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Added value of whole genome sequencing for *Mycobacterium tuberculosis* drug resistance detection

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One of the major advantages of whole genome sequencing (WGS)-enabled genome-wide analysis over conventional genomic tests is the ability to recognize mutations associated with phenotypic resistance. Contemporary drug resistance databases, including the CRyPTIC Consortium catalogue (Allix-Beguec, Arandjelovic et al. 2018), ReseqTB (Ezewudo, Borens et al. 2018) , tbvar (Joshi, Dhiman et al. 2014) , TBDreaMDB (Sandgren, Strong et al. 2009) and others (Coll, Preston et al. 2014, Coll, Phelan et al. 2018) provide a solid foundation from which to predict genomic drug resistance. Here, we present results of routine WGS of *M. tuberculosis* isolates performed in a clinical diagnostic setting in Australia and quantify the "added value" of WGS resistance detection over other approaches. In the context of a low TB burden, high resource setting, we aimed to compare and correlate *Mycobacterium tuberculosis* drug resistance detected using traditional pDST, in parallel with WGS derived resistome assessment and commercial tests for genotypic DST (gDST).

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Methods

Clinical isolates. All isolates referred to the Mycobacterial Reference Laboratory at the Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology in Sydney, Australia between February 2016 and June 2019 for confirmatory identification and characterization were cultured and prospectively sequenced.

Drug susceptibility testing. Phenotypic DST was performed using the modified proportion method in the BACTEC MGIT 960 system (Rusch-Gerdes, Pfyffer et al. 2006) with the following drug concentrations: low and high isoniazid (INH) (0.1 mg/L and 0.4 µg/mL, respectively), rifampicin (RIF) (1 µg/mL), pyrazinamide (PZA) (100 µg/mL) and ethambutol (EMB) (5 µg/mL).

Whole genome sequencing. All *M. tuberculosis* isolates were subjected to WGS as previously described (Votintseva, Pankhurst et al. 2015). Briefly, mycobacterial DNA was extracted and normalized to 0.2 ng/µL to be used as input for the Nextera XT library preparation kit (Illumina, San Diego, California). Libraries were batched and sequenced with an Illumina NextSeq500 (Illumina, San Diego, California) using 2x150bp paired-end chemistry. Sequencing reads were mapped against reference genome of *M. tuberculosis* H37Rv (NCBI Genbank accession no. NC000962.3).

WGS resistome analysis. Single nucleotide polymorphisms (SNPs) were identified using Snippy v3.1. Mutations potentially conferring resistance to antimycobacterial drugs were identified using tabix and in-house Python scripts and the CRYPTIC catalogue (Allix-Beguec et al. 2018). Further phylogenetic allocation was performed using the RedDog package. Mfasta files were used to construct pairwise SNP distances matrix with snp-dists v0.6.

Commercial genotypic drug susceptibility tests. WGS resistome analysis was also compared with GeneXpert MTB/RIF (Cepheid, USA), as well as GenoType MTBDR*plus* (v 2.0) and GenoType MTBDRsl (v 2.0) (HAIN Lifescience, Germany). These assays are able to confirm the presence of *M. tuberculosis* as well as detect common mutations conferring resistance to RIF (GenXpert, GenoType MTBDR*plus*), INH, ciprofloxacin and injectable drugs (GenoType MTBDR*plus* and GenoType MTBDR*sl*).

Results

M. tuberculosis isolates in NSW were predominantly from East-African Indian (EAI) and Beijing lineages (30.0% and 30.4%, respectively). Pairwise SNP distances between isolates showed a high level of strain diversity, especially for EAI and European American lineage strains. Nearly all strains isolated (1103/1107; 99.6%) had pDST results for all first line drugs, with the vast majority (88.5%) being pan-susceptible. Mono-resistance was generally rare, except with isoniazid where 91 (8.3%) strains were resistant; 43/91 (47%) belonged to the Beijing lineage. Twenty-nine strains (2.6%) were identified as multi-drug resistant (MDR). Beijing lineage strains were associated with significantly higher frequency of resistance to first line drugs than other lineages ($p<0.05$).

WGS resistome analysis was performed in all 1107 isolates, with slightly more resistance detected than with pDST (13.0%; 144/1107 vs 12.4%; 137/1104). Mutations in the Rifampicin Resistance Determining Region (RRDR) of *rpoB* accounted for 30/32 (93.8%) of rifampicin resistance with Ser450Leu (Ser531Leu) being the most frequent mutation (13/32; 40.6%). Upon re-testing, all rifampicin resistant isolates with *rpoB* mutations were phenotypically resistant at the WHO recommended breakpoint of 1ug/mL. Figure 1 provides an overview of all mutations associated with first line drug resistance detected in different lineages. The overall rate of MDR-TB was low (2.6%). Most MDR strains (21/29; 72.4%) belonged to the Beijing lineage with no evidence of local MDR-TB transmission.

Isolates with either *katG* Ser315 (n=75) or *inhA* (n=5/6) mutations almost universally had high-level isoniazid resistance (>0.4mg/L), while those with only *fabG1* C-15T mutations (n=24/26) had low level (0.1mg/L) resistance. No isolates with these high confidence resistance mutations were phenotypically susceptible to isoniazid. In 23 strains pyrazinamide resistance was suggested by the detection of *pncA* mutations but only 18 (78.3%) could be phenotypically confirmed using MGIT 960 and/or Wayne's assay. Phenotypic ethambutol resistance was uncommon (n=18; 1.7%). Only four codons in *embB* were associated with resistance: *embB* Met306Val (n=13), Met306Ile (n=5), Met306Leu (n=1), Phe330Val (n=1) and Gln497Arg (n=1).

Table 1 summarises the diagnostic accuracy of WGS resistome predictions compared to pDST. Importantly, WGS derived genomic resistance across all lineages provided significant gains in comparison to WHO recommended commercial gDST assays ($p=0.0137$, paired *t*-test), but gains were variable in different lineages. The largest percentage gains were observed in European American and EAI lineages.

Table 1: The number of isolates testing as susceptible or resistant by phenotypic drug susceptibility testing (pDST) to four first line drugs are listed. The corresponding sensitivity and specificity values have been calculated and shown with confidence intervals (CI) of 95%, as well as positive predictive value (PPV) and negative predictive value (NPV) of WGS compared with pDST.

Drugs	pDST	Wild type (n)	Mutation (n)	Total # isolates	Sensitivity % (CI)	specificity % (CI)	PPV % (CI)	NPV % (CI)
RIF	Susceptible	1072	0	1072	100	100	100	100
	Resistant	0	31	31	(88.78-100)	(99.66-100)		
INH	Susceptible	966	12	978	93	98.8	90.7	99.2
	Resistant	8	117	125	(87.78-97.2)	(97.93-99.79)	(84.72-94.49)	(98.45-99.59)
PZA	Susceptible	1076	6	1082	85.71	98.8	75	99.72
	Resistant	3	18	21	(63.66-96.95)	(97.93-99.38)	(57.0-87.16)	(99.2-99.9)
EMB	Susceptible	1082	3	1085	100	99.72	85.71	100
	Resistant	0	18	18	(81.47-100)	(99.19-99.14)	(65.9-94.89)	

Discussion

This study quantified lineage specific improvements in drug-resistance detection offered by routine WGS resistome assessment in *M. tuberculosis*. The low incidence of TB in NSW, coupled with high levels of culture confirmed cases (>85%), and integration of WGS into routine reference laboratory workflows enabled this comparison. Both pDST and WGS accurately identified all culture confirmed MDR-TB isolates, but as previously reported routine WGS improved the median reporting time from 16 days using pDST to 11 days (Martinez, Bustamante et al. 2016). In contrast to findings in TB endemic areas, NSW isolates were genetically very diverse across all four major lineages, suggesting importation of cases from a wide variety of overseas locations rather than local transmission (Gurjav, Outhred et al. 2016, Martinez, Hennessy et al. 2018). The comparable contribution of Beijing and EAI lineages is broadly reflective of migration patterns into NSW and Australia (Gurjav, Outhred et al. 2016), as well as the global situation (Luo, Comas et al. 2015).

Routine WGS resistome analysis provided significant improvements in genomic resistance detection over commercial gDST across all *M. tuberculosis* lineages. WGS resistome analysis detected an additional ~1% of cases with likely drug resistance not detected by pDST, explained by mutations previously associated with treatment failure and mixed bacterial populations. The overall sensitivity of WGS resistome prediction, including the lower sensitivity for pyrazinamide resistance, is consistent with other WGS studies performed in low incidence settings (Pankhurst, Del Ojo Elias et al. 2016, Cabibbe, Trovato et al. 2018). The study also demonstrated the strength of *M. tuberculosis* WGS to identify hetero-resistance and large deletions missed by other modalities.

In conclusion, we demonstrated the feasibility of routine WGS resistome analysis in a programmatic setting with significant benefit for drug resistance detection compared to commercial gDST; guiding expedited person-centered care. More high quality sequences with associated pDST results will facilitate the identification of novel genes and pathways associated with drug resistance, and will also enable predictions of resistance to newer antituberculosis drugs such as bedaquiline, linezolid and delamanid.

Figure 1.

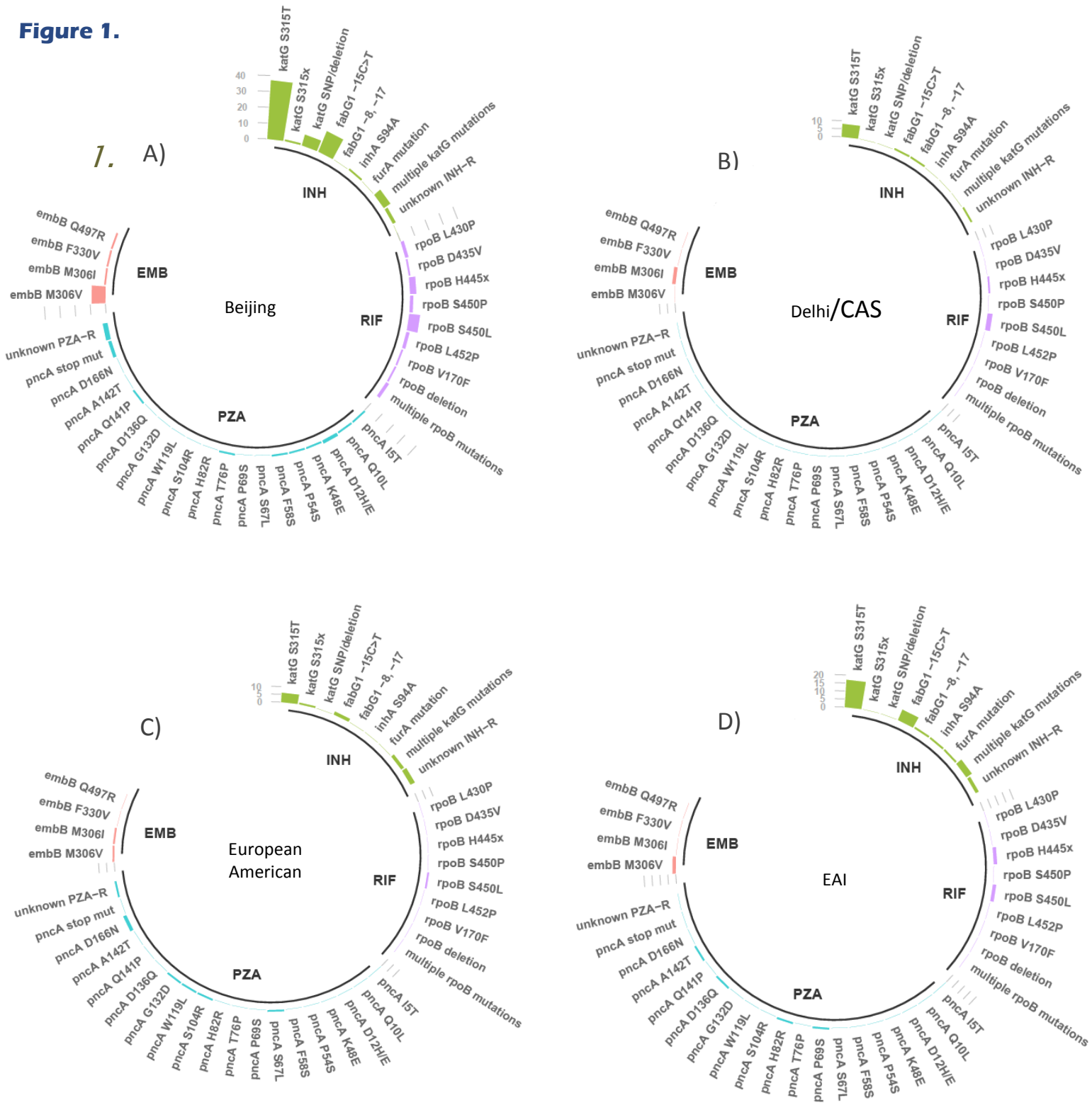

Figure 1: Distribution of mutations associated with first line drug resistance across four major lineages of *M. tuberculosis.* Circular barplots of resistance mutations and deletions in Beijing lineage (A), Delhi/CAS (B), European American (C), and EAI lineage (D). For each plot, the number of mutations in *katG*, *fabG1*, *inhA*, and *furA* associated with isoniazid (INH) resistance are shown in green; *rpoB* mutations conferring rifampicin (RIF) resistance in purple; *pncA* mutations for pyrazinamide (PZA) resistance in blue, and *embB* mutations in pink for ethambutol (EMB) resistance. Scale bars have been adjusted for each lineage according to the number of mutations present. Refer to main text for *E.coli* nomenclature for *rpoB* mutations.

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Staff Profile

Dr Carl Suster

Carl Suster completed his PhD in experimental particle physics at The University of Sydney as a member of the ATLAS Collaboration at CERN, the European Organization for Nuclear Research. His research focused on studying the production of the top quark, the most massive observed elementary particle, via weak interaction processes in proton collisions at the Large Hadron Collider (LHC). The research involved statistical analysis of large simulated and experimental datasets to distinguish the target interaction from other processes that mimic its signature.

In 2020 Carl joined the Discipline of Biomedical Informatics and Digital Health at the Sydney School of Medical Sciences. His work centred on the detection and management of sepsis in patients who present to emergency departments. He analysed electronic medical record (eMR) datasets to build sepsis screening models used to develop pilot programs of improved sepsis clinical decision support tools in adult and paediatric settings. He also supported investigations into the effectiveness of timely and appropriate interventions in patients with suspected sepsis.

Carl has recently joined CIDM-PH as a postdoctoral fellow in infectious disease informatics. Drawing on his experience working with complex datasets, his research initially will address the characterisation of ongoing outbreaks by combining genomic and epidemiological information with an aim to provide public health and clinical decision support.

Dr Carl Suster

Postdoctoral Fellow in Infectious Diseases Informatics

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NEWS

MBI announced as Flagship Centre

As part of their 2025 Strategy, the Faculty of Medicine and Health embarked on a review of its Research Centres to determine where they should strategically focus their research support and investment. MBI is excited to announce that we have been selected as one of six Flagship Centres. We look forward to our world-leading research community continuing their research into the health and socioeconomic consequences of emerging and re-emerging infectious diseases. If you would like to join our community and stay up to date with our activities please visit our website

https://www.sydney.edu.au/marie-bashir-institute/about-us/become-a-member.html

Prof Tania Sorrell & Prof Ben Marais

MBI Co-Directors

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UPCOMING EVENTS

SEMINAR

'SARS-CoV-2 neutralizing antibodies; longevity, breadth, and evasion by emerging viral variants'

A/Prof Fabienne Brilot-Turville,
Principal Research Fellow,
University of Sydney

Friday 7th May 2021

1 - 2pm

Westmead Education & Conference Centre, Westmead Hospital

Free ticket, **registration** essential for zoom link and in-person attendance:

https://fabiennebrilotturvilleseminar.eventbrite.com.au

Event Enquiries:

WSLHD-CIDM-PH@health.nsw.gov.au

CIDM centre for infectious diseases & microbiology Public Health

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