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Commentary

TB Transmission: Closing the Gaps

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Molecular tools, including RFLP, spoligotyping, and MIRU-VNTR, have greatly enhanced our understanding of tuberculosis transmission [1, 2]. In settings where molecular tools are regularly employed, MIRU-VNTR is perhaps the most frequently used. The size of PCR amplicons spanning either 12, 15, or 24 repeat loci is used to infer a fingerprint for a given isolate, allowing surveillance programs to identify clusters of isolates potentially related by recent transmission. However, the increasing use of genomics in national TB surveillance programs is confirming what many in the TB community have long suspected – that MIRU-VNTR clusters often do not represent epidemiologically linked, recently transmitted cases, particularly for *Mycobacterium tuberculosis* isolates not belonging to Lineage 4.

In a publication in *EBioMedicine*, Wyllie et al. benchmark MIRU-VNTR against genomics using a dataset of over 2000 prospectively collected UK TB isolates, revealing that only 20% of isolates with identical MIRU-VNTR profiles were likely the result of recent transmission when the genomic data were considered [3]. While single nucleotide variant (SNV) distances were typically <10 between Lineage 4 isolates with identical MIRU-VNTR fingerprints, clustered isolates in other lineages were often >100 SNVs apart. An analysis of isolates from recent immigrants to the UK versus those who were born in the country or had been there for more than two years also revealed the extent to which MIRU-VNTR overestimates clustering – despite identical MIRU-VNTR profiles, isolates in recent immigrants exhibit SNV distances incompatible with recent transmission. Ultimately, Wyllie et al. confirm that MIRU-VNTR overestimates TB transmission in certain settings, particularly amongst individuals from countries where lineages other than Lineage 4 dominate [3]. While some of these insights are not new [4] – it is common practice to run a more variable set of MIRU loci for Lineage 2 strains to better capture relatedness – the scale of these analyses reveals just how much more powerful genomics is at identifying potential recent transmission and raises important questions about the future of MIRU-VNTR in well-resourced settings.

While universal MIRU-VNTR of all isolates received by a reference laboratory can reveal unsuspected clustered cases [5], its utility in real-time investigation is unclear. Fingerprinting requires DNA from culture-positive isolates with results often taking upwards of a month to arrive, and there is limited evidence to suggest that MIRU-VNTR directly impacts case-finding and outbreak management in a meaningful way. While we have previously shown that TB program staff report high confidence in interpreting MIRU-VNTR data [6], anecdotal evidence suggests that some of the intricacies of interpretation, particularly around identical patterns in recent immigrants, are not always clear to all parties involved in an investigation. Together with Wyllie et al.'s data demonstrating the clear superiority of genomics at revealing true recent transmission, these observations suggest that settings currently relying on MIRU-VNTR for insights into local epidemiology would be better served by implementing a real-time genomics platform instead. Whereas MIRU-VNTR is restricted to identifying clusters, relying on contact investigation to draw inferences around transmission, genomics' resolution can be leveraged to identify directional transmission events, greatly facilitating investigations in challenging situations, where populations might be hard to reach, where contacts go unnamed, or where survey instruments might fail to yield actionable information. Thus, the limited resources available to local TB prevention programs can be more strategically deployed to mitigate ongoing transmission.

Implementing routine genomics is not simple however [7]. Beyond the oft-cited economical and operational obstacles, there are substantial interpretive challenges. It is common practice to use SNV thresholds to define linkage by recent transmission [8], such as the five SNV threshold used by Wyllie et al. Such thresholds are sensitive to the bioinformatics pipeline used to analyze the data [8], and they assume a constant, low substitution rate. If the organism has accumulated an unusual number of SNVs – there is evidence that substitution rates may vary in active disease as a result of host factors, such as co-morbidities, and possibly also in latent infection – a case may not be linked to its transmission cluster. Furthermore, inferring the underlying phylogeny and associated transmission networks requires additional analyses. Recent approaches to this problem take advantage of state-of-the-art phylogenetic modelling and integration of relevant biological and epidemiological parameters, such as the pathogen's substitution rate or infectious period of the host [9]; however, our knowledge about the ranges of those parameters is still limited. While an operational definition of transmission based on

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SNV threshold is a useful placeholder for public health agencies engaged in routine genomic surveillance, further work is needed to address gaps in our understanding of the genomic, clinical, and epidemiological aspects of TB transmission if we are to truly leverage genomics as a tool to advance TB elimination efforts.

Ultimately, Wyllie et al. bring us one step closer to closing the gaps between contact investigation, genotyping, and genomic epidemiology, presenting evidence to help TB molecular surveillance programs to choose the best tool for their needs. This is particularly relevant in the era of TB elimination in low-burden countries, where TB is not seen as a priority area for public health funding. With the promising reports of genomics as replacement for phenotypic drug sensitivity testing [10] and the possibility of interrogating the pathogen genome directly from sputum samples [7], we envision a future in which TB genomic epidemiology will be integral to local and global tuberculosis surveillance and prevention programs.

Disclosure

The authors declare no conflicts of interest.

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