Author/s:
Dorji, T;Horan, K;Sherry, NL;Tay, EL;Globan, M;Viberg, L;Bond, K;Denholm, JT;Howden, BP;Andersson, P

Title:
Whole genome sequencing of drug-resistant Mycobacterium tuberculosis isolates in Victoria, Australia

Date:
2024-01-01

Citation:
Dorji, T., Horan, K., Sherry, N. L., Tay, E. L., Globan, M., Viberg, L., Bond, K., Denholm, J. T., Howden, B. P. & Andersson, P. (2024). Whole genome sequencing of drug-resistant Mycobacterium tuberculosis isolates in Victoria, Australia. International Journal of Infectious Diseases, 138, pp.46-53. https://doi.org/10.1016/j.ijid.2023.11.010.

Persistent Link:
https://hdl.handle.net/11343/344371

International Journal of Infectious Diseases 138 (2024) 46–53

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Whole genome sequencing of drug-resistant *Mycobacterium tuberculosis* isolates in Victoria, Australia

Thinley Dorji[1], Kristy Horan[2], Norelle L. Sherry[1,2], Ee Laine Tay[3], Maria Globan[4], Linda Viberg[4], Katherine Bond[4,5], Justin T. Denholm[5,6,7], Benjamin P. Howden[1,2,8,#,*], Patiyan Andersson[1,2,#]

[1] *Department of Microbiology and Immunology at The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia*
[2] *Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology at The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia*
[3] *Communicable Disease Epidemiology and Surveillance, Health Protection Branch, Public Health Division, Department of Health, Melbourne, Australia*
[4] *Mycobacterium Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Royal Melbourne Hospital at The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia*
[5] *Royal Melbourne Hospital, Melbourne, Australia*
[6] *Victorian Tuberculosis Program. Melbourne Health at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia*
[7] *Department of Infectious Diseases at The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia*
[8] *Centre for Pathogen Genomics, University of Melbourne, Australia*

ARTICLE INFO

Article history:
Received 11 August 2023
Revised 31 October 2023
Accepted 6 November 2023

Keywords:
Multi-drug resistant tuberculosis
MDR-TB
Genomic epidemiology
Transmission networks
Australia

ABSTRACT

Objectives: Whole genome sequencing (WGS) can identify clusters, transmission patterns, and drug resistance mutations. This is important in low-burden settings such as Australia, as it can assist in efficient contact tracing and surveillance.

Methods: We conducted a retrospective cohort study using WGS from 155 genomically defined drug-resistant *Mycobacterium tuberculosis* (DR-TB) isolates collected between 2018-2021 in Victoria, Australia. Bioinformatic analysis was performed to identify resistance-conferring mutations, lineages, clusters and understand how local sequences compared with international context.

Results: Of the 155 sequences, 42% were identified as lineage 2 and 35% as lineage 1; 65.8% (102/155) were isoniazid mono-resistant, 8.4% were multi-drug resistant TB and 5.8% were pre-extensively drug-resistant / extensively drug-resistant TB. The most common mutations were observed in *katG* and *fabG1* genes, especially at Ser315Thr and *fabG1* -15 C>T for first-line drugs. Ser450Leu was the most frequent mutation in *rpoB* gene. Phylogenetic analysis confirmed that Victorian DR-TB were associated with importation events. There was little evidence of local transmission with only five isolate pairs.

Conclusion: Isoniazid-resistant TB is the commonest DR-TB in Victoria, and the mutation profile is similar to global circulating DR-TB. Most cases are diagnosed among migrants with limited transmission. This study highlights the value of WGS in identification of clusters and resistance-conferring mutations. This information is crucial in supporting disease mitigation and treatment strategies.

Introduction

Globally, millions of people are infected with *Mycobacterium tuberculosis*, with most cases concentrated in developing countries. The burden of tuberculosis (TB) is low in most industrialised countries, and clusters of cases mostly arise from index cases who acquired the disease overseas. In Australia, TB is mostly diagnosed among migrants from high TB-burden countries, healthcare workers, and among communities of First Nations Peoples [1]. The high frequency among migrant populations is primarily due to reactivation of latent TB. Substantial progress has been made in TB case reduction in Australia, with case notification rates decreasing from 40 per 100,000 in the 1960s, to six cases per 100,000 in 1980s, and plateauing since then [2].

The Australian Government endorsed the World Health Organization (WHO)'s *'TB elimination action framework for low incidence countries'* in 2014. This framework aims to reduce the number of

* Corresponding author.
E-mail address: bhowden@unimelb.edu.au (B.P. Howden).
Joint senior author.

https://doi.org/10.1016/j.ijid.2023.11.010

TB cases to <10 TB cases/million population by 2035 to achieve pre-elimination phase [3]. While this has been achieved among the non-indigenous Australian population, it is yet to be reached among the First Nations People and people born overseas [2], indicating the need for further optimisation of existing surveillance and screening systems to better capture these high-risk groups.

Currently, phenotypic drug susceptibility testing (pDST) is the gold standard test for the diagnosis of drug-resistant TB (DR-TB). However, it is impeded by long duration required for culture and testing [4]. Whole genome sequencing (WGS) can identify resistance-conferring mutations with high concordance with pDST, and is increasingly used in parallel with phenotypic tests for the identification of drug resistance [5]. Additionally, mutation profiles derived *in silico* facilitate inter-laboratory comparison. Genomic analysis can also identify clusters of strains with high genetic similarity [5], which in conjunction with epidemiological data can characterise disease transmission, enhancing contact tracing efforts toward elimination of the disease. This is particularly important in the context of DR-TB, as recent studies demonstrate that the increasing burden of drug resistance is due to the transmission of drug-resistant strains, rather than *de novo* development of resistance [6].

Recently, a study describing the genomic epidemiology of TB in Victoria was published by Dale et al. [7] but did not examine drug resistance of the TB cases in depth. Therefore, to better understand the dynamics of resistance-conferring mutations and phylogenetic relationships among genomic drug-resistant strains, we performed a detailed genomic analysis of DR-TB identified between 2018 and 2021 in the state of Victoria, Australia.

Methodology

Setting, study design, and data sources

In the state of Victoria, Australia (population 6.5 million in 2021) all TB cases are notifiable by legislation. Positive TB cultures are referred to the Victorian Infectious Disease Reference Laboratory, where species identification is confirmed, and routine pDST for first-line antimicrobials is performed (rifampicin, isoniazid, pyrazinamide, and ethambutol). Since mid-2018, all non-duplicate TB isolates have undergone WGS at the Microbiological Diagnostic Unit Public Health Laboratory. The genomic antimicrobial resistance (AMR) data are then reported to referring labs, clinicians, and the state Department of Health/Victorian Tuberculosis Program.

This retrospective cohort study includes all genomically defined DR-TB cases diagnosed between January 01, 2018 and December 31, 2021. The resistance pattern was defined as per the updated WHO classification [8], but *in silico* identification of resistance mutations was used in this study. Rifampicin-resistant TB (RR-TB) was defined as resistance to rifampicin and any other TB agent except for isoniazid. Isoniazid-resistant TB (HR-TB) was defined as resistant to isoniazid and other TB drug except for rifampicin. Multidrug resistant TB (MDR-TB) was defined as resistance to both rifampicin and isoniazid. Pre-extensively drug-resistant (pre-XDR-TB) was defined as MDR-TB also resistant to fluoroquinolones. Extensively drug resistant TB (XDR-TB) was defined as a case of pre-XDR-TB also resistant to one of the Group A drugs (bedaquiline, linezolid, levofloxacin, and moxifloxacin). However, since the genomic AMR has not been validated for all second-line drugs, we have grouped these together as pre-XDR/XDR-TB. “Other resistant TB” in this study was defined as resistance to any TB agents but not fulfilling above definitions. De-identified epidemiological and sample data required for analysis was provided by the Victorian Tuberculosis Program. This is presented in the form of frequency and proportions, analysed using R-studio (version 2023.06.1).

DST and whole genome sequencing

The culture, drug susceptibility testing, and DNA extraction were performed as previously described [7]. Library preparations were performed using NexteraXT DNA preparation kit (Illumina) and sequenced on an Illumina NextSeq platform. WGS was performed as per the manufacturer’s protocol.

Drug resistance mutations and phylogenetic analysis

TB-Profiler was used for lineage prediction and identification of resistance-conferring mutations against TB drugs [9]. Single nucleotide polymorphisms (SNP)-based analysis was performed with the reference strain, *M. tuberculosis* H37Rv (GenBank accession no. NC_000962.3) using default settings in bohra (https://github.com/kristyhoran/bohra). The maximum likelihood phylogenetic tree was built in IQ-tree with GTR-G4 model and bootstrap of 1000 [10]. SNPs in the repetitive region and PE/PPE were excluded from the phylogenetic tree. Single linkage clustering was performed on the SNP-distance matrix using a cut-off of $\leq$ 5 SNPs. Drug-sensitive samples from study period (n = 1197) were included in the assessment of clustering of drug-resistant isolates. Phylogenies were visualised using midpoint rooting in ggtree [11]. All data analyses were performed using R-studio (version 2023.06.1).

International dataset

The Victorian genomic data were compared to publicly available international DR-TB sequences. All DR-TB isolates from the CRyPTIC (Comprehensive Resistance Prediction for Tuberculosis International Consortium) study [12] were curated to compare with our data. In addition, the sequences were identified from publications in National Center for Biotechnology Information (NCBI) using the Medical Subject Headings terms “whole genome sequencing” AND “drug resistant tuberculosis” AND “Not drug sensitive OR drug susceptible”. We also searched for sequences from India and Vietnam as there were no DR-TB sequences from these countries in the CRyPTIC data. We then randomly selected 1800 international sequences. To provide further temporal context, 55 Victorian DR-TB genome sequences from 2010 to 2017 were also included in the phylogenetic analysis.

Results

The study included 155 cases of DR-TB *Mycobacterium tuberculosis sensu stricto* with a temporal distribution of 37 (23.9%) in 2018, 47 (30.3%) in 2019, 46 (29.7%) in 2020 and 25 (16.1%) in 2021. This corresponded to 8.2% (37/449) of the annual proportions of all notified cases in the state of Victoria in 2018, 10.4% (47/454) in 2019, 9.2% (46/501) in 2020 and 5.3%) in 2021 (25/472) [12].

Demographic characteristics of patients

The median age of the patients was 37 years (interquartile range 33, range: 5-90 years) with most (43%) in the age groups of 26-50 years (Table 1). In total, 43 % (n = 61) were diagnosed with DR-TB within five years of arrival in Australia. Of the total patients, 46% (n = 71) were diagnosed with pulmonary TB, while 18% (n = 28) had a mix of both pulmonary and extrapulmonary TB. The extrapulmonary TB accounted for 36% (n = 48) of all cases. Most patients (n = 150, 97%) had not been previously treated for TB. At the time of analysis, 79% (120/155) of the patients had completed treatment, while 12 patients died before completing the treatment.

Table 1
Socio-demographic and clinical characteristics of genomic drug TB sequences.

Characteristic	MDR/Pre-XDR-TB/XDR-TB, N = 22[a]	Non-MDR-TB, N = 133[1]	Overall, N = 155[1]	*P*-value[b]
Age of patients				0.007
≤ 25 years	7 (32%)	29 (22%)	36 (23%)	
26-50 years	13 (59%)	53 (40%)	66 (43%)	
51-75 years	0 (0%)	39 (30%)	39 (25%)	
> 75 years	2 (9.1%)	11 (8.3%)	13 (8.4%)	
Unknown	0	1	1	
Sex				0.7
Female	11 (50%)	61 (46%)	72 (46%)	
Male	11 (50%)	72 (54%)	83 (54%)	
Continent of birth				0.3
Africa	3 (14%)	6 (4.5%)	9 (5.8%)	
Asia	16 (76%)	113 (85%)	129 (84%)	
Australia	1 (4.8%)	9 (6.8%)	10 (6.5%)	
Europe	1 (4.8%)	5 (3.8%)	6 (3.9%)	
Unknown	1	0	1	
Years of arrival in Australia				0.2
< 1 year	1 (4.8%)	10 (8.3%)	11 (7.8%)	
1-5 years	12 (57%)	38 (32%)	50 (35%)	
6-10 years	4 (19%)	21 (18%)	25 (18%)	
10-15 years	1 (4.8%)	14 (12%)	15 (11%)	
> 15 years	3 (14%)	37 (31%)	40 (28%)	
Unknown	1	13	14	
Previously treated for TB				0.021
No	19 (86%)	131 (98%)	150 (97%)	
Yes	2 (9.1%)	2 (1.5%)	4 (2.6%)	
Unknown	1 (4.5%)	0 (0%)	1 (0.6%)	
Year of diagnosis				0.7
2018	6 (27%)	31 (23%)	37 (24%)	
2019	5 (23%)	42 (32%)	47 (30%)	
2020	6 (27%)	40 (30%)	46 (30%)	
2021	5 (23%)	20 (15%)	25 (16%)	
Lineage				0.004
Lineage 1	2 (9.1%)	52 (39%)	54 (35%)	
Lineage 2	16 (73%)	49 (37%)	65 (42%)	
Lineage 3	1 (4.5%)	19 (14%)	20 (12.9%)	
Lineage 4	3 (14%)	13 (9.8%)	16 (10.3%)	
Symptoms present				0.5
No	3 (14%)	10 (7.5%)	13 (8.4%)	
Yes	19 (86%)	122 (92%)	141 (91%)	
Unknown	0 (0%)	1 (0.8%)	1 (0.6%)	
Disease site				>0.9
Extra pulmonary	8 (36%)	48 (36%)	56 (36%)	
Pulmonary	10 (45%)	61 (46%)	71 (46%)	
Pulmonary plus Other sites	4 (18%)	24 (18%)	28 (18%)	
Treatment outcome				>0.9
Completed treatment	15 (79%)	105 (79%)	120 (79%)	
Defaulted	0 (0%)	2 (1.5%)	2 (1.3%)	
Died from another cause	0 (0%)	4 (3.0%)	4 (2.6%)	
Died of TB	1 (5.3%)	7 (5.3%)	8 (5.3%)	
Transferred overseas	1 (5.3%)	8 (6.0%)	9 (5.9%)	
Under treatment	2 (11%)	7 (5.3%)	9 (5.9%)	
Unknown	3	0	3	

[a] n (%)
[b] Fisher's exact test; Pearson's chi-squared test
Footnotes: MDR/pre-XDR-TB/XDR-TB: multi-drug resistant TB/pre-extensively drug resistant TB /extensively drug resistant TB. The pre-XDR-TB and XDR-TB have been combined as pre-XDR/XDR-TB since the genomic detection of bedaquiline/ linezolid resistance has not been validated. Non-MDR-TB refers to all sequences not fulfilling the definition of MDR-TB or pre-XDR/XDR-TB

Phylogenetic analysis of drug-resistant Mycobacterium tuberculosis in Victoria

The phylogenetic analysis showed an early divergence of lineage 1 from the other three lineages, which all shared a common ancestor before subsequently diverging (Figure 1). Lineage 2 (East Asian lineage, 42%) was the predominant lineage in this dataset, followed by lineage 1 (Indo-Oceanic lineage, 35%), lineage 3 (East-African Indian, 12.9%), and lineage 4 (Euro-American, 10.3%).

Genomic analysis detected 102 HR-TB (65.8%), 13 MDR-TB (8.4%), nine pre-XDR/XDR-TB (5.8%), five RR-TB (3.2%), and 26 isolates (16.8%) resistant to other TB drugs. HR-TB (43/102) was commonly seen among lineage 1 compared to other lineages. In contrast, MDR-TB (8/13) and pre-XDR/XDR-TB (8/9) were seen more among lineage 2. The sequences of patients from Asia were uniformly distributed over all lineages while sequences from Africa were commonly seen among lineage 3 and 4. Conversely, sequences from Oceania were more frequently observed among lineage 2.

Clustering

The median pairwise core-SNP distance among the 155 isolates in this dataset was 1169 SNPs (range: 0-1934 SNPs). Cluster analysis performed using ≤5 SNPs distance threshold and single linkage clustering revealed the presence of five genomic clusters, with two isolates each, all observed in Beijing sub-lineage 2.2.1. Cluster 1 included two pre-XDR/XDR-TB cases with SNP distance of

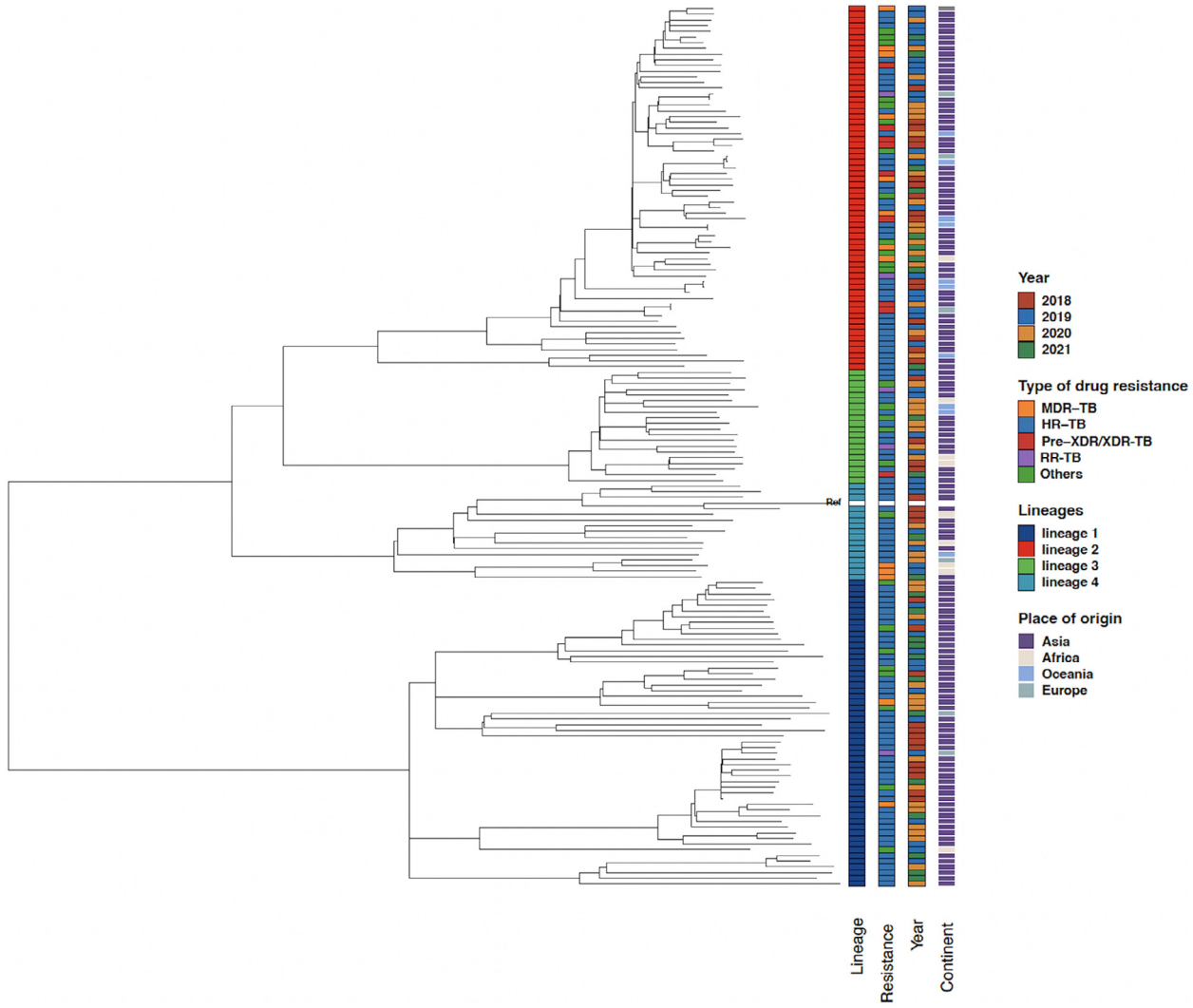

Figure 1. Maximum likelihood phylogeny of circulating 155 DR-TB in Victoria for 2018-2021 with lineage, resistance profile defined as per the World Health Organization, year of diagnosis and continent of birth. MDR-TB (Multi-drug resistant TB), HR-TB (Isoniazid resistant TB), RR-TB (Rifampicin resistance TB), Pre-XDR/XDR-TB (Pre-extensively resistant/Extensively resistant TB). Others include resistance to other TB drugs The pre-XDR-TB and XDR-TB have been combined as pre-XDR/XDR-TB since the genomic detection of bedaquiline/ linezolid resistance has not been validated.

0. Clusters 2 and 3 comprised of HR-TB, with SNP distances of 0 and 3 respectively. Epidemiologically, there was no contact among cases in cluster 1, while latter two clusters were among members from same household. Cluster 4 included two unrelated HR-TB cases (SNP distance 3) who separately had history of multiple trips to Asia, where they likely acquired their infections independently. The fifth cluster encompassed one RR-TB (this sequence was resistant to rifampicin and fluoroquinolone) and one fluoroquinolone-resistant case, with a pairwise SNP distance of 4. There was no known contact between these two cases.

The inclusion of drug-sensitive TB (DS-TB) samples lead to addition of one DS-TB case with one of the above clusters. Moreover, a new cluster was formed consisting of a sensitive and streptomycin-resistant TB cases.

Comparison with international data

We incorporated 1800 randomly selected DR-TB sequences from CRyPTIC and other publicly available DR-TB sequences for the phylogenetic analysis. In addition, 55 Victorian DR-TB genome sequences from 2010 to 2017 were also included in the phylogeny for temporal context. Victorian sequences were interspersed among the global circulating lineages without forming a separate clade. The majority of Victorian sequences notably shared common ancestors with sequences from Asian countries, aligning closely with the sequences from their country of origin and its neighbouring regions. This could be due to the fact that most of our cases were born in Asia, suggesting that the Victorian cases might have acquired the infection in country of birth or during visits. Therefore, it is most likely that drug-resistant Victorian strains resulted from multiple separate incursions from high TB-burden areas (Supplementary Figure 1).

Resistance pattern and lineages based on country of origin

The majority (83.2%, 129/155) of DR-TB in Australia were isolated among people born in Vietnam (n = 31, 20%), India (n = 31, 20%), China (n = 19, 12.3%), and Philippines (n = 14, 9%) (Supplementary Figure 2). HR-TB was predominantly seen among people born in India (20/102, 19.6%) and Vietnam (19/102, 18.6%) (Supplementary Table 1). MDR-TB was detected among people from Vietnam (4/13, 30.8%) and pre-XDR/XDR-TB from India (4/9, 44.4%).

Lineage 1 and lineage 4 were predominantly seen among those born in Vietnam (Supplementary Table 2). Lineage 2 was seen among those born in China, and lineage 3 from India.

Intersection of resistance pattern

The resistance pattern of sequences was further explored using an upset plot (Supplementary Figure 3). Forty-five isolates were resistant to ≥3 TB drugs, commonly seen among sub-lineages 2.2.1 (n = 23, 51%) and sub-lineage 2.2.1.1 (n = 8, 17.8%). Five of these sequences were resistant to seven or more drugs.

Drug resistance mutations against first-line drugs

A total of 27 sequences (17.4%) were resistant to rifampicin (including RR-TB, MDR-TB, and pre-XDR/XDR-TB). Of this, 26 sequences had mutations in the *rpoB,* and one had a deletion in position 1302_1310 (Supplementary Figure 4). Two pre-XDR/XDR-TB sequences had putative compensatory mutations in the *rpoC* (Ile491Thr). With the exception of Ile480Val, all mutations in *rpoB* were in the rifampicin resistance determining region (RRDR, codon 426-452). The most common site of mutation was in codon 450 (14/26), Ser450Leu (n = 13) and Ser450Trp (n = 1), followed by Leu452Pro (3/26). Genotypic rifampicin resistance was supported by phenotypic resistance in 96.3% (26/27) of cases.

Isoniazid resistance mutation was present in 124 isolates (80%). Most commonly observed in *katG* gene (n = 82), with 87.8% (72/82) at Ser315Thr. This mutation was present in 88.9% pre-XDR/XDR-TB (8/9) and 84.6% MDR-TB (11/13) isolates. The second most common loci (n = 39) were the *fabG1* gene with most mutations (n = 37) in the 15C>T promoter region. Among 124 isolates, nine sequences had double mutations in different genes and all of them exhibited phenotypic resistance to isoniazid (Supplementary Table 3). Overall, phenotypic resistance was observed in 84.7% (105/124) of cases displaying isoniazid conferring mutations.

Pyrazinamide resistance was seen among 19 sequences: 16 with mutations in *pncA* gene and one duplication in codons 234_258. Mutations in *pncA* gene were diverse without hotspots. Phenotypic pyrazinamide resistance was seen in 73.7% (14/19) of cases. Ethambutol resistance was detected in 23 sequences, with mutations in *embB* and promotor region of *embA.* Genotypic ethambutol resistance was supported by phenotypic resistance in 69.9% (16/23) of cases.

Drug resistance mutation against second-line drugs

Streptomycin resistance was detected in 81 sequences due to mutations in: *rpsL* (n = 47), *rrs* (n = 17), and *gid* (n = 17)) genes. All mutations in the *rpsL* gene were in the coding region, while it was in the promotor region for *rrs* gene. Mutations in *gid* gene were due to deletions, except for one isolate (Supplementary Table 3). One sequence displayed kanamycin resistance due to a -12C>T promotor mutation in *eis* gene. Thirteen sequences had mutations in the *gyrA* gene associated with fluoroquinolone resistance, all of which occurred in quinolone resistance determining region (QRDR) (74-113). We did not detect any resistance-conferring mutations in *gyrB* gene. Phenotyping fluoroquinolone resistance was detected in 90% (9/10) isolates with genotyping resistance. Ethionamide resistance was present in 48 sequences. Most mutations were found in the promotor region of *fabG1* (n = 39), predominantly at -15C>T (n = 37). Phenotypic concordance for ethionamide was 76.5% (26/34). Furthermore, we identified three sequences with mutations in *thyA* and one sequence with mutations in *folC* gene associated with resistance to para-aminosalicylic acid. There were also two sequences with delamanid resistance-conferring mutation in *ddn* gene. The pDST was not done for this latter second-line drugs.

We also had sequences, where predicted genotypic resistance was not supported by phenotypic data as described in Supplementary Table 4. Whilst most of the observed mutations were classified as associated with resistance as per the WHO catalogue of mutations, there were some that are of uncertain significance [13].

Mutations by lineages

AMR mutational profiles differed by the lineages (Figure 2). Mutations in *rpoB* genes such as Leu452Pro and Leu430Pro, as well as *katG* mutations such as Thr380Ile, Thr394Ala and Asn238Lys were exclusively present in lineage 2. Other mutations such as *rpoB* Ser450Leu, *katG* Ser315Thr, and *rpsL* Lys43Arg were more commonly seen among lineage 2 compared to other lineages. Conversely, mutations such as *fabG1* -15C>T and *gid* deletions were more common among lineage 1. Notably, no fluoroquinolone resistance associated mutations were detected in lineage 1 or lineage 4.

Discussion

WGS is a key surveillance tool that can assist in elimination of the TB disease [14]. We report a comprehensive view of the AMR characteristics of DR-TB in Victoria, Australia, and their genomic context locally and internationally.

In this study, only a small number of clustered pairs were observed, likely a consequence of effective contact tracing and interruption of transmission of disease. Most DR-TB cases in this study had no previous exposure to anti-TB drugs, implying that they acquired their infection directly as primary DR-TB. While there were some DS-TB isolates that joined these clusters in an expanded analysis, this is likely more indicative of common exposure to strain types characteristic of specific geographical locations. Early recognition of these international strains with concerning AMR characteristics is an important surveillance task, and future enhancement of interjurisdictional and international exchange of genomic data would support efforts towards TB elimination [15].

Comparison with an international dataset showed that the Victorian cases were distributed amongst international lineages and subclades indicative of recurrent importation to Australia, and consistent with published data. Bright et al. [2] described that in Australia in 2018, 89% of all TB cases detected among immigrants were from 30 high TB-burden countries. The DR-TB lineages detected in Victoria are consistent with lineages prevalent in the patient's country of origin; lineage 3 was most commonly seen among patients from India [16], lineage 2 from China [17] and lineage 1 from Vietnam [18].

In this dataset, lineage 2 was most common, contrasting with a previous study in Victoria where lineage 1 dominated [7]. This is likely a consequence of the selection of only resistant strains in the current study, as previously noted in other studies [19]. Compared to other lineages, lineage 2 has been associated with higher levels of drug resistance and higher transmissibility [19]. A greater diversity of mutations was observed among lineage 2 strains compared to other lineages in this dataset, but this could in part be due to the proportionally higher representation of the lineage 2.

Studies have shown that WGS has the potential to deliver drug resistance results earlier and cheaper than routine diagnostics in high-income countries [20]. *In silico* determination of resistance has high sensitivity for the detection of resistance to rifampicin (97.5%), isoniazid (97.1%), ethambutol (94.6%) and pyrazinamide (91.3%) [12].

DR-TB cases in Victoria primarily consist of HR-TB, while MDR-TB and pre-XDR/XDR-TB make up small portion of cases in this

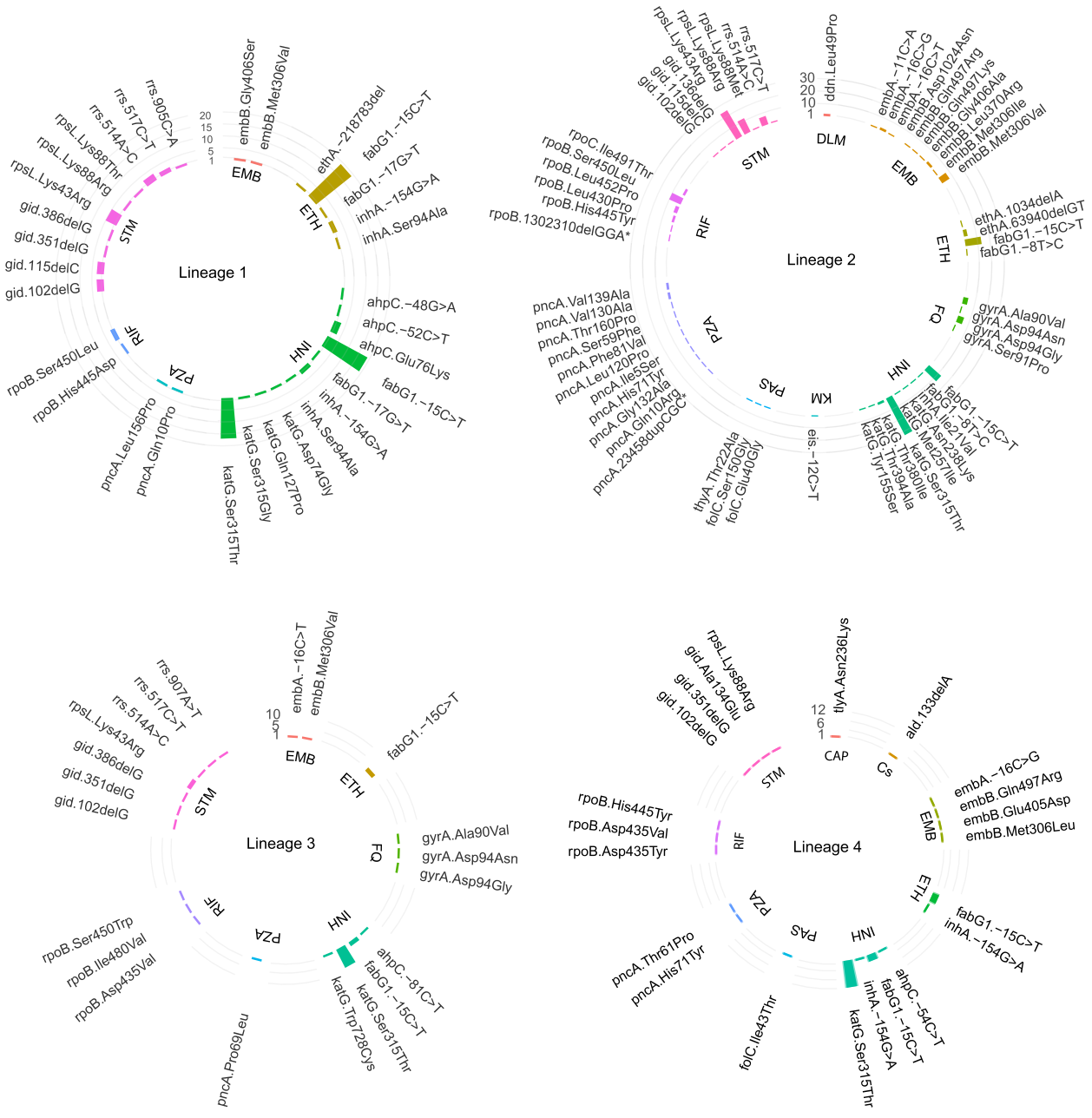

Figure 2. Distribution of mutations for all TB drugs by lineages. The genes associated with resistance is stratified by drug with different colours. CAP, capreomycin; Cs, cycloserine; D, delamanid; EMB, ethambutol; ETH, ethionamide; FQ, fluoroquinolone; INH, isoniazid; PZA, pyrazinamide; KM, kanamycin; PAS, para-amino salicylic acid; RIF, rifampicin. STM, streptomycin.

low-incidence setting. The high proportion of isoniazid mono-resistance observed is consistent with national and global data [21]. High prevalence of isoniazid resistance has been attributed to treatment of TB with non-rifampicin-containing regimens in 1960s [22] and its use as monotherapy in the initial days of treatment. *katG* Ser315Thr, known to confer high-level isoniazid resistance [23], was the most frequent mutation detected in our study, and was commonly seen among lineage 2 in agreement with a previous study from Victoria [24]. The second most common mutation *fabG1,* was observed mostly among lineage 1 and mutations in this gene provides low to moderate level resistance [23]. Mutations associated with isoniazid resistance in this study is similar to studies from other states of Australia [25].

Congruent to other studies, our results show that RR-TB is mainly associated with mutations in the 81 base-pair RRDR of *rpoB,* especially in the codons 450 and 452 [26]. Mutations such as Ser450Leu in *rpoB* confer high-level resistance [23] while imposing a low fitness cost [27], contributing to selection of this mutation. Moreover, this mutation is also associated with smear positivity and active transmission of the disease [19].

Resistance to fluoroquinolones is usually associated with mutations in the QRDR of *gyrA* gene. Comparable to other studies, *gyrA* Asp94Gly, associated with high-level resistance to moxifloxacin [28], was the most common mutation observed in our study. The second common mutation *gyrA* Ala90Val, is associated with low-level moxifloxacin resistance [28].

We observed 45 isolates with resistance to ≥3 TB drugs, and a small number of samples with resistance to at least seven drugs. Unlike pDST, where each drug is tested separately, WGS simultaneously provides information on the resistance profile for most TB

drugs, which can help in individualising patient therapy. This can be particularly relevant to *inhA* mutations which provide low-level isoniazid resistance and can be treated with high dose of isoniazid [29]. This optimisation of treatment reduces the use of second-line drugs, leaving these as alternative options for future use.

WGS can provide information on the genetic provenance of the strain and comprehensive characterisation of the AMR elements with a single test, as well as assist in the elucidation of the disease transmission. However, it is currently challenged by need for high-quality isolates requiring culture before sequencing. Alternatives are being explored, including DNA extraction directly from clinical samples using biotinylated RNA baits, but remains limited by the high cost, especially for resource-limited high TB-burden countries [30].

Conclusion

This work highlights the value of genomics-informed surveillance of DR-TB in providing high-resolution information on the local and international context of strains, and a comprehensive view of AMR determinants. In Victoria, DR-TB is driven by multiple introductions of internationally circulating resistant strains from high burden countries with minimal local transmission.

Declarations of competing interest

The authors have no competing interests to declare.

Funding

This work was supported by the National Health and Medical Research Council, Australia (NHMRC) Practitioner Fellowship to BPH (APP1105905). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Ethical approval

The study was approved by the Human Research Ethics Committee of University of Melbourne, approval reference number 2022-12981-25709-6.

Acknowledgements

We are grateful to Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), Mycobacterium Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory (VIDRL) and Department of Health, Victoria for providing access to the data used in this study, and their continued collaboration and contributions.

Authors contributions

TD, KH, BPH and PA conceived, performed the statistical analyses and wrote first draft of the paper. NLS, KB and JTD contributed to analysis plan and revision of the paper. ET, MG and LV contributed data for the study and revision of paper.

Data availability

The sequences used in this study are deposited in the EMBL-EBL European Nucleotide Archive (ENA) PRJNA857537. The individual Bio Sample number of this project and publicly available data used are in the supplementary table number 5, submitted in a separate sheet.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.11.010.

References

[1] Konstantinos A. Diagnostic tests: testing for tuberculosis. *Aust Prescr* 2010;**33**:12–18. doi:10.18773/austprescr.2010.005.
[2] Bright A, Denholm J, Coulter C, Waring J, Stapledon R. Tuberculosis notifications in Australia, 2015–2018. *Commun Dis Intell (2018)* 2020;**44**:1–39 PubMed PMID:33278873. doi:10.33321/cdi.2020.44.88.
[3] Lönnroth K, Migliori GB, Abubakar I, D'Ambrosio L, de Vries G, Diel R, Douglas P, et al. Towards tuberculosis elimination: an action framework for low-incidence countries. *Eur Respir J* 2015;**45**:928–52. doi:10.1183/09031936.00214014.
[4] van Klingeren B, Dessens-Kroon M, van der Laan T, Kremer K, van Soolingen D. Drug susceptibility testing of Mycobacterium tuberculosis complex by use of a high-throughput, reproducible, absolute concentration method. *J Clin Microbiol* 2007;**45**:2662–8. doi:10.1128/JCM.00244-07.
[5] Dohál M, Dvořáková V, Šperková M, Pinková M, Spitaleri A, Norman A, et al. Whole genome sequencing of multidrug-resistant Mycobacterium tuberculosis isolates collected in the Czech Republic, 2005–2020. *Sci Rep* 2022;**12**:7149. doi:10.1038/s41598-022-11287-5.
[6] Nsofor CA, Jiang Q, Wu J, Gan M, Liu Q, Zuo T, et al. Transmission is a noticeable cause of resistance among treated tuberculosis patients in Shanghai, China. *Sci Rep* 2017;**7**:7691. doi:10.1038/s41598-017-08061-3.
[7] Dale K, Globan M, Horan K, Sherry N, Ballard S, Tay EL, et al. Whole genome sequencing for tuberculosis in Victoria, Australia: a genomic implementation study from 2017 to 2020. *Lancet Reg Health West Pac* 2022;**28**:100556. doi:10.1016/j.lanwpc.2022.100556.
[8] World Health Organization *WHO announces updated definitions of extensively drug resistant tuberculosis*. Geneva: World Health Organization; 2021.
[9] Phelan JE, O'Sullivan DM, Machado D, Ramos J, Oppong YEA, Campino S, et al. Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs. *Genome Med* 2019;**11**:41. doi:10.1186/s13073-019-0650-x.
[10] Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;**32**:268–74. doi:10.1093/molbev/msu300.
[11] Yu G. Using ggtree to Visualize Data on Tree-Like Structures. *Curr Protoc Bioinformatics* 2020;**69**:e96. doi:10.1002/cpbi.96.
[12] CRyPTIC Consortium and the 100,000 Genomes ProjectPrediction of susceptibility to first-line tuberculosis drugs by DNA sequencing. *N Engl J Med* 2018;**379**:1403–15. doi:10.1056/NEJMoa1800474.
[13] World Health Organization *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance*. Geneva: World Health Organization; 2021.
[14] Dunstan SJ, Williamson DA, Denholm JT. Understanding the global tuberculosis epidemic: moving towards routine whole-genome sequencing. *Int J Tuberc Lung Dis* 2019;**23**:1241–2. doi:10.5588/ijtld.19.0604.
[15] Donnan EJ, Marais BJ, Coulter C, Waring J, Bastian I, Williamson DA, et al. The use of whole genome sequencing for tuberculosis public health activities in Australia: a joint statement of the National tuberculosis Advisory Committee and Communicable Diseases Genomics Network. *Commun Dis Intell (2018)* 2023;**47**:1–11. doi:10.33321/cdi.2023.47.8.
[16] Singh AV, Singh S, Yadav A, Kushwah S, Yadav R, Sai DK, et al. Genetic variability in multidrug-resistant Mycobacterium tuberculosis isolates from patients with pulmonary tuberculosis in North India. *BMC Microbiol* 2021;**21**:123. doi:10.1186/s12866-021-02174-6.
[17] Liu Q, Ma A, Wei L, Pang Y, Wu B, Luo T, et al. China's tuberculosis epidemic stems from historical expansion of four strains of Mycobacterium tuberculosis. *Nat Ecol Evol* 2018;**2**:1982–92. doi:10.1038/s41559-018-0680-6.
[18] Le Hang NT, Hijikata M, Maeda S, Miyabayashi A, Wakabayashi K, Seto S, et al. Phenotypic and genotypic features of the Mycobacterium tuberculosis lineage 1 subgroup in central Vietnam. *Sci Rep* 2021;**11**:13609. doi:10.1038/s41598-021-92984-5.
[19] Goig GA, Menardo F, Salaam-Dreyer Z, Dippenaar A, Streicher EM, Daniels J, et al. Effect of compensatory evolution in the emergence and transmission of rifampicin-resistant Mycobacterium tuberculosis in Cape Town, South Africa: a genomic epidemiology study. *Lancet Microbe* 2023;**4**:e506–15. doi:10.1016/S2666-5247(23)00110-6.
[20] Pankhurst LJ, Del Ojo Elias C, Votintseva AA, Walker TM, Cole K, Davies J, et al. Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. *Lancet Respir Med* 2016;**4**:49–58. doi:10.1016/S2213-2600(15)00466-X.
[21] Jenkins HE, Zignol M, Cohen T. Quantifying the burden and trends of isoniazid resistant tuberculosis, 1994–2009. *PLoS One* 2011;**6**:e22927. doi:10.1371/journal.pone.0022927.
[22] Bainomugisa A, Lavu E, Hiashiri S, Majumdar S, Honjepari A, Moke R, et al. Multi-clonal evolution of multi-drug-resistant/extensively drug-resistant Mycobacterium tuberculosis in a high-prevalence setting of Papua New Guinea for over three decades. *Microb Genom* 2018;**4**:e000147. doi:10.1099/mgen.0.000147.

[23] Finci I, Albertini A, Merker M, Andres S, Bablishvili N, Barilar I, et al. Investigating resistance in clinical Mycobacterium tuberculosis complex isolates with genomic and phenotypic antimicrobial susceptibility testing: a multicentre observational study. *Lancet Microbe* 2022;**3**:e672–82. doi:10.1016/S2666-5247(22)00116-1.

[24] Lavender C, Globan M, Sievers A, Billman-Jacobe H, Fyfe J. Molecular characterization of isoniazid-resistant Mycobacterium tuberculosis isolates collected in Australia. *Antimicrob Agents Chemother* 2005;**49**:4068–74. doi:10.1128/AAC.49.10.4068-4074.2005.

[25] Lam C, Martinez E, Crighton T, Furlong C, Donnan E, Marais BJ, et al. Value of routine whole genome sequencing for Mycobacterium tuberculosis drug resistance detection. *Int J Infect Dis* 2021;**113**:S48–54. doi:10.1016/j.ijid.2021.03.033.

[26] Caws M, Duy PM, Tho DQ, Lan NTN, Hoa DV, Farrar J. Mutations prevalent among rifampin- and isoniazid-resistant Mycobacterium tuberculosis isolates from a hospital in Vietnam. *J Clin Microbiol* 2006;**44**:2333–7. doi:10.1128/JCM.00330-06.

[27] Gagneux S, Long CD, Small PM, Van T, Schoolnik GK, Bohannan BJ. The competitive cost of antibiotic resistance in Mycobacterium tuberculosis. *Science* 2006;**312**:1944–6. doi:10.1126/science.1124410.

[28] Chien JY, Chiu WY, Chien ST, Chiang CJ, Yu CJ, Hsueh PR. Mutations in gyrA and gyrB among fluoroquinolone- and multidrug-resistant Mycobacterium tuberculosis isolates. *Antimicrob Agents Chemother* 2016;**60**:2090–6. doi:10.1128/AAC.01049-15.

[29] Warren RM, Streicher EM, Gey van Pittius NC, Marais BJ, van der Spuy GD, Victor TC, et al. The clinical relevance of Mycobacterial pharmacogenetics. *Tuberc (Edinb Scotl)* 2009;**89**:199–202. doi:10.1016/j.tube.2009.03.001.

[30] Brown AC, Bryant JM, Einer-Jensen K, Holdstock J, Houniet DT, Chan JZ, et al. Rapid whole-genome sequencing of Mycobacterium tuberculosis isolates directly from clinical samples. *J Clin Microbiol* 2015;**53**:2230–7. doi:10.1128/JCM.00486-15.