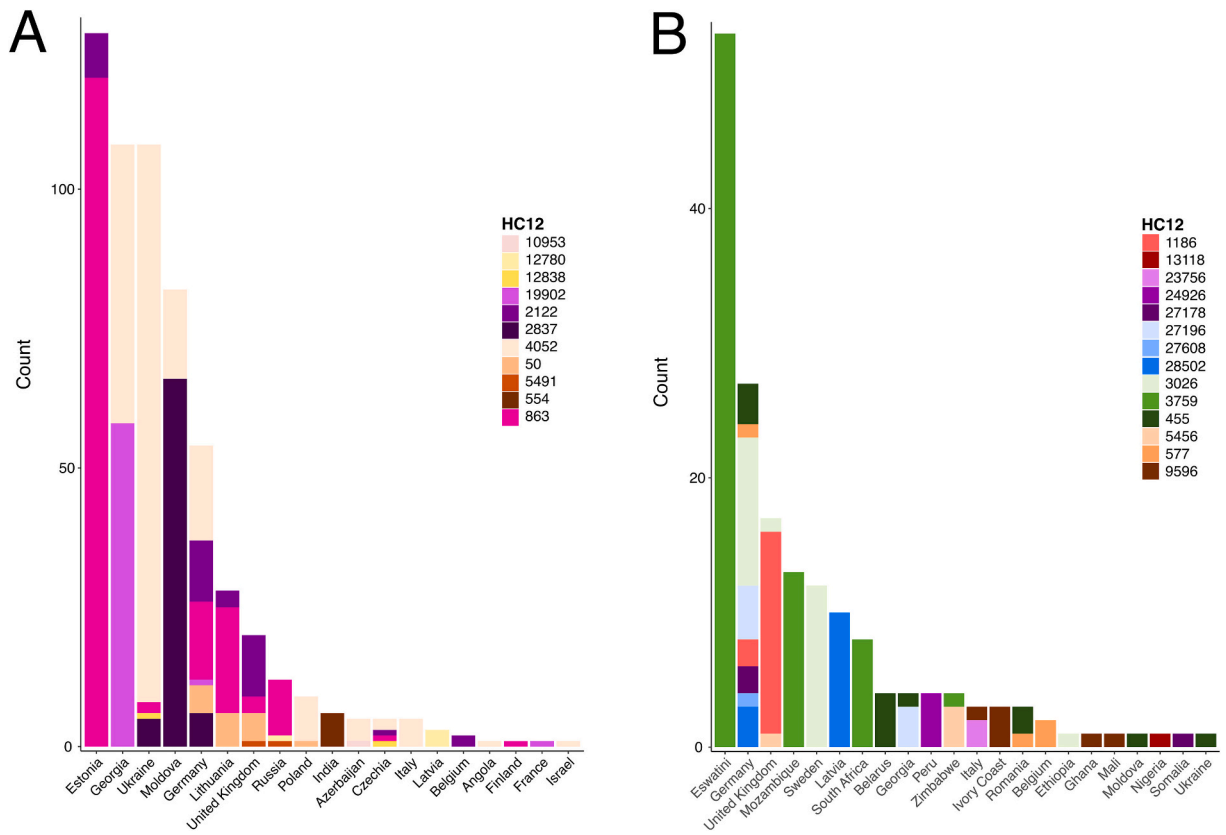


**Fig. 8.** Abundance of *Mycobacterium tuberculosis* HC12 groups by country. Common hierarchical clusters of genomes within 12 allelic differences (HC12) that were shared isolates from Ireland were analysed with regard to abundance per country and a comparison was performed with respect to Lineage 2 and Lineage 4. A, Lineage 2 HC12 group composition for each country. B, Lineage 4 HC12 group composition for each country.

TB. This is in line with the consensus statement from the TBnet consortium that "Dedicated care for TB prevention and treatment in migrant populations within the EU/EEA &UK is essential." [40].

There are a number of limitations to this study that should be acknowledged. Routine genome sequencing of *M. tuberculosis* isolates in Ireland and other countries is a more recent practice and therefore, genome sequences were not available for all years examined in this study. Nevertheless, the distribution of individual Lineages among the genome-sequenced Irish isolates is comparable to what has previously been reported in Ireland whereby Lineage 4 was dominant at 63 % of total *M. tuberculosis* isolates [20] compared to 64.37 % of genomes in this study, and Lineages 1, 2 and 3 were each present at levels below 20 % of total isolates.

In addition, classification of isolates into RR-/MDR−/pre-XDR-/XDR-TB drug-resistance types is based on genetically-encoded resistance rather than phenotypic resistance. However, the resistance mutations used to classify isolates in this study were derived from the World Health Organization's catalogue of confidence-graded *M. tuberculosis* complex genetic markers of phenotypic resistance [41].

Country of isolation data were not available for all of the *M. tuberculosis* genomes analysed from other countries. For Lineage 2 and 4 genomes, data completeness for country of isolation was 83.10 % and 57.81 %, respectively. The geographical analysis of international isolates that were closely related to isolates from Ireland had to be limited to data for which country of isolation was known.

Future work that follows from this study includes further sampling and genome sequencing of *M. tuberculosis* isolates from countries with clusters that are phylogenetically linked to isolates in Ireland. This would help identify where progression from drug susceptible to the different drug-resistant phenotypes among isolates is taking place and would further refine the translocation pathway of resistant strains to Ireland. An examination of clinical and/or surveillance data would assist in demonstrating the route specific strains of *M. tuberculosis* have followed. Other potential work includes modelling the impact of new control measures on the future incidence of DR-TB in Ireland.

## 5. Conclusions

In summary, Lineages 2 and 4 were the dominant lineages among DR-TB isolates of *M. tuberculosis* detected in Ireland. Many of the RR-/MDR-TB/pre-XDR-TB isolates in Ireland belonged to large clusters that were concentrated overseas. The main geographical origins of related Lineage 2 DR-TB isolates in Ireland were former Soviet Union countries including Estonia, Georgia, Ukraine, Moldova, and Lithuania. Lineage 4 was genetically more diverse than Lineage 2 with DR-TB isolates in Ireland being part of clusters centred in Southern Africa and Western Europe including in Eswatini, Mozambique, Germany, Sweden and the UK.

### Recommendations

- Demographic information should be collected and maintained for all isolates of *M. tuberculosis* including country and year of collection data
- DS and DR isolates of *M. tuberculosis* should be routinely genome sequenced with prompt accessibility of sequences for scientists and clinicians across jurisdictions (similar to COVID-19 and influenza viruses)
- In addition to TB in individuals from WHO-designated high MDR-TB burden countries, TB cases in persons from countries with large phylogenetic clusters of DR-TB also require vigilance

- More broadly, it is essential that TB control is optimised in HBCs to prevent acquisition or transmission of DR-TB given that MDR- and XDR-TB strains can translocate readily to low incidence countries with travel and population movement

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2025.111132.

## CRediT authorship contribution statement

**Cian Ennis:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Gaetan Thilliez:** Writing – review & editing, Supervision, Resources, Methodology. **Ronan F. O'Toole:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Ethics approval

Ethics approval was obtained from the DCU Research Ethics Committee (DCUREC/2024/117).

## Funding

CE was supported by a PhD Scholarship from the Life Sciences Institute, Dublin City University.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This research was supported by use of high-performance computing resources provided through the Irish Centre for High-End Computing (ICHEC) and LuxProvide in Luxembourg.

## Data availabilitySequence read files for the *Mycobacterium tuberculosis* isolates analysed in this work are available through the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) as listed in Supplementary Table S1.

Sequence read files for the *Mycobacterium tuberculosis* isolates analysed in this work are available through the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) as listed in Supplementary Table S1.

## References

[1] C.J.L. Murray, K.S. Ikuta, F. Sharara, L. Swetschinski, G. Robles Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool, S.C. Johnson, A.J. Browne, M.G. Chipeta, F. Fell, S. Hackett, G. Haines-Woodhouse, B.H. Kashef Hamadani, E.A.P. Kumaran, B. McManigal, S. Achalapong, R. Agarwal, S. Akech, S. Albertson, J. Amuasi, J. Andrews, A. Aravkin, E. Ashley, F.-X. Babin, F. Bailey, S. Baker, B. Basnyat, A. Bekker, R. Bender, J.A. Berkley, A. Bethou, J. Bielicki, S. Boonkasidecha, J. Bukosia, C. Carvalheiro, C. Castañeda-Orjuela, V. Chansamouth, S. Chaurasia, S. Chiurchiù, F. Chowdhury, R. Clotaire Donatien, A.J. Cook, B. Cooper, T. R. Cressey, E. Criollo-Mora, M. Cunningham, S. Darboe, N.P.J. Day, M. De Luca, K. Dokova, A. Dramowski, S.J. Dunachie, T. Duong Bich, T. Eckmanns, D. Eibach, A. Emami, N. Feasey, N. Fisher-Pearson, K. Forrest, C. Garcia, D. Garrett, P. Gastmeier, A.Z. Giref, R.C. Greer, V. Gupta, S. Haller, A. Haselbeck, S.I. Hay, M. Holm, S. Hopkins, Y. Hsia, K.C. Iregbu, J. Jacobs, D. Jarovsky, F. Javanmardi, A. W.J. Jenney, M. Khorana, S. Khusuwan, N. Kissoon, E. Kobeissi, T. Kostyanev, F. Krapp, R. Krumkamp, A. Kumar, H.H. Kyu, C. Lim, K. Lim, D. Limmathurotsakul, M.J. Loftus, M. Lunn, J. Ma, A. Manoharan, F. Marks, J. May, M. Mayxay, N. Mturi, T. Munera-Huertas, P. Musicha, L.A. Musila, M.M. Mussi-Pinhata, R.N. Naidu, T. Nakamura, R. Nanavati, S. Nangia, P. Newton, C. Ngoun, A. Novotney, D. Nwakanma, C.W. Obiero, T.J. Ochoa, A. Olivas-Martinez, P. Olliaro, E. Ooko, E. Ortiz-Brizuela, P. Ounchanum, G.D. Pak, J.L. Paredes, A.Y. Peleg, C. Perrone, T. Phe, K. Phommasone, N. Plakkal, A. Ponce-de-Leon, M. Raad, T. Ramdin, S. Rattanavong, A. Riddell, T. Roberts, J.V. Robotham, A. Roca, V.D. Rosenthal, K. E. Rudd, N. Russell, H.S. Sader, W. Saengchan, J. Schnall, J.A.G. Scott, S. Seekaew, M. Sharland, M. Shivamallappa, J. Sifuentes-Osornio, A.J. Simpson, N. Steenkeste, A.J. Stewardson, T. Stoeva, N. Tasak, A. Thaiprakong, G. Thwaites, C. Tigoi, C. Turner, P. Turner, H.R. Van Doorn, S. Velaphi, A. Vongpradith, M. Vongsouvath, H. Vu, T. Walsh, J.L. Walson, S. Waner, T. Wangrangsimakul, P. Wannapinij, T. Wozniak, T.E.M.W. Young Sharma, K.C. Yu, P. Zheng, B. Sartorius, A.D. Lopez, A. Stergachis, C. Moore, C. Dolecek, M. Naghavi, Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, Lancet 399 (2022) 629–655, https://doi.org/10.1016/S0140-6736(21)02724-0.

[2] World Health Organization, Global Tuberculosis Report. https://www.who.int/publications-detail-redirect/9789240037021, 2021 (accessed April 9, 2024).

[3] J.P. Cegielski, E. Kurbatova, M. Van Der Walt, J. Brand, J. Ershova, T. Tupasi, J. C. Caoili, T. Dalton, C. Contreras, M. Yagui, J. Bayona, C. Kvasnovsky, V. Leimane, L. Kuksa, M.P. Chen, L.E. Via, S.H. Hwang, M. Wolfgang, G.V. Volchenkov, T. Somova, S.E. Smith, S. Akksilp, W. Wattanaamornkiet, H.J. Kim, C. Kim, B. Y. Kazennyy, T. Khorosheva, K. Kliiman, P. Viiklepp, R. Jou, A.S.-E. Huang, I. A. Vasilyeva, O.V. Demikhova, Multidrug-Resistant Tuberculosis Treatment Outcomes in Relation to Treatment and Initial Versus Acquired Second-Line Drug Resistance, Clin. Infect. Dis. (2015), https://doi.org/10.1093/cid/civ910 civ910.

[4] World Health Organization, WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment, 2022 update. https://www.who.int/publications/i/item/9789240063129, 2022 (accessed April 25, 2025).

[5] World Health Organization, Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis. https://www.who.int/publications-detail-redirect/9789240013131, 2020 (accessed April 15, 2024).

[6] World Health Organization, Global TB Rep. 2024 (2024). https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024 (accessed March 17, 2025).

[7] R. Diel, J. Vandeputte, G. De Vries, J. Stillo, M. Wanlin, A. Nienhaus, Costs of tuberculosis disease in the European Union: a systematic analysis and cost calculation, Eur. Respir. J. 43 (2014) 554–565, https://doi.org/10.1183/09031936.00079413.

[8] R.F. O'Toole, Antibiotic resistance acquisition versus primary transmission in the presentation of extensively drug-resistant tuberculosis, Int. J. Mycobacteriol. 11 (2022) 343–348, https://doi.org/10.4103/ijmy.ijmy_187_22.

[9] Health Protection Surveillance Centre, Report on the Epidemiology of Tuberculosis in Ireland 1998. https://www.hpsc.ie/a-z/vaccinepreventable/tuberculosistb/tbdataandreports/annualreports/, 1998 (accessed April 15, 2024).

[10] Health Protection Surveillance Centre, Tuberculosis in Ireland: Trends in Surveillance Data. https://www.hpsc.ie/a-z/vaccinepreventable/tuberculosistb/tbdataandreports/annualreports/, 2024 (accessed April 15, 2024).

[11] A. Hanway, C.M. Comiskey, K. Tobin, R.F. O'Toole, Relating annual migration from high tuberculosis burden country of origin to changes in foreign-born tuberculosis notification rates in low-medium incidence European countries, Tuberculosis (Edinb.) 101 (2016) 67–74, https://doi.org/10.1016/j.tube.2016.07.019.

[12] World Health Organization, WHO global lists of high burden countries for tuberculosis (TB), TB/HIV and multidrug/rifampicin-resistant TB (MDR/RR-TB), 2021–2025. https://www.who.int/news/item/17-06-2021-who-releases-new-global-lists-of-high-burden-countries-for-tb-hiv-associated-tb-and-drug-resistant-tb, 2021 (accessed April 15, 2024).

[13] A. Vasiliu, N. Köhler, E. Altpeter, T.R. Ægisdóttir, M. Amerali, W.A. De Oñate, Á. Bakos, S. D'Amato, D.M. Cirillo, R. Van Crevel, E. Davidaviciene, I. Demuth, J. Domínguez, R. Duarte, G. Günther, J.-P. Guthmann, S. Hatzianastasiou, L. H. Holm, Z. Herrador, U. Hribar, C. Huberty, E. Ibraim, S. Jackson, M. Jensenius, K. S. Josefsdottir, A. Koch, M. Korzeniewska-Kosela, L. Kuksa, H. Kunst, C. Lienhardt, B. Mahler, M.J. Makek, I. Muylle, J. Normark, A. Pace-Asciak, G. Petrović, D. Pieridou, G. Russo, O. Rzhepishevska, H.J.F. Salzer, M.S. Marques, D. Schmid, I. Solovic, M. Sukholytka, P. Svetina, M. Tyufekchieva, T. Vasankari, P. Viiklepp, K. Villand, J. Wallenfels, S. Wesolowski, A.-M. Mandalakas, L. Martinez, D. Zenner, C. Lange, on behalf of the TBnet, tuberculosis incidence in foreign-born people residing in European countries in 2020, Eurosurveillance 28 (2023), https://doi.org/10.2807/1560-7917.ES.2023.28.42.2300051.

[14] P. Palittapongarnpim, P. Tantivitayakul, P. Aiewsakun, S. Mahasirimongkol, B. Jaemsai, Genomic interactions between Mycobacterium tuberculosis and humans, Annu. Rev. Genomics Hum. Genet. 25 (2024) 183–209, https://doi.org/10.1146/annurev-genom-021623-101844.

[15] G.A. Goig, E.M. Windels, C. Loiseau, C. Stritt, L. Biru, S. Borrell, D. Brites, S. Gagneux, Ecology, global diversity and evolutionary mechanisms in the Mycobacterium tuberculosis complex, Nat. Rev. Microbiol. (2025), https://doi.org/10.1038/s41579-025-01159-w.

[16] S. Niemann, R. Diel, G. Khechinashvili, M. Gegia, N. Mdivani, Y.-W. Tang, Mycobacterium tuberculosis Beijing lineage favors the spread of multidrug-resistant tuberculosis in the republic of Georgia, J. Clin. Microbiol. 48 (2010) 3544–3550, https://doi.org/10.1128/JCM.00715-10.

[17] N, National Institute for public health and the environment, Beijing/W genotype Mycobacterium tuberculosis and drug resistance, Emerg. Infect. Dis. 12 (2006) 736–743, https://doi.org/10.3201/eid1205.050400.

[18] S. Yen, J.E. Bower, J.T. Freeman, I. Basu, R.F. O'Toole, Phylogenetic lineages of tuberculosis isolates in New Zealand and their association with patient demographics, Int. J. Tuberc. Lung Dis. 17 (2013) 892–897, https://doi.org/10.5588/ijtld.12.0795.

[19] M. Keikha, M. Majidzadeh, Beijing genotype of Mycobacterium tuberculosis is associated with extensively drug-resistant tuberculosis: a global analysis, New Microbes and New Infections 43 (2021) 100921, https://doi.org/10.1016/j.nmni.2021.100921.
[20] M.M. Fitzgibbon, N. Gibbons, E. Roycroft, S. Jackson, J. O'Donnell, D. O'Flanagan, T.R. Rogers, A snapshot of genetic lineages of Mycobacterium tuberculosis in Ireland over a two-year period, 2010 and 2011, Eurosurveillance 18 (2013), https://doi.org/10.2807/ese.18.03.20367-en.
[21] S. Chen, Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp, iMeta 2 (2023) e107, https://doi.org/10.1002/imt2.107.
[22] P. Ewels, M. Magnusson, S. Lundin, M. Käller, MultiQC: summarize analysis results for multiple tools and samples in a single report, Bioinformatics 32 (2016) 3047–3048, https://doi.org/10.1093/bioinformatics/btw354.
[23] Z. Zhou, J. Charlesworth, M. Achtman, HierCC: a multi-level clustering scheme for population assignments based on core genome MLST, Bioinformatics 37 (2021) 3645–3646, https://doi.org/10.1093/bioinformatics/btab234.
[24] Z. Zhou, N.-F. Alikhan, K. Mohamed, Y. Fan, the A.S. Group, M. Achtman, D. Brown, M. Chattaway, T. Dallman, R. Delahay, C. Kornschober, A. Pietzka, B. Malorny, L. Petrovska, R. Davies, A. Robertson, W. Tyne, F.-X. Weill, M. Accou-Demartin, N. Williams, The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity, Genome Res. 30 (2020) 138–152, https://doi.org/10.1101/gr.251678.119.
[25] N.P. Dyer, B. Päuker, L. Baxter, A. Gupta, B. Bunk, J. Overmann, M. Diricks, V. Dreyer, S. Niemann, K.E. Holt, M. Rahman, P.E. Brown, R. Stark, Z. Zhou, S. Ott, U. Nübel, EnteroBase in 2025: exploring the genomic epidemiology of bacterial pathogens, Nucleic Acids Res. 53 (2025) D757–D762, https://doi.org/10.1093/nar/gkae902.
[26] D. Bespiatykh, J. Bespyatykh, I. Mokrousov, E. Shitikov, A Comprehensive Map of *Mycobacterium tuberculosis* Complex Regions of Difference, mSphere 6 (2021) e0053521, https://doi.org/10.1128/mSphere.00535-21.
[27] Y. Zhang, C. Chen, J. Liu, H. Deng, A. Pan, L. Zhang, X. Zhao, M. Huang, B. Lu, H. Dong, P. Du, W. Chen, K. Wan, Complete genome sequences of Mycobacterium tuberculosis strains CCDC5079 and CCDC5080, which belong to the Beijing family, J. Bacteriol. 193 (2011) 5591–5592, https://doi.org/10.1128/JB.05452-11.
[28] K.W.C. Leong, S.S. Gautam, M. Pradhan, Y.I. Singh, R. Kc, S.K. Rajbhandari, G. R. Ghimire, K. Adhikari, U. Shrestha, R. Chaudhary, G. Ghimire, S. Khadka, R. F. O'Toole, Comparative genomic analyses of multi-drug resistant Mycobacterium tuberculosis from Nepal and other geographical locations, Genomics 114 (2022) 110278, https://doi.org/10.1016/j.ygeno.2022.110278.
[29] N.J. Croucher, A.J. Page, T.R. Connor, A.J. Delaney, J.A. Keane, S.D. Bentley, J. Parkhill, S.R. Harris, Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using gubbins, Nucleic Acids Res. 43 (2015) e15, https://doi.org/10.1093/nar/gku1196.
[30] T.M. Walker, C.L. Ip, R.H. Harrell, J.T. Evans, G. Kapatai, M.J. Dedicoat, D.W. Eyre, D.J. Wilson, P.M. Hawkey, D.W. Crook, J. Parkhill, D. Harris, A.S. Walker, R. Bowden, P. Monk, E.G. Smith, T.E. Peto, Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study, Lancet Infect. Dis. 13 (2013) 137–146, https://doi.org/10.1016/S1473-3099(12)70277-3.
[31] V. Nikolayevskyy, K. Kranzer, S. Niemann, F. Drobniewski, Whole genome sequencing of *Mycobacterium tuberculosis* for detection of recent transmission and tracing outbreaks: a systematic review, Tuberculosis 98 (2016) 77–85, https://doi.org/10.1016/j.tube.2016.02.009.
[32] S.E. Hasnain, R.F. O'Toole, S. Grover, N.Z. Ehtesham, Whole genome sequencing: a new paradigm in the surveillance and control of human tuberculosis, Tuberculosis (Edinb.) 95 (2015) 91–94, https://doi.org/10.1016/j.tube.2014.12.007.
[33] A.M. Kozlov, D. Darriba, T. Flouri, B. Morel, A. Stamatakis, RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference, Bioinformatics 35 (2019) 4453–4455, https://doi.org/10.1093/bioinformatics/btz305.
[34] P. Supply, M. Marceau, S. Mangenot, D. Roche, C. Rouanet, V. Khanna, L. Majlessi, A. Criscuolo, J. Tap, A. Pawlik, L. Fiette, M. Orgeur, M. Fabre, C. Parmentier, W. Frigui, R. Simeone, E.C. Boritsch, A.-S. Debrie, E. Willery, D. Walker, M. A. Quail, L. Ma, C. Bouchier, G. Salvignol, F. Sayes, A. Cascioferro, T. Seemann, V. Barbe, C. Locht, M.-C. Gutierrez, C. Leclerc, S.D. Bentley, T.P. Stinear, S. Brisse, C. Médigue, J. Parkhill, S. Cruveiller, R. Brosch, Genomic analysis of smooth tubercle bacilli provides insights into ancestry and pathoadaptation of Mycobacterium tuberculosis, Nat. Genet. 45 (2013) 172–179, https://doi.org/10.1038/ng.2517.
[35] J.C.S. Ngabonziza, C. Loiseau, M. Marceau, A. Jouet, F. Menardo, O. Tzfadia, R. Antoine, E.B. Niyigena, W. Mulders, K. Fissette, M. Diels, C. Gaudin, S. Duthoy, W. Ssengooba, E. André, M.K. Kaswa, Y.M. Habimana, D. Brites, D. Affolabi, J. B. Mazarati, B.C. De Jong, L. Rigouts, S. Gagneux, C.J. Meehan, P. Supply, A sister lineage of the Mycobacterium tuberculosis complex discovered in the African Great Lakes region, Nat. Commun. 11 (2020) 2917, https://doi.org/10.1038/s41467-020-16626-6.
[36] E. Roycroft, R.F. O'Toole, M.M. Fitzgibbon, L. Montgomery, M. O'Meara, P. Downes, S. Jackson, J. O'Donnell, I.F. Laurenson, A.M. McLaughlin, J. Keane, T. R. Rogers, Molecular epidemiology of multi- and extensively-drug-resistant Mycobacterium tuberculosis in Ireland, 2001-2014, J. Inf. Secur. 76 (2018) 55–67, https://doi.org/10.1016/j.jinf.2017.10.002.
[37] S.S. Gautam, G. Haug, L.A. Cooley, M. Mac Aogáin, R.F. O'Toole, Intercontinental translocation of latent multidrug-resistant tuberculosis to Australia demonstrated by whole genome sequencing, Med. J. Aust. 210 (2019) 236–236.e1, https://doi.org/10.5694/mja2.50044.
[38] A. Schwalb, K. Kayumba, R.M.G.J. Houben, G.H. Bothamley, Recent travel and tuberculosis in migrants: data from a low-incidence country, Clin. Infect. Dis. 78 (2024) 742–745, https://doi.org/10.1093/cid/ciad672.
[39] M. Pareek, C. Greenaway, T. Noori, J. Munoz, D. Zenner, The impact of migration on tuberculosis epidemiology and control in high-income countries: a review, BMC Med. 14 (2016) 48, https://doi.org/10.1186/s12916-016-0595-5.
[40] H. Kunst, B. Lange, O. Hovardovska, A. Bockey, D. Zenner, A.B. Andersen, S. Hargreaves, M. Pareek, J.S. Friedland, C. Wejse, G. Bothamley, L. Guglielmetti, D. Chesov, S. Tiberi, A. Matteelli, A.M. Mandalakas, J. Heyckendorf, J. Eimer, A. Malhotra, J. Zamora, A. Vasiliu, C. Lange, for the TBnet, tuberculosis in adult migrants in Europe: a TBnet consensus statement, Eur. Respir. J. 65 (2025) 2401612, https://doi.org/10.1183/13993003.01612-2024.
[41] Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, 2nd ed, 2025. https://www.who.int/publications/i/item/9789240082410 (accessed May 8, 2025).