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Exploring programmatic indicators of tuberculosis control that incorporate routine whole genome sequencing in low incidence settings: a comprehensive (2017-2021) patient cohort analysis

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Tuberculosis (TB) remains a leading infectious disease threat worldwide with more than 10 million people developing TB every year and causing ~1.5 million deaths annually.[1] Australia has a low burden of TB with an annual incidence of ~6 cases per 100,000 population and ~2-3% of RR/MDR-TB cases per year.[2] Although TB case numbers in New South Wales (NSW) remain relatively small compared to high incidence settings,[3] TB control is a constitutionally mandated public health priority in Australia and the country has formally committed to TB elimination.

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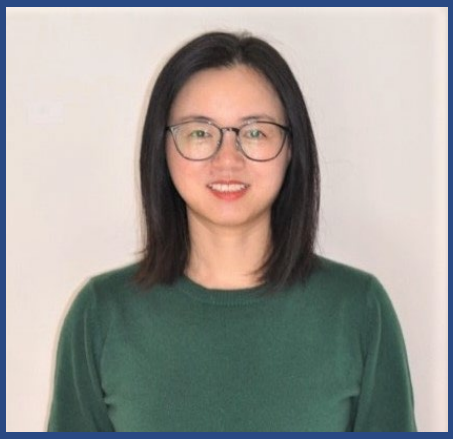

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NSW began routine whole genome sequencing (WGS) of all culture-confirmed TB cases in 2016 and standardised processes were implemented from 2017. The utility of WGS for timely recognition of drug resistance and accurate transmission tracking for individual cases and TB outbreaks has been recognised in many settings,[4-7] but the use of routine WGS in advising and evaluating the overall performance of TB control programs has yet to be established. One suggested metric to consider is the calculation of "locally transmitted TB incidence", which aims to encourage progress towards achieving "zero TB transmission" in low TB incidence settings.[8] The calculation of locally transmitted TB incidence, based on standardized cluster definitions and epidemiological verification, presents an opportunity to establish programmatic indicators and evaluate progress towards achieving the ambitious goal of "zero TB transmission".

In this study, we employed data from routine *M. tuberculosis* cultures that were prospectively sequenced by the NSW TB control program to explore genomically-informed TB control programmatic indicators. A total of 1831 *M. tuberculosis* strains were successfully sequenced, representing 64.8% of all TB cases notified between 1st January 2017 and 31st December 2021 in NSW, 84.8% of all bacteriologically confirmed cases, and 96.2% of all culture confirmed cases. Cases with successfully sequenced *M. tuberculosis* strains were highly representative of the microbiologically-confirmed TB patient cohort in NSW. Routine pDST data were available for 1829/1831 (99.9%) isolates, with 198 (198/1829, 10.8%) resistant to one or more first-line TB drug (Figure 1a).

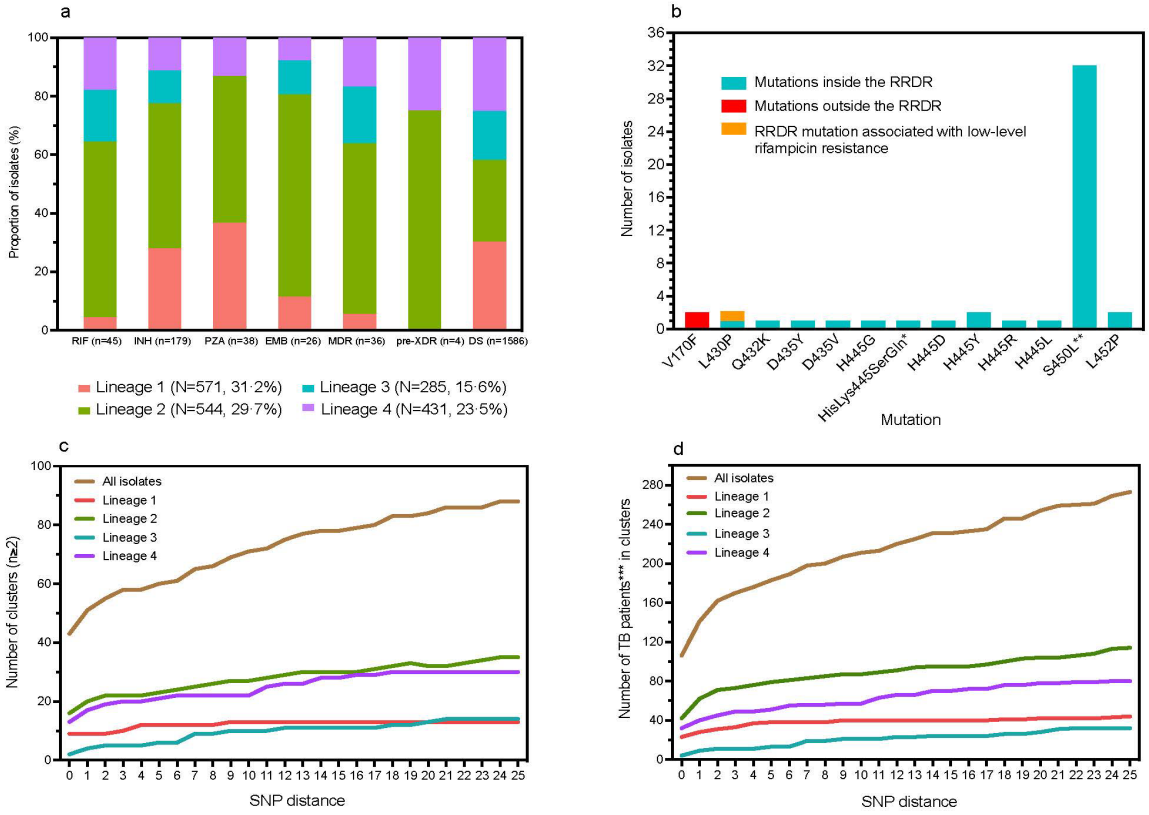

Figure 1: Overview of M. tuberculosis drug resistance profiles, and transmission clusters identified over the 5-year study period

a): M. tuberculosis lineage-specific pDST profiles. Numbers (n) in brackets next to drugs (X axis) indicate the total number of isolates with phenotypic resistance against the specified drug. Numbers (N) in brackets next to lineages (legend) indicate the total number of isolates per lineage. b): Frequency of mutations inside or outside the RRDR and its association with phenotypic rifampicin resistance. All mutations identified for rifampicin resistance were group 1 (Assoc w R) mutations according to the 2021 WHO drug resistant TB mutation catalogue. Mutations outside RRDR (indicated by the red bar) would be missed by Gene Xpert. c): Number of M. tuberculosis transmission clusters identified. 'SNP distance' defined as the number of SNP differences from 0-25 and 'SNP cluster' defined using variable pairwise SNP distance cluster definition. d): Number of TB cases contained in these clusters.

*EMB: ethambutol; INH: isoniazid; MDR: multidrug-resistant; PZA: pyrazinamide; pDST: phenotypic drug susceptibility testing; RIF: rifampicin; RRDR: Rifampicin Resistance Determining Region used for rifampicin resistance detection by Xpert MTB/RIF® / ULTRA® and Hain GenoType MTBDRplus V2; SNP: single nucleotide polymorphism; TB: tuberculosis; XDR: extensively drug resistant. *delCACAinsTCCC p.HisLys445SerGln; **with compensatory mutations in rpoC P1040R (n=3, MDR-TB), I491T (n=3, 2 MDR-TB and 1 RR-TB); ***Number of isolates reflects the number of TB patients in identified transmission clusters.*

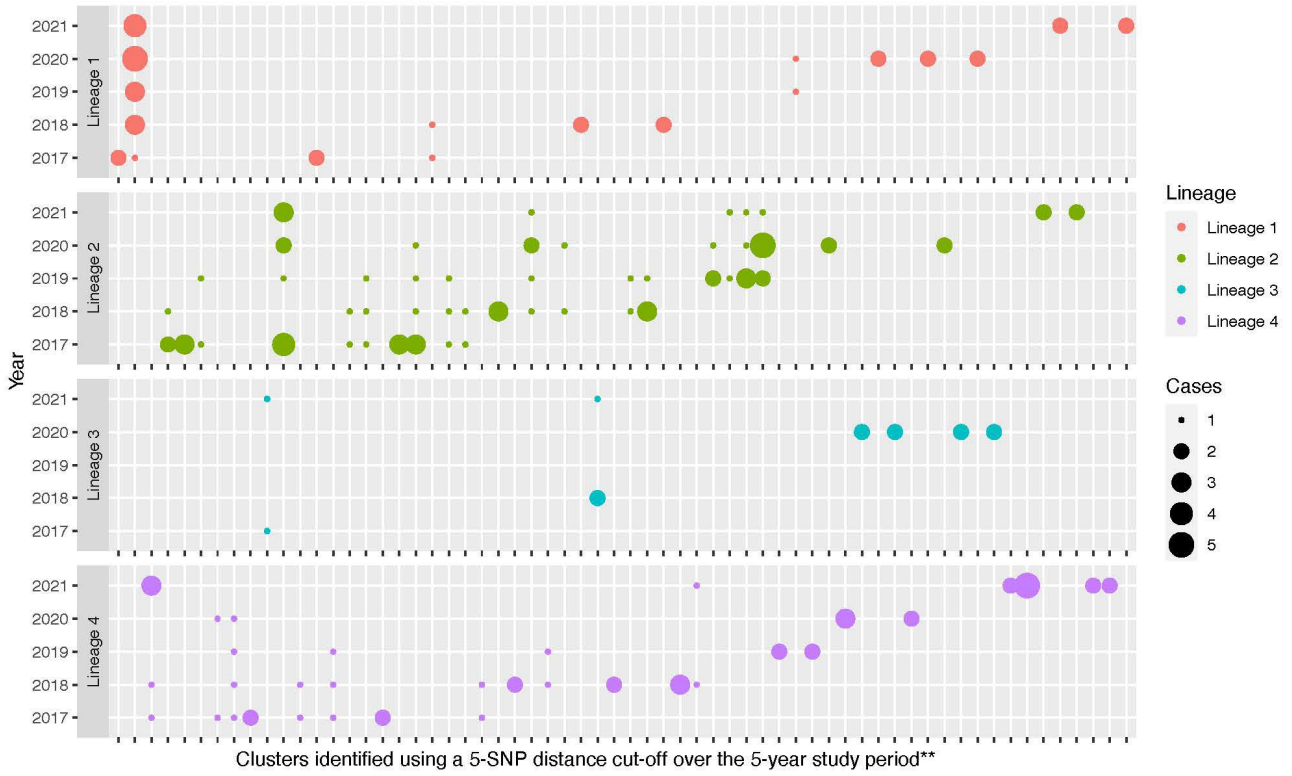

Figure 2: All M. tuberculosis transmission clusters identified over the 5-year study period*

Y-axis indicates the study year (2017-2021) and the X-axis the sequential clusters identified in that year.
**Using a five SNP distance cut-off; programmatically the focus should be on larger and/or multi-year clusters that indicate failed containment.*
***The clusters on the X-axis are presented in the order they were identified, based on the isolate in cluster with the earliest collection date. Single cases (small dots) included were part of multi-year clusters dispersed over the period indicated.*
SNP: single nucleotide polymorphism.

SNP-based clustering has been used to track transmission chains with high fidelity,[9] but the use of standard SNP thresholds for transmission inference has been questioned.[10] In our study, we assessed pairwise SNP clustering over a wide range (0-25) of SNP differences to ascertain the most appropriate and useful metrics for objective programmatic performance review and comparison (Figure 1c, Figure 1d). Application of a large and inclusive 25 SNP threshold that is not highly reflective of likely recent transmission identified 88 clusters, representing 15.0% (273/1821) of all sequenced TB cases, with an estimated RRT of 10.2% (25-SNP RRT). Application of a traditional 5-SNP threshold identified 62 clusters with 183 clustered TB cases and an estimated RRT of 6.8% (5-SNP RRT). A more conservative 2-SNP cut-off identified 55 clusters and 160 clustered TB cases, with an estimated RRT of 5.8% (2-SNP RRT). In NSW currently, all clusters with ≤5 SNPs are currently investigated. Our findings revealed a significant proportion of clustered TB cases (101/183, 55.2%) with 0 SNP differences, indicating likely recent transmission within Australia, which justify targeted intervention.

As a standard and reproducible TB control metric we propose a rolling 5-year cluster assessment, using a 5-SNP cluster threshold, alongside classical epidemiological investigation to monitor and track likely local transmission. Using a 5-SNP cut-off (Figure 2), eight large clusters (defined as ≥5 cases per cluster) were identified that included 60 TB cases. We also examined protracted multi-year clusters as an indication of sub-optimal local TB control, which can serve an additional programmatic performance indicators. Such clusters included cases that had been detected in at least three out of five consecutive years. In total, 29.0% (18/62) of all clusters (using a 5-SNP cut-off) were multi-year clusters, containing 83 TB cases over the 5-year period of study. Figure 3 provides a 'birds eye' overview of lineage-specific clusters suggestive of local TB transmission over the study period, including 35 (35/183, 19.1%) drug resistant isolates; one was MDR and 34 were isoniazid mono-resistant. We also performed expanded cluster assessment, using 0 SNP, 2 SNP, and 5 SNP cut-offs, over a rolling 2-year period. The proportion of TB isolates in large clusters were identical using a 2-SNP or 5-SNP cut-off over the rolling 2-year review. We believe that use of a 5-SNP cut-off for cluster identification, with analysis over the rolling 5-year review period, provides a highly informative overview of likely local transmission in a low incidence setting, like Australia.

An important WGS surveillance function is the ability to detect resistance to existing TB drugs not detected by current methods, as well as resistance to novel TB drugs where detection methods are still in development. These include rifampicin resistance mutations located outside the RRDR, which would be missed by Xpert MTB/RIF®. For instance, two strains had a *rpoB* V170F mutation outside the RRDR (Figure 1b) that was associated with phenotypic resistance but not detected by Xpert MTB/RIF®. In addition, one phenotypically susceptible strain had a *rpoB* L430P mutation located within the RRDR, which has been associated with low-level rifampicin resistance.[11] Monitoring the local frequency of these mutations has important surveillance value. Apart from mutation surveillance, gDST also provides clinically useful information. More than 15.0% of strains had genomic markers of isoniazid resistance that were undetectable by commercial molecular tests, including Gene Xpert XDR (Cepheid) and Genotype MTBDR*plus* v2 (HAIN LifeSciences). These included atypical *katG-furA* (including a large deletion), *fabG1-inhA* and *ahpC* mutations. WGS based gDST assesses the complete repertoire of drug resistance genes, including uncommon isoniazid resistance conferring mutations (e.g., *katG* S315N; *fabG1* L203L) that may be associated with low- or high-level resistance.

The discrepancies observed among isolates that tested phenotypically susceptible to first-line TB drugs, but carried mutations associated with drug resistance, require careful consideration. Routine gDST identified likely drug resistance mutations in an additional 6.8% of *M. tuberculosis* isolates compared to routine pDST (Table 1), which is usually defined as the 'reference standard'. This highlights the potential value of gDST in identifying drug resistance missed by other molecular tests, which would be greatly increased in the settings where routine pDST is not performed. gDST also identified genomic markers of fluoroquinolone resistance in cases that were phenotypically susceptible to all first-line drugs and therefore not phenotypically tested for second-line drug resistance. The limitations of culture and pDST as the only reference standard are well recognised, given instances of suboptimal accuracy.[1, 12] Integration of routine gDST for rapid and comprehensive drug resistance detection can assist optimal personalised treatment strategies for TB patients.

In conclusion, routine WGS of *M. tuberculosis* offers important benefits for transmission and drug resistance surveillance. Monitoring of genomic case clusters is useful to guide public health interventions and may provide useful transmission programmatic indicators for enhanced TB control. It may also assist to measure progress towards 'zero TB transmission' in low TB incidence countries like Australia.[8] As a programmatic tool we propose a rolling 5-year review of transmission clusters, using a standard 5-SNP threshold with additional assessment as relevant, to monitor local TB transmission and guide public health intervention. In addition, routine gDST provides valuable information to assist patient management and serves an important drug resistance surveillance function.

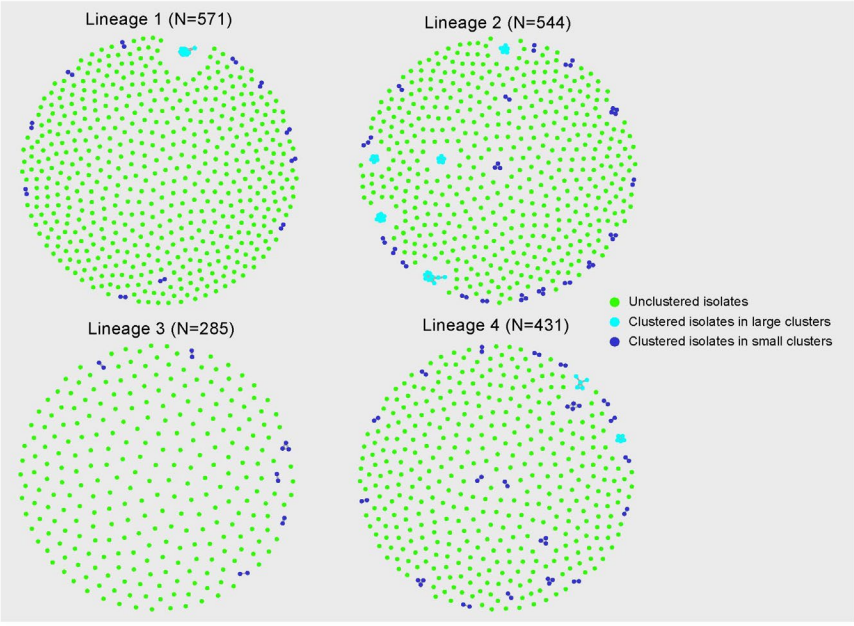

Figure 3: 'Birds eye' overview of lineage-specific tuberculosis cases and likely local M. tuberculosis transmission observed in New South Wales, Australia, over the 5-year study period (2017-2021)*

Single dots within each lineage circle represents one sequenced M. tuberculosis isolate (TB patient). The varying density of dots reflects variation in the number of lineage specific TB cases (Table S4). For clustered cases the size of the cluster is explained in the legend with large clusters representing ≥5 cases/isolates and small clusters <5 cases/isolates.

**Assessment of M. tuberculosis transmission clustering using a 5 SNPs cluster cut-off. SNP: single nucleotide polymorphism; TB: tuberculosis.*

Table 1: Overview of gDST accuracy for first-line drug resistance detection and consideration of its 'perceived added value' in the study context*

	pDST n (%)	gDST n (%)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Perceived added value of gDST[#]		
							All discordant[##]	gDST only[###]	Frequency of perceived value add[####]
RIF	45 (2.5%)	46 (2.5%)	100 (92.1-100)	99.9 (99.7-100	97.8 (88.7-99.9)	100 (99.8-100)	1	0	2.2% (1/45)
INH	179 (9.8%)	195 (10.6%)	99.4 (96.9-100)	99.0 (98.4-99.4)	91.3 (86.5-94.5)	99.9 (99.7-100)	11	1	6.7% (12/179)
PZA**	38 (2.1%)	48 (2.6%)	84.2 (69.6-92.6)	99.1 (98.6-99.5)	66.7 (52.5-78.3)	99.7 (99.3-99.9)	16***	0	0% (0/38)
EMB**	26 (1.4%)	31 (1.7%)	100 (87.1-100)	99.7 (99.4-99.9)	83.9 (67.4-92.9)	100 (99.8-100)	5	0	19.2% (5/26)
MDR	40 (2.2%)	42 (2.3%)	100 (91.2-100)	99.9 (99.6-100)	95.2 (84.2-99.2)	100 (99.8-100)	2	0	5.0% (2/40)
Total	198/1829 (10.8%)	221/1829 (12.1%)	96.5 (92.9-98.3)	98.2 (97.4-98.7)	86.4 (81.3-90.3)	99.6 (99.1-99.8)	12	1	6.8% (13/192[####])

*CI: confidence interval; DST: drug susceptibility testing; gDST: genotypic DST; pDST: phenotypic DST; EMB: ethambutol; INH: isoniazid; PZA: pyrazinamide; RIF: rifampicin; NPV: Negative Predictive Value; PPV: Positive Predictive Value. *Compared to pDST (regarded as the reference standard); **pDST result was unavailable for 1 isolate; ***Overall poor confidence in individual mutations associated with PZA resistance.*

[#]Reflects gDST 'value add' in the presence of routine pDST; would be greatly increased in settings without routine pDST. The 'value add' include discordant results with mutations that are graded as "Assoc w R" in 2021 WHO catalogue and specimens with failed pDST; [##]'All discordant' indicate strains with resistance mutations that are graded as "Assoc W R" and "Assoc w R -Interim" in 2021 WHO catalogue on gDST and that tested susceptible on pDST; [###]pDST failed; [####]Fraction with resistance mutations not detected by routine pDST. The number of discordant pairs observed included six isolates that tested PZA resistant by pDST but had no recognised resistance conferring mutations detected by gDST. These resistant strains 'missed by gDST' were subtracted from the perceived added value calculation (198-6=192). The mutations that contributed to the 6.8% 'value add' estimate for first-line drug resistance detection, included rpoB L430P (n=1), katG S315T (n=1), fabG1 -15 c>t (n=5), fabG1 -8 t>a (n=5), and fabG1 -8 t>c (n=1).

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UPCOMING EVENTS

CIDM-PH AI WEBINAR

NAVIGATING THE DATA JUNGLE: AI FOR INFECTIOUS DISEASES DIAGNOSTICS

Friday, 30th August 2024

1pm – 4.30pm (AEST)

In a rapidly evolving landscape of infectious diseases and microbiology, artificial intelligence (AI) emerges as a powerful ally. This webinar aims to explore AI's transformative potential to rapidly bridge the gap between cutting-edge research and improved clinical practice.

PROGRAM & REGISTRATION AVAILABLE SOON

CIDM-PH Annual Colloquium

Friday, 29 November 2024

9am – 4pm (AEST)

Westmead Education & Conference Centre, Westmead Hospital

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