

Evidence for asymptomatic TB transmission in rural South Africa

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Biological Sciences - Article

Keywords:

Posted Date: June 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9569436/v1>

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Additional Declarations: There is **NO** Competing Interest.

Abstract

Whether asymptomatic TB contributes to *M. tuberculosis* (Mtb) transmission is critical to global public health strategy. To address this, we evaluated microbiologic, radiographic, molecular and spatial data from a population-based TB study in South Africa. We found that 82% of microbiologically-confirmed pulmonary TB occurred in asymptomatic individuals. Microbiologic and radiologic correlates of infectiousness, including measures of bacterial burden and lung cavitation, were similar between asymptomatic and symptomatic TB detected in the community. Drug-resistant variants, ranging from mono- to multi-drug resistance, were detected in people with asymptomatic TB. Analysis of Mtb strains showed that half of genotypic transmission clusters involved exclusively asymptomatic people. Our results demonstrate that most prevalent pulmonary TB is asymptomatic and that asymptomatic TB, including that involving drug-resistant Mtb, is transmissible. Overall, these findings suggest that symptoms do not reliably identify people with transmissible TB and that screening strategies to detect asymptomatic disease will be required to end TB.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains the world's leading infectious cause of death¹. For much of the last 50 years, public health strategies have been designed to address TB in two distinct forms: latent TB, defined as asymptomatic, immunologically contained and non-transmissible; and active TB, defined as symptomatic, featuring bacterial replication and tissue destruction, and transmissible². Recently, however, analysis of national TB prevalence data has drawn attention to the many people with bacteriologically-confirmed Mtb in their sputum who do not report any of the "typical" TB symptoms (including but not limited to cough, night sweats, fever and/or loss of weight)³⁻⁵. Since symptom screening has typically been the gateway to TB testing in public health systems, this has raised questions about the importance of asymptomatic TB, which can only be identified through symptom-agnostic screening, both to the individual in terms of its morbidity and to society in terms of its transmissibility⁶⁻⁹.

Whilst cough is considered to be an important mechanism to effectively aerosolize infectious particles for Mtb transmission, emerging evidence from bioaerosol studies indicates that tidal breathing may release higher amounts of infectious organism into the air potentially providing a mechanism for patients without overt cough to be infectious¹⁰. Mathematical modeling studies have also suggested that longer durations of exposure to asymptomatic individuals may translate to an equivalent or higher likelihood of Mtb transmission in contacts of people with asymptomatic TB than in contacts of people with symptomatic TB^{11,12}. Implications of asymptomatic TB extend beyond transmission. For example, there may be a risk of developing drug resistance in scenarios in which individuals with asymptomatic TB screen falsely negative due to lack of symptoms and receive regimens for preventative therapy (TPT) which may be inadequate to fully eradicate their disease². This scenario also raises concern for subsequent transmission of these drug-resistant asymptomatic strains. With the proportion of cases of

asymptomatic TB ranging from 50–80% in previous studies^{3–5,13,14}, the need to understand this entity is growing and the importance of addressing these knowledge gaps to inform public health policy was highlighted in the World Health Organization's Global Tuberculosis Report 2024^{15,16}.

Overall, a better understanding of the frequency of asymptomatic TB, its radiographic and microbiological features, especially those known to confer transmission risk like bacterial burden and presence of cavitory lung disease^{17–19} and whether it is implicated in community transmission will be crucial for designing more effective global TB care and prevention strategies. Here we analyze epidemiological, clinical, molecular and spatial data from a population-based TB screening survey conducted between 2018 and 2020 in a rural South African setting with high HIV- and TB-prevalence with the aim of understanding the TB transmission dynamics in this setting.

Results

Most prevalent TB was asymptomatic community-detected TB, which had comparable measures of infectiousness to symptomatic community-detected TB

Half of the eligible adolescent and adult residents (18,041/36,097) of a demographic and health surveillance area in rural KwaZulu-Natal, South Africa participated in TB screening during a multi-disease survey^{20,21} (Fig. 1a). Of these, 10,682 participants (59.2%) were triaged for sputum collection because they reported having one of the World Health Organization Four Screening Symptoms²² (W4SS: cough of any duration, night sweats, unexplained weight loss or fever) and/or because they had a computer assisted diagnostic abnormality score above the triaging threshold (> 25 CAD4TBv5, Delft) or lung field abnormalities identified by an expert radiologist²³. Sputum from 9,337 participants (87.4% of those triaged) was collected and processed to evaluate for molecular (Xpert Ultra, Cepheid) or microbiological evidence of Mtb (MGIT, BD). At the time of the survey, 0.4% (71/18,041) of participants were defined as having clinic-diagnosed TB because they reported current treatment for TB that had been diagnosed after presentation to local clinics or hospitals and 1.0% (174/18,041) of participants were defined as having community-detected TB because their sputum demonstrated microbiological evidence of Mtb and they were not currently being treated for TB (Fig. 1a). Male sex, age over 25 years, living with HIV, history of a previous episode of TB and active use of tobacco were overrepresented among people with clinic-diagnosed and community-detected TB, when each group was compared to people without evidence of TB (Table 1, **Table S1**). People with community-detected TB were less likely to be living with HIV, had higher CD4 cell counts if they were, and were less likely to have a previous episode of TB than people with clinic-diagnosed TB.

Table 1

Clinical features of participants with clinic-diagnosed and community-detected TB compared to people without TB

Male sex	Clinic-diagnosed TB N = 71¹	Community- detected TB N = 174¹	No TB N = 17,796¹
	31 (44%)*	80 (46%)*	5,701 (32%)
Age category, years			
15–25	9 (13%) [^]	29 (17%) [^]	5,259 (30%)
26–50	45 (63%)*	69 (40%)*	6,906 (39%)
50+	17 (24%)	76 (44%)*	5,631 (32%)
HIV positive ²	45 (64%)*	76 (44%)* [#]	5,975 (34%)
On antiretroviral therapy ³	40 (89%)	61 (80%)	4,913 (82%)
Undetectable HIV viral load ⁴	30 (67%)	50 (67%)*	4,741 (79%)
CD4 count, median (IQR) ⁵	357 (253, 581)*	581 (366, 727)* [#]	696 (485, 918)
Diabetes mellitus ⁶	8 (11%)	24 (14%)	1,705 (9.6%)
Previous TB ⁷	29 (41%)*	38 (23%)* [#]	1,935 (11%)
Tobacco use, current ⁸	9 (13%)	29 (17%)*	1,263 (7.1%)
Any W4SS TB symptom at TB screening ⁹	-	31 (18%)*	1,449 (8.2%)
Subjective wellness, EQ-5D-3L ¹⁰ , median (IQR)	-	95 (80, 100)	90 (80, 100)
* p-value for univariate logistic regression < 0.05 for comparison to No TB group, # p-value for univariate logistic regression < 0.05 for comparison to Clinic-diagnosed TB group, ^ reference group for univariate logistic regression.			

¹n (%); Median (Q1, Q3). ²HIV results available for groups n = 70, 174, 17708. ³Self reported, for HIV positive only, n = 45, 76, 5975. ⁴Viral load < 40, for HIV positive only. ⁵For HIV positive only. ⁶HbA1c > 6.5 or on treatment for previously diagnosed diabetes, n = 70, 174, 17709. ⁷Reported having previously been treated for TB (not including current episode), n = 70, 166, 16891. ⁸Self reported current use of cigarettes or tobacco products, n = 71, 174, 17779. ⁹Not reported for the Clinic-diagnosed TB group because duration of TB treatment for current episode varied at the time of the survey, n = --, 174, 17776. ¹⁰Subjective wellness, n = --, 152, 15162.

Notably, 82% (143/174) of people with community-detected TB were defined as asymptomatic because they did not report any of the four symptoms measured in the W4SS at the time of TB screening. People with asymptomatic and symptomatic community-detected TB were similar with respect to sex, age, HIV status, and diabetes status (Table 2). Compared to people with symptomatic community-detected TB, people with asymptomatic community-detected TB were less frequently underweight, less likely to have had previous TB and less likely to use tobacco (Table 2). On an independently measured scale of perceived health status (EQ-5D-3L), people with community-detected TB reported levels of subjective wellbeing (median 95 [IQR 80–100]) that were similar to people without evidence of TB (90 [80–100], Table 1). However, people with asymptomatic community-detected TB reported higher levels of subjective wellbeing (98 [80–100]) than people with symptomatic community-detected TB (80 [70–95], $p = 0.011$ **Table 2**).

Table 2
Clinical features of people with asymptomatic and symptomatic community-detected TB.

Characteristic	Asymptomatic TB ¹ (n = 143)	Symptomatic TB ¹ (n = 31)	p-value ²
Male sex	63 (44%)	17 (55%)	0.3
Age category, years			
15–25	25 (17%)	4 (13%)	ref
26–50	60 (42%)	9 (29%)	> 0.9
50+	58 (41%)	18 (58%)	0.3
BMI Category ³			
Normal: 18.6–24.9	78 (55%)	15 (50%)	ref
Underweight: Below 18.5	9 (6.3%)	6 (20%)	0.038
Overweight: 25-29.9	25 (17%)	5 (17%)	> 0.9
Obese: Over 30	31 (22%)	4 (13%)	0.5
HIV positive	59 (41%)	17 (55%)	0.2
On antiretroviral therapy ⁴	46 (78%)	15 (88%)	0.5
Undetectable viral load ⁵	39 (67%)	11 (65%)	0.8
CD4 count, median (IQR) ⁶	556 (360, 720)	598 (388, 927)	0.6
Diabetes mellitus ⁷	21 (15%)	3 (9.7%)	0.6
Previous TB ⁸	27 (20%)	11 (35%)	0.06
Tobacco use ⁹	19 (13%)	10 (32%)	0.01
Subjective wellness, EQ-5D-3L ¹⁰	98 (80, 100)	80 (70, 95)	0.006
Sputum Xpert Ultra MTB/RIF cycle threshold, median (IQR) ¹¹	28.2 (23.8, 29.7)	27.9 (23.4, 30.6)	0.7

¹n (%); Median (Q1, Q3). ²Univariate logistic regression. ³BMI n = 143, 30. ⁴Self-reported, HIV positive only n = 59, 17. ⁵VL < 40, HIV positive only. ⁶HIV positive only. ⁷HbA1c > 6.5 or on treatment for previously diagnosed diabetes. ⁸Self reported having previously been treated for TB (not including current episode), n = 135, 31, ⁹Self reported current use of cigarettes or tobacco products, n = 143, 31. ¹⁰Wellness index n = 131, 21. ¹¹Reported only in samples > trace positive, n = 62, 12. ¹² Liquid Culture time to positivity n = 81, 21. ¹³CXR Abnormality n = 137, 30

Characteristic	Asymptomatic TB ¹ (n = 143)	Symptomatic TB ¹ (n = 31)	p-value ²
Sputum Liquid Culture (MGIT) days to positivity, median ¹²	15.3 (12.6, 17.5)	16.4 (13.0, 18.2)	0.5
CXR abnormality score (CAD4TBv5), median IQR ¹³	63 (43, 76)	64 (54, 87)	0.6
¹ n (%); Median (Q1, Q3). ² Univariate logistic regression. ³ BMI n = 143, 30. ⁴ Self-reported, HIV positive only n = 59, 17. ⁵ VL < 40, HIV positive only. ⁶ HIV positive only. ⁷ HbA1c > 6.5 or on treatment for previously diagnosed diabetes. ⁸ Self reported having previously been treated for TB (not including current episode), n = 135, 31, ⁹ Self reported current use of cigarettes or tobacco products, n = 143, 31. ¹⁰ Wellness index n = 131, 21. ¹¹ Reported only in samples > trace positive, n = 62, 12. ¹² Liquid Culture time to positivity n = 81, 21. ¹³ CXR Abnormality n = 137, 30			

When people with clinic-diagnosed, asymptomatic community-detected and symptomatic community-detected TB were mapped according to the GIS coordinates of their homes (with random error introduced to protect confidentiality), all three groups were noted to reside throughout the demographic surveillance area (Fig. 1b) with asymptomatic community-detected TB clearly outnumbering both other groups. With regards to clinical measures that have been shown to impact infectiousness, people with asymptomatic TB had similar sputum bacterial load as people with symptomatic community-detected TB, whether measured by the Xpert Ultra MTB/RIF semi-quantitative grade (Fig. 1c) and qPCR cycle threshold (Table 2) or by the liquid Mtb culture time-to-positivity in days (Fig. 1d, Table 2). The presence of any lung field abnormality on digital chest x-rays was similar between people with symptomatic and asymptomatic community-detected TB (76% and 81% respectively, $p = 0.600$, Fig. 1e). Specific abnormalities reported were also similar between the two groups, including the presence of lung cavitation, which has been associated with infectiousness and was identified in 26% of people with asymptomatic TB and 20% of people with symptomatic TB (Fig. 1e, $p = 0.400$); the only exception to this was calcification which was less frequent in asymptomatic than symptomatic community-detected TB (0.7% vs 16%, $p = 0.003$). Severity of computer-assisted TB abnormality scores (CAD4TBv5) was similar (Fig. 1f, Table 2) in people with symptomatic and asymptomatic TB. These results showing similar measures of microbiologic and radiologic disease severity between asymptomatic and symptomatic community-detected TB were robust when sensitivity analyses using more two more stringent definitions of bacteriologically confirmed TB were performed (**Tables S2.1-2.3**).

Mtb isolates from asymptomatic TB demonstrate drug-resistance and are included in transmission clusters

Mtb was cultured from 106 sputum specimens (4 from people with clinic-diagnosed TB and 102 from people with community-detected TB), and 105 of these isolates were successfully sequenced revealing 102 isolates which resolved to a single sequence (Fig. 2a) and 3 mixed infections (**Table S3**, excluded

from subsequent analyses). Mtb isolates represented lineages 1 to 4 (Fig. 2a) and no lineage or sub-lineage was associated with asymptomatic TB or any of the clinical features associated with infectiousness (bacterial burden, cavity or radiologic severity, **Table S4**). Drug resistance mutations were present in 19% (20/105) of isolates across all 4 lineages (Fig. 2a) and ranged in severity from isoniazid mono-resistance to multi-drug resistance (MDR) and pre-extensive drug resistance (pre-XDR, Fig. 2b). Ninety percent (18/20) of drug-resistant isolates were cultured from people with asymptomatic TB including 82% (9/11) with MDR or pre-XDR TB. Phenotypic drug resistance testing was concordant with genotypic data; but sputum GeneXpert Ultra RIF/Mtb only identified rifampin resistance in 23% (3/13) individuals in whom rifampin resistance was present according to both genotypic/phenotypic analysis; consistent with the pauci-bacillary nature of many of the samples (**Figure S1**). No association was noted between lineage and presence of drug-resistance when considering phenotypic drug resistance (**Table S5**).

Whole genome assembly and clustering analysis using a conventionally accepted relatedness threshold of 12 single nucleotide polymorphisms^{24,25} revealed that 17/102 isolates clustered into eight small transmission clusters (a-h, Fig. 2a). Pair-wise comparison of clustered strains revealed a moderate correlation between geospatial and SNP distance (Fig. 2c). Mapping the clusters to the location of their homes (Fig. 2d), revealed that the members of only one cluster (1/8, 12.5% of the clusters) resided in the same household (cluster b), 37.5% (3/8) resided in the same neighborhood (< 2.5km, clusters a, c and d) and 50% (4/8) had no clear geospatial relationship (> 2.6km, clusters e, f, g and h). Notably, 50% of the clusters (4/8) included only asymptomatic community-detected TB (including one cluster of isoniazid-mono-resistant (cluster c) and one cluster of multi-drug resistant strains (cluster g), 25% (2/8) included only symptomatic community-detected TB, 12.5% (1/8) included individuals of mixed symptom status, and 12.5% (1/8) included only individuals with clinic-diagnosed TB. The largest asymptomatic cluster consisted of 3 adults who lived in separate but geographically proximal households and whose chest x-rays revealed a range of radiographic abnormalities (Fig. 2e).

While a minority of Mtb isolates fell within clusters defined by a 12 SNP genetic distance, visual inspection of the phylogenetic tree suggested that more closely related Mtb strains were isolated from patients with the same symptom status, suggesting a potential relationship between strain and symptomatology. Thus we formally tested the hypothesis that closely related strains are more likely to be isolated from patients with the same symptom status. To test for more recently evolved Mtb traits rather than lineage or sublineage effects, we focused on isolates that differed by fewer than 50 SNPs, where the *in vivo* mutation rate of Mtb is $\sim 0.24\text{--}0.5$ SNP/genome/year. For each isolate, we identified all isolates within a 50 SNP distance and calculated the frequency of symptom status match, which was then linearly weighted by genetic similarity; results for all isolates were aggregated into a mean match frequency. Statistical significance was measured by comparing observed mean match frequencies to those from 10,000 permutations of randomised symptom labels. Closely related strains were significantly more likely to come from individuals with the same symptom status (empirical p-value = 0.045, Fig. 1f). Because Mtb strains from people with symptomatic TB were underrepresented in the Vukuzazi dataset, we attempted to break asymptomatic clusters by incorporating a publicly available

dataset of 122 clinic-diagnosed strains from the nearby district hospital (Hlabisa Hospital) isolated during an overlapping time period (2016–2019)²⁶ which we assumed were from people who presented with symptoms because they were diagnosed with drug-resistant TB in a clinical setting (**Figure S2**). Again, however, strains isolated from asymptomatic patients were more frequently closely related to other asymptomatic isolates (empirical p-value = 0.025, Fig. 1f). These data suggest that there may be bacterial genetic determinants of symptom status, but this study was not sufficiently powered to conduct a genome-wide association study for causal variants and qualitative assessment of variants in asymptomatic strains did not identify a dominant signal.

Discussion

In a large community-based multi-modal survey in rural KwaZulu-Natal, South Africa, we found that community-detected asymptomatic TB accounted for the vast majority of prevalent bacteriologically-confirmed disease. Additionally, we found that microbiologic and radiographic features known to correlate with infectiousness, specifically bacterial burden and presence of lung cavity, were similar in asymptomatic and symptomatic community-detected TB. Finally, we found molecular and spatial evidence consistent with asymptomatic TB transmission in the community, including transmission of drug-resistant strains. Taken together these findings suggest that asymptomatic TB contributes to Mtb transmission and that improved approaches for its detection and treatment will be required to quell the epidemic.

We found that 82% of community-detected TB in a TB-endemic region of rural South Africa was asymptomatic. This very high proportion of asymptomatic TB exceeds levels that have been reported in most other settings^{27–31}. A meta-analysis of all TB prevalence surveys conducted in Africa and Asia since 2000 found that approximately half of all prevalent TB was asymptomatic and the contemporaneous South African National TB Prevalence survey also reported that just over half of prevalent TB was asymptomatic^{5,27}. Possible reasons for the higher proportion of asymptomatic TB in our setting include our primary definition of microbiologically-confirmed TB which included people with ‘trace’ amounts of Mtb DNA detected in their sputum; nonetheless, when more stringent definitions of microbiological confirmation were used in sensitivity analyses, the very high proportion of asymptomatic disease persisted. Another possible explanation is that participants under-reported their symptoms or the screening questions from the WHO’s 4 symptom screen (W4SS) were insensitive in detecting actual symptoms. Inconsistent asking and reporting of symptoms and the fluctuating nature of symptoms are well-recognized problems that have complicated definition of asymptomatic TB in individuals and across populations^{32,33}. Despite these complexities, in this study people who met the study definition of asymptomatic TB reported very high scores on an independently collected and validated quality of life and functional status tool; these scores were equivalent to people without any evidence of TB and higher than people with community-detected TB who reported symptoms, suggesting that the people defined in this study as asymptomatic did indeed feel well. The most likely explanation for the very high proportion of asymptomatic TB is that this study collected sputum from over half of the screened population, which

is much higher than is typically possible in prevalence surveys, which collect sputum from 8–20% of screened participants who are identified as high risk based on chest x-ray images suggestive of active TB. Three South African studies that have used universal sputum screening instead of chest x-ray triaged sputum collection have also shown very high rates of asymptomatic TB that are comparable to this study^{13,34,35}. Although this study used an intentionally low chest x-ray triaging score, nonetheless 40% of the population who were asymptomatic and had completely normal chest x-rays did not have sputum collected which may have caused us to miss cases of asymptomatic TB in people with normal chest x-rays and resulted in an underestimation of the true frequency of asymptomatic TB. Taken together, the findings of this study and those of other studies that have conducted universal sputum screening suggest that asymptomatic TB makes up a major proportion of prevalent TB, especially in high burden settings, with the true burden of disease likely underestimated by routine symptom-screen based or chest x-ray triaged surveillance methods.

Regardless of symptom status, we found that people with community-detected asymptomatic and symptomatic TB had similar patterns of bacterial burden and radiographic pathology relevant to transmission. Although we did not perform smear microscopy, which has been shown to correlate with bacterial burden and infectiousness, we did perform Xpert and analyze PCR cycle thresholds, which have been shown to correlate with smear status³⁶. Additionally, we performed mycobacterial culture which is the bacteriologic gold-standard for TB diagnosis and confirms the presence of viable organism in the sputum. People with asymptomatic and symptomatic community-detected TB had similar amounts of bacilli in their respiratory secretions, as measured by both Xpert PCR cycle thresholds and Mtb culture time-to-positivity, suggesting similar potential for generation of infectious aerosols. Independent of smear status, the presence of cavitation on chest radiography has been shown to be associated with increased infectiousness in studies that measure household transmission of Mtb infection^{17–19}. It is thought that people with cavitary lung disease are more likely to have Mtb infected lung tissue anatomically contiguous to bronchial airways and therefore more likely to transmit these organisms into exhaled or expectorated air. Our study found that over a quarter of people with asymptomatic TB had cavity on chest x-ray and that rates of cavitation were similar in people with asymptomatic and symptomatic TB detected through community screening. Although interpretation of radiographic features by radiologists are subjective and can have poor inter-reader correlation, our finding of radiologic similarity between asymptomatic and symptomatic community-detected TB was strengthened by use of artificial intelligence assisted radiologic algorithms which have been shown to perform similarly to human radiologists³⁷ and in this study found comparable severity of radiologic abnormalities suggestive of TB in both groups. These results coupled with recent evidence that tidal breathing may account for substantially more exhaled bacilli than coughing¹⁰ suggests that even in the absence of cough, people with asymptomatic TB likely contribute to the presence of viable Mtb in the air, and therefore, to transmission.

Ninety percent of drug-resistant Mtb strains identified in this study were cultured from asymptomatic individuals. Asymptomatic drug resistance ranged from isoniazid mono-resistance to multi-drug

resistance and pre-extensive drug resistance (pre-XDR). These results suggest that people living with asymptomatic TB may unknowingly harbor drug-resistant strains which go unrecognized and may be transmitted while they feel well and go about normal daily activities. Importantly, many of the rifampin-resistant Mtb strains in this study were not initially identified by GeneXpert Ultra, the first-line screening test which detects drug resistance by PCR, because they were pauci-bacillary and did not contain sufficient Mtb DNA for amplification of the *rpoB* target. This finding highlights the fact that more sensitive tools to detect drug-resistance will be required if asymptomatic TB is to be targeted. Our findings suggest that high rates of asymptomatic TB may increase the risk of drug resistance because lack of symptoms may lead to failure to identify active TB in people being assessed for initiation of TB preventative therapy (TPT) due to recent TB exposure or because they are living with HIV. Inadvertent treatment of asymptomatic TB with TPT drug regimens may risk incomplete treatment and increase the risk of acquired isoniazid resistance^{38,39}.

Using a SNP threshold of 12, we found that 65% (11/17) of clustered isolates were derived from people with asymptomatic TB, and half of the clusters (4/8) include only people with asymptomatic TB. We cannot exclude the existence of missing symptomatic members of these transmission clusters, but the data are consistent with the idea that asymptomatic TB itself may be responsible for transmission. This finding is also supported by prior work. For example, a study conducted in Valencia, Spain which integrated genomic and epidemiological data found that people who transmit TB were more likely to have done so before the reported date of symptom onset⁴⁰. Additional evidence that supports transmission from asymptomatic TB is a randomized controlled trial of community-based active case finding in Vietnam that showed a decrease in child Mtb infection rates in communities which had undergone sequential rounds of diagnosis and treatment of TB regardless of the symptom status⁴¹. Bezerra et al., also found evidence for likely transmission of asymptomatic TB in their recent study in which two-thirds of participants screened for TB in a Brazilian prison were asymptomatic and their contacts and contacts of symptomatic TB participants had similar increased risks of QuantiFERON TB test positivity^{42,43}. Additionally, a recent large molecular epidemiology study from China attributed 25–50% of transmission events to asymptomatic TB⁴⁴. Mapping of the transmission clusters identified in our study showed that most people with closely related strains did not live together, and instead resided in the same neighborhood or had no apparent geospatial connection other than residence within the AHRI demographic surveillance area. These data suggest that sharing air in community settings is a more important driver of transmission in this population than sharing air within the home. This finding is supported by molecular epidemiology, social contact and mathematical modeling studies which suggest that in high-burden settings more transmission occurs in public settings where people come into casual contact with each other than in households^{45–48}. The evidence presented in this study therefore contributes to a growing body of evidence that implicates asymptomatic tuberculosis in transmission events that occur outside of households in community settings.

Our data suggest that more closely related Mtb strains are significantly more likely to be isolated from individuals with the same symptom status suggesting potential mycobacterial genetic contributors to

clinical presentation. Natural selection on pathogens is driven by the need to optimize transmission and there are many solutions to this evolutionary challenge including the evolution of attenuated virulence enabling the infected host to remain in the population, and the emergence of diagnostic escape variants. It is possible that similar forces have acted on *Mtb*, perhaps in relative recent times, to select for strains causing less pronounced symptoms. Stringently testing this hypothesis requires larger sample sizes. Here we tried to increase sample size by incorporating publicly available data from Hlabisa hospital, but we could not fully control for difference in catchment area or recruitment period. Certainly, larger, well-annotated strain sets will be required for identification of bacterial genetic contributors via genome-wide association approaches.

This study has several limitations. Only 50% of the eligible population were enrolled and screened for TB; this may have introduced selection bias and resulted in incomplete transmission clusters. Sputum was not collected universally from all individuals enrolled, and only one sample was collected; these limitations mean that individuals with normal chest x-rays and asymptomatic TB or individuals with paucibacillary TB may have been missed. These limitations may have caused us to underestimate the prevalence of asymptomatic TB or to overestimate its microbiologic or radiologic severity. The inclusion of GeneXpert 'trace' sputum-positive individuals within the primary definition of microbiologically-confirmed TB may have been overly permissive, however sensitivity analyses excluding this group confirmed the results, and a recent longitudinal study of individuals with GeneXpert 'trace' sputum found that half progress to more severe disease within a year, suggesting that many of them do have an early form of asymptomatic TB^{49,50}. The fundamental unreliability of symptom screen data is an important limitation of this study. As previously discussed, it is difficult to know whether people who deny symptoms on the WHO4SS are truly free of symptoms, fail to recognize them perhaps due to their subtly or relapsing/remitting nature, or fail to disclose them due to factors related to the interviewer^{33,51}. Prior TB or tobacco use may make someone more likely to recognize symptoms and may result in misclassification of symptomatic and asymptomatic disease at the time of screening. Data was not collected on use of TB preventative therapy, therefore acquisition of resistance could not be linked to exposure to isoniazid mono-therapy. An additional limitation of the study was that people with clinic-diagnosed TB were identified during the survey and not at the time of their TB diagnosis, which limited our ability to compare their pre-treatment symptom status, subjective wellness score, bacterial burden and chest x-ray findings to the people with community-detected TB.

Despite these limitations, this study's strength is in its population-based design that includes very complete multimodal data on over 18,000 precisely defined participants. Together, these data suggest that asymptomatic TB represents a clinically and epidemiologically significant stage of disease, with implications for transmission, antimicrobial resistance and global TB elimination efforts. We found that most people with community-detected TB were asymptomatic and reported high health-related quality of life. People with asymptomatic TB had similar bacterial burden, radiological severity, and rates of cavitation as people with symptomatic community-detected TB suggesting that both groups were similarly infectious. *Mtb* strains isolated from people with asymptomatic TB clustered together, in some

cases implicating transmission among neighbors or community contacts and suggesting that there may be bacterial determinants of symptom status. These findings suggest that asymptomatic TB represents a poorly characterized and understood reservoir that may contribute to overall TB transmission and fuel drug-resistance in communities. Our results support the need to reconsider current diagnosis and screening paradigms and to expand strategies for earlier detection and treatment of asymptomatic TB to end the TB epidemic. These findings also have important implications for human and pre-clinical studies of TB pathogenesis, immunology and vaccine design.

Methods

Study design and participants

This analysis used data collected during the Vukuzazi study, a population-based cross sectional health surveillance study conducted between May 25, 2018 and March 13, 2020. Vukuzazi was based in the Africa Health Research Institute (AHRI) health and demographic surveillance system area (HDSS) located in rural uMkhanyakude district of KwaZulu-Natal, South Africa and utilised methods described elsewhere^{20,21,52}. Briefly, the surveillance region covered an area of 438km² and comprised 36 097 eligible participants aged ≥15 years. Eligible participants were invited to mobile health camps where multimorbidity screening, sample collection and consent for biobanking was conducted. All participants who provided written informed consent were included in this study.

TB screening protocol

Tuberculosis screening was based on the World Health Organization (WHO) TB prevalence surveys methodology⁵³ and screened for active pulmonary TB, without specific tests for extrapulmonary TB, as described elsewhere²¹. Briefly, TB screening included: TB symptom assessment, chest x-ray (CXR) evaluation, and single spot sputum collection for microbiological investigation. Symptom screening was based on the WHO four symptom screen tool (W4SS) which evaluated presence of cough, fever, night sweats, and weight loss. Digital CXRs were obtained on-site using a mobile unit (Canon CXDI-NE) and then uploaded uncompressed in DICOM format into cloud storage. Images were reviewed immediately on-site using the commercially available Computer Assisted Diagnosis for TB version 5 (CAD4TBv5) algorithm, which runs a black box algorithm that outputs numbers on a 0-100 scale. For this study, a value of ≥25 was used as the lowest-feasible threshold to indicate abnormal lung field as defined in a pilot testing phase²³. Chest-X-rays were also evaluated off-site within seven days by an experienced radiologist who assessed lung fields and reported on presence or absence of specific radiographic findings including; normal lung fields, lung fields suggestive of TB, presence of cavity, consolidation, nodules and/or fibrosis²³.

Anyone who reported TB symptoms, who was unable to undergo CXR due to pregnancy or physical disability, or who had any degree of lung field abnormality by CXR (i.e. CAD score of at least 25 or abnormality reported by radiologist) were triaged for a single spot sputum to be collected for

microbiological investigation. Most participants had sputum collected the same day – if triaged by CAD4TBv5 score or symptoms – but if the CAD4TBv5 score was <25 and subsequently the radiologist read showed abnormal lung fields, participants were followed up for sputum collection. Participants were coached to produce a deep sputum specimen with a minimum of 5ml of sputum advised for collection. However, if unable to produce sputum participants were encouraged to fill the cup with a combination of sputum and saliva. All specimens were processed in the laboratory regardless of volume or appearance.

Simultaneous to TB screening, participants underwent detailed characterization of their TB and HIV treatment history, HIV status (4th generation antibody/antigen test and if positive CD4 T cell count and HIV-1 viral load), diabetes status (glycosylated haemoglobin, HBA1c), height and weight, current tobacco use, and alcohol use. A validated quality of life questionnaire (EQ-5D-3L)⁵⁴ was added to the survey in November 2018.

Laboratory testing: Sputum samples were transported from the field to the BSL-3 laboratory in Durban at room temperature. Within 24-hours of collection, sputum samples were decontaminated using the NALC/NaOH method: N-Acetyl-L-Cysteine (NALC) is a mucolytic agent, and sodium hydroxide (NaOH) acts as a bactericidal/contaminant removal agent. After 15 minutes the sample was neutralised by a buffer solution and concentrated by refrigerated centrifugation, increasing recovery of mycobacteria. The sediment was then suspended in a 5mL phosphate buffer and used for molecular and culture techniques or stored for whole genome sequencing.

Laboratory testing: Xpert MTB/RIF Ultra

An Xpert MTB/RIF Ultra assay (Xpert; Cepheid, Sunnyvale, CA, USA), a nucleic acid amplification test, was performed on a decontaminated 600uL aliquot derived from all sputum specimens collected. Semi-quantitative results were reported as: Mtb not detected, trace detected, detected very low, detected low, detected medium, detected high, invalid, error or no result. Xpert cycle threshold (Ct) values were reported as the lowest Ct value for any *rpoB*³⁶²⁹. Xpert assays also assess genomic evidence of rifampicin resistance (*rpoB*) in samples in which Mtb DNA is detected at greater than trace levels; trace results indicate detection of the multicopy targets IS6110 and IS1081 but not *rpoB*, so trace samples are reported “indeterminate” for rifampin resistance.

Laboratory testing: MGIT liquid culture

Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson, Sparks, MD, USA) liquid culture for isolation of Mtb was performed on a decontaminated 500uL aliquot of all sputum samples specimens collected in the Vukuzazi study. 7mL MGIT 7H9 broth base tubes were prepared with growth supplement (oleic acid, albumin, dextrose and catalase) and antimicrobial supplement PANTA (polymixin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) before the sputum specimens were inoculated. MGIT cultures were retained for a period of six weeks (42 days) or until a positive culture was detected. Following a positive flag within 42 days, confirmatory tests were performed: a blood agar

plate for purity, Ziehl-Neelsen stain for acid-fast bacilli and morphology, and an Xpert MTB/RIF Ultra. On this basis, results were categorised as: Positive Mtb; Positive NTM; Culture contaminated or Negative.

Phenotypic drug resistance testing

During the Vukuzazi study all Mtb culture positive samples underwent phenotypic DST, using the proportion method on solid agar, for a standard panel of drugs including rifampicin, isoniazid, ethambutol, streptomycin and ofloxacin. In January 2021, after the study had ended, study isolates that had resistance to one or more drug were further tested for sensitivity to levofloxacin, moxifloxacin, bedaquiline and linezolid in accordance with updated WHO definitions for TB drug resistance⁵⁵. Resistance to isoniazid was defined as high level resistance (minimum inhibitory concentration, or MIC 1.0 µg/ml) and resistance to other drugs was defined according to WHO recommended MIC thresholds, as follows: Isoniazid 1.0 µg/ml, Rifampicin 1.0 µg/ml, Ethambutol 7.5 µg/ml, Streptomycin 2.0 µg/ml, Kanamycin 5.0 µg/ml, Ofloxacin 2.0 µg/ml, Levofloxacin 1.0 µg/ml, Moxifloxacin 0.5 µg/ml, Bedaquiline 1.0 µg/ml, Linezolid 1.0 µg/ml.

Whole genome sequencing

Frozen stored samples described above, were thawed and reinoculated onto 7H11 solid agar. Mycobacterial DNA was extracted from 7H11 by heat killing the culture, removal of the glycerol medium, bead beating and lysozyme addition, and finally AMPure XP bead (Beckman Coulter, Atlanta, Georgia) clean up. The concentration of DNA was determined using the Qubit dsDNA HS Assay kit (Invitrogen, Carlsbad, CA). Some cultures were re-cultured, and their DNA re-extracted up to three times to ensure the minimum required DNA yield, 20 ng/µL. Samples were diluted to 0.2ng/µl and the Nextera XT DNA Library preparation kit and the Nextera XT Index kit (Illumina, San Diego, CA) were used to prepare the libraries, according to the manufacturer's protocol. Indexed amplicons were purified using AMPure XP beads and fragment analysis was performed using the LabChip GX Touch (Perkin Elmer, Waltham, US). Each library was pooled and diluted to an equimolar concentration of 4nM followed by denaturation and dilution to the final loading concentration. The library was spiked with 10% PhiX which serves as an internal control to account for low diversity libraries and run on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using the MiSeq v3 600 cycle reagent kit.

TB outcome definitions

Based on the results of their Vukuzazi TB screening, participants were categorized according to the following definitions of TB outcomes:

Microbiologically confirmed TB

Mtb detected in sputum by either Xpert or culture. In addition to the primary definition, we defined two alternate, more stringent, case definitions for microbiologically confirmed TB: 1) culture positive and/or Xpert positive greater than "trace" on semi-quantitative grading (this alternative definition allowed

individuals with Xpert Ultra “trace” as their only microbiological evidence of Mtb to be excluded), and 2) culture positive regardless of Xpert result.

Community-detected TB

Participants who were not on TB treatment at the time of the survey and had microbiologically confirmed TB were defined as having community-detected TB.

Clinic-diagnosed TB

Participants who were on TB treatment at the time of the survey were defined as having clinic-diagnosed TB. This included anyone who reported taking TB treatment, regardless of microbiological confirmation or clinical documentation, and made no distinction between pulmonary or extrapulmonary TB.

Prevalent TB

Prevalent TB included clinic-diagnosed and community-detected TB.

No TB

All other people surveyed who had neither clinic-diagnosed nor community-detected TB were considered to be without evidence of TB, regardless of their radiological findings.

Asymptomatic and symptomatic TB

Among people with community-detected microbiologically confirmed TB, people who experienced one or more W4SS were defined as symptomatic and those who answered no to all W4SS questions were defined as asymptomatic.

Prior TB

Previous TB was defined as reporting a history of receiving TB treatment. This was not confirmed with any documentation, and no distinction was made between treatment for pulmonary or extrapulmonary TB.

Statistical analysis

We first compared demographic and clinical characteristics of those with clinically diagnosed and community diagnosed TB comparing them to those without TB using univariate logistic regression. Univariate logistic regression was also used to compare variables from asymptomatic and symptomatic community diagnosed individuals with TB. To test the robustness of the findings we conducted two sensitivity analyses, one excluding GeneXpert trace results, and the second including only those with culture positive TB. All statistical tests were two-sided, with the threshold for statistical significance set at $\alpha = 0.05$. Due to the low proportion of missingness we used complete case analysis for all comparisons. All analyses were performed using R version 4.5.2 (R Core Team, 2025).

Bioinformatics analysis: whole genome assembly

Whole genome assembly was performed using MTBseq⁵⁶, an automated pipeline for reference-based whole genome assembly and variant calling from Illumina high throughput sequencing data of MTBC isolates was used to perform quality control assessments of the raw input data, produce a reference-based consensus whole genome sequences (reference was H37Rv) and calculate the SNP distance matrix.

The default median coverage of 30 was considered adequate for whole genome assembly and annotation. Where necessary isolates were re-cultured, re-extracted and re-sequenced to achieve the desired coverage. FASTQ files for all multiply-sequenced isolates were concatenated for analysis.

Bioinformatics analysis: drug resistance and lineage annotation

TB Profiler⁵⁷ (version 4.4.2 in combination with the WHO 2021 catalogue (tbdb version c2fb9a2) was used to process high throughput sequencing data to predict lineage, sub-lineage and mutations associated with drug resistance. Trimmomatic⁵⁸ was used for automated quality control, BWA⁵⁹ to align to the reference genome (H37Rv), freebayes (or GATKv4/bcftools) to call single nucleotide variants and delly⁶⁰ to call larger deletions.

Bioinformatics analysis: phylogeny

Using the aligned FASTA output from MTBseq, we generated a maximum likelihood phylogenetic tree with IQTree2⁶¹. IQTree2 implements ModelFinder to find the best evolutionary model assumptions⁶², and uses tree search and ultra-fast bootstrapping to produce a phylogenetic tree file. Interactive Tree of Life (IToL) was used to visualise phylogenetic trees which were annotated with files from TB Profiler. Mixed infections were excluded from the phylogeny.

Anonymized maps

Maps were generated with QGIS, a free and open-source geographical information systems (GIS) software application. People with TB were marked on maps using GPS coordinates of their homes with random variation introduced and a circle of large enough diameter to obscure identity and protect privacy. Major and minor roads were annotated on a basemap sourced from Google Maps.

Symptom status match analysis

To calculate the observed match statistic, we took the mean match statistic for all isolates on the tree. Isolate match statistics were calculated by counting the fraction of isolates matching the index isolate's symptom status weighted by their SNP distance from the index isolate; match fractions were calculated for each asymptomatic isolate (unidirectional analysis—study and Hlabisa strains) or all isolates (bidirectional analysis—study strains) in the tree and consolidated into the mean observed match frequency for the tree. Weights were calculated by a linear decay with maximum weight at 0 distance

and no weight at >50 distance. SNP distances for matching analysis were calculated by calling variants with breseq for this study, according to previously published work⁶³, and baseline patient samples collected from Hlabisa Hospital from PRJNA559528²⁶. To model the null distribution, symptom labels were randomly permuted (n=10,000) and the observed match statistic was recalculated as above. The empirical p-value was calculated as the number of permutations greater than the observed statistic divided by the total number of permutations.

Declarations

Ethics

The University of KwaZulu-Natal Biomedical Research Ethics Committee, the London School of Hygiene & Tropical Medicine Ethics Committee, the Partners Institutional Review Board approved the Vukuzazi study protocol, from which all data and samples for this study were derived. All participants provided informed consent at mobile clinic camps.

Data sharing

All raw next-generation sequencing (NGS) data generated and analysed in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number SRP677838. These data are publicly available and can be accessed via the NCBI SRA portal (<https://www.ncbi.nlm.nih.gov/sra>). All corresponding BioSample accession numbers (**Table S6**) and Sequence read archive accession (SRA) numbers are included in the supplementary material (**Table S7**). A total of 106 BioSample accession numbers are reported, additionally a total of 160 SRA accessions are reported. This discrepancy is reflective of multiple sequencing runs performed for selected samples where improved sequencing depth or coverage was required.

Other data and related documents, including the study protocol, informed consent forms, de-identified participant data, and a data dictionary defining each field, can be accessed via the Africa Health Research Institute Data Repository (please email RDMServiceDesk@ahri.org) after publication, upon approval of the proposed analyses by the Vukuzazi Scientific Steering Committee and completion of a data access agreement.

Funding statement

The work reported herein was made possible through funding by the South African Medical Research Council through its Division of Research Capacity Development under the Bongani Mayosi National Health Scholars Programme from funding received from the Public Health Enhancement Fund / South African National Department of Health. The content hereof is the sole responsibility of the authors and does not necessarily represent the official views of the SAMRC.

Additional funders included, Bill & Melinda Gates Foundation (INV-070251), the South African Department of Science and Innovation, South African Population Research Infrastructure Network, the Burroughs Wellcome Pathogenesis of Infectious Disease Fellowship (1022002). This research was funded in part by Wellcome Strategic Core award: [227167/A/23/Z].

The funders of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

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Contributors

YM and US contributed equally to data analysis, data visualization and interpretation, article writing, review and editing of this manuscript. YM and EBW conceptualized the project, designed the study and the analysis. EBW, TN, MS, KH, WH, AG, SO, SM, TS and the Vukuzazi Team conducted the survey. SM, KB, TS, JG, TdO conducted laboratory experiments and testing. YM, EBW, SO, US, PK, NM, SEJ, JL, AD, RW, PC, CF, SN analysed the data. YM, EBW, US, PK, RL, SN, SO, PC, HC, LM, AR performed data visualization and interpretation. YM, US and EBW wrote the article. All authors reviewed and edited the manuscript, had access to all data reported in this article and had final responsibility for the decision to submit for publication.

Declaration of interest

We declare no competing interests.

Acknowledgements

The authors thank the members of the community and study participants in the Vukuzazi study as well as the entire team that conducted the study (listed in the Supplementary Materials).

References

1. World Health Organization. Global tuberculosis report 2025. (Geneva, 2025).
2. Esmail, H., Macpherson, L., Coussens, A. K. & Houben, R. Mind the gap - Managing tuberculosis across the disease spectrum. *EBioMedicine* 78, 103928 (2022).
<https://doi.org/10.1016/j.ebiom.2022.103928>
3. Teo, A. K. J., MacLean, E. L. & Fox, G. J. Subclinical tuberculosis: a meta-analysis of prevalence and scoping review of definitions, prevalence and clinical characteristics. *Eur Respir Rev* 33 (2024).
<https://doi.org/10.1183/16000617.0208-2023>
4. Stuck, L. *et al.* Prevalence of subclinical pulmonary tuberculosis in adults in community settings: an individual participant data meta-analysis. *Lancet Infect Dis* 24, 726–736 (2024).

[https://doi.org/10.1016/S1473-3099\(24\)00011-2](https://doi.org/10.1016/S1473-3099(24)00011-2)

5. Frascella, B. et al. Subclinical Tuberculosis Disease-A Review and Analysis of Prevalence Surveys to Inform Definitions, Burden, Associations, and Screening Methodology. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 73, e830–e841 (2021).
<https://doi.org/10.1093/cid/ciaa1402>
6. Nguyen, H. V., Tiemersma, E., Nguyen, N. V., Nguyen, H. B. & Cobelens, F. Disease Transmission by Patients With Subclinical Tuberculosis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 76, 2000–2006 (2023). <https://doi.org/10.1093/cid/ciad027>
7. Coussens, A. K. et al. Classification of early tuberculosis states to guide research for improved care and prevention: an international Delphi consensus exercise. *Lancet Respir Med* 12, 484–498 (2024).
[https://doi.org/10.1016/S2213-2600\(24\)00028-6](https://doi.org/10.1016/S2213-2600(24)00028-6)
8. Kendall, E. A., Shrestha, S. & Dowdy, D. W. The Epidemiological Importance of Subclinical Tuberculosis. A Critical Reappraisal. *Am J Respir Crit Care Med* 203, 168–174 (2021).
<https://doi.org/10.1164/rccm.202006-2394PP>
9. Kranzer, K. et al. The benefits to communities and individuals of screening for active tuberculosis disease: a systematic review. *The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease* 17, 432–446 (2013).
<https://doi.org/10.5588/ijtld.12.0743>
10. Dinkele, R. et al. Aerosolization of Mycobacterium tuberculosis by Tidal Breathing. *Am J Respir Crit Care Med* 206, 206–216 (2022). <https://doi.org/10.1164/rccm.202110-2378OC>
11. Emery, J. C. et al. Estimating the contribution of subclinical tuberculosis disease to transmission: An individual patient data analysis from prevalence surveys. *eLife* 12 (2023).
<https://doi.org/10.7554/eLife.82469>
12. Ryckman, T. S., Dowdy, D. W. & Kendall, E. A. Infectious and Clinical Tuberculosis Trajectories: Bayesian modeling with case finding implications. 2022.2006.2027.22276965 (2022).
<https://doi.org/10.1101/2022.06.27.22276965> medRxiv
13. Mendelsohn, S. C. et al. Screening for asymptomatic tuberculosis among adults with household exposure to pulmonary tuberculosis: a prospective observational cohort study. *Lancet Glob Health* 13, e1869–e1879 (2025). [https://doi.org/10.1016/S2214-109X\(25\)00276-1](https://doi.org/10.1016/S2214-109X(25)00276-1)
14. MacPherson, P. et al. Community-based active-case finding for tuberculosis: navigating a complex minefield. *BMC Glob Public Health* 2, 9 (2024). <https://doi.org/10.1186/s44263-024-00042-9>
15. World Health Organization. Asymptomatic tuberculosis and implications for programmatic action. (2024).
16. Organization, W. H. Report of the WHO consultation on asymptomatic tuberculosis, Geneva, Switzerland, 14–15 October 2024. (Geneva, 2025).
17. Long, R., Divangahi, M. & Schwartzman, K. Chapter 2: Transmission and pathogenesis of tuberculosis. *Canadian Journal of Respiratory, Critical Care, and Sleep Medicine* 6, 22–32 (2022).
<https://doi.org/10.1080/24745332.2022.2035540>

18. Urbanowski, M. E., Ordonez, A. A., Ruiz-Bedoya, C. A., Jain, S. K. & Bishai, W. R. Cavitary tuberculosis: the gateway of disease transmission. *Lancet Infect Dis* 20, e117–e128 (2020).
[https://doi.org/10.1016/S1473-3099\(20\)30148-1](https://doi.org/10.1016/S1473-3099(20)30148-1)
19. Melsew, Y. A. et al. Risk factors for infectiousness of patients with tuberculosis: a systematic review and meta-analysis. *Epidemiol Infect* 146, 345–353 (2018).
<https://doi.org/10.1017/S0950268817003041>
20. Wong, E. B. et al. Convergence of infectious and non-communicable disease epidemics in rural South Africa: a cross-sectional, population-based multimorbidity study. *Lancet Glob Health* 9, e967–e976 (2021). [https://doi.org/10.1016/S2214-109X\(21\)00176-5](https://doi.org/10.1016/S2214-109X(21)00176-5)
21. Gunda, R. et al. Cohort Profile: The Vukuzazi ('Wake Up and Know Yourself' in isiZulu) population science programme. *International journal of epidemiology* 51, e131–e142 (2022).
<https://doi.org/10.1093/ije/dyab229>
22. Hamada, Y., Lujan, J., Schenkel, K., Ford, N. & Getahun, H. Sensitivity and specificity of WHO's recommended four-symptom screening rule for tuberculosis in people living with HIV: a systematic review and meta-analysis. *Lancet HIV* 5, e515–e523 (2018). [https://doi.org/10.1016/S2352-3018\(18\)30137-1](https://doi.org/10.1016/S2352-3018(18)30137-1)
23. Fehr, J. et al. Computer-aided interpretation of chest radiography reveals the spectrum of tuberculosis in rural South Africa. *NPJ Digit Med* 4, 106 (2021). <https://doi.org/10.1038/s41746-021-00471-y>
24. Xiao, Y. X., Chan, T. H., Liu, K. H. & Jou, R. Define SNP thresholds for delineation of tuberculosis transmissions using whole-genome sequencing. *Microbiol Spectr* 12, e0041824 (2024).
<https://doi.org/10.1128/spectrum.00418-24>
25. Van der Roest, B. R. et al. Phylodynamic assessment of SNP distances from whole genome sequencing for determining Mycobacterium tuberculosis transmission. *Scientific reports* 15, 10694 (2025). <https://doi.org/10.1038/s41598-025-94646-2>
26. Nimmo, C. et al. Dynamics of within-host Mycobacterium tuberculosis diversity and heteroresistance during treatment. *EBioMedicine* 55, 102747 (2020).
<https://doi.org/10.1016/j.ebiom.2020.102747>
27. Moyo, S. et al. Prevalence of bacteriologically confirmed pulmonary tuberculosis in South Africa, 2017–19: a multistage, cluster-based, cross-sectional survey. *Lancet Infect Dis* 22, 1172–1180 (2022). [https://doi.org/10.1016/S1473-3099\(22\)00149-9](https://doi.org/10.1016/S1473-3099(22)00149-9)
28. Nguyen, H. V. et al. The second national tuberculosis prevalence survey in Vietnam. *PloS one* 15, e0232142 (2020). <https://doi.org/10.1371/journal.pone.0232142>
29. Lansang, M. A. D. et al. High TB burden and low notification rates in the Philippines: The 2016 national TB prevalence survey. *PloS one* 16, e0252240 (2021).
<https://doi.org/10.1371/journal.pone.0252240>
30. Aung, S. T., Nyunt, W. W., Moe, M. M., Aung, H. L. & Lwin, T. The fourth national tuberculosis prevalence survey in Myanmar. *PLOS Glob Public Health* 2, e0000588 (2022).

<https://doi.org/10.1371/journal.pgph.0000588>

31. Kapata, N. et al. The Prevalence of Tuberculosis in Zambia: Results from the First National TB Prevalence Survey, 2013–2014. *PloS one* 11, e0146392 (2016).
<https://doi.org/10.1371/journal.pone.0146392>
32. Stuck, L. et al. How "Subclinical" is Subclinical Tuberculosis? An Analysis of National Prevalence Survey Data from Zambia. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 75, 842–848 (2022). <https://doi.org/10.1093/cid/ciab1050>
33. McCreesh, N. et al. Reported Tuberculosis Symptoms: An Inadequate Classifier of Disease State. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 82, e589–e594 (2026). <https://doi.org/10.1093/cid/ciaf611>
34. Mendelsohn, S. C. et al. Validation of a host blood transcriptomic biomarker for pulmonary tuberculosis in people living with HIV: a prospective diagnostic and prognostic accuracy study. *Lancet Glob Health* 9, e841–e853 (2021). [https://doi.org/10.1016/S2214-109X\(21\)00045-0](https://doi.org/10.1016/S2214-109X(21)00045-0)
35. Scriba, T. J. et al. Biomarker-guided tuberculosis preventive therapy (CORTIS): a randomised controlled trial. *Lancet Infect Dis* 21, 354–365 (2021). [https://doi.org/10.1016/S1473-3099\(20\)30914-2](https://doi.org/10.1016/S1473-3099(20)30914-2)
36. Martin-Higuera, M. C., Rivas, G., Rolo, M., Munoz-Gallego, I. & Lopez-Roa, P. Xpert MTB/RIF Ultra CT value provides a rapid measure of sputum bacillary burden and predicts smear status in patients with pulmonary tuberculosis. *Scientific reports* 13, 1591 (2023). <https://doi.org/10.1038/s41598-023-28869-6>
37. Qin, Z. Z. et al. Tuberculosis detection from chest x-rays for triaging in a high tuberculosis-burden setting: an evaluation of five artificial intelligence algorithms. *Lancet Digit Health* 3, e543–e554 (2021). [https://doi.org/10.1016/S2589-7500\(21\)00116-3](https://doi.org/10.1016/S2589-7500(21)00116-3)
38. Li, T. L. et al. Acquired Resistance to Isoniazid During Isoniazid Monotherapy in a Subject with Latent Infection Following Household Rifampicin-Resistant Tuberculosis Contact: A Case Report. *Infect Drug Resist* 14, 1505–1509 (2021). <https://doi.org/10.2147/IDR.S304799>
39. Kendall, E. A. et al. Isoniazid or rifampicin preventive therapy with and without screening for subclinical TB: a modeling analysis. *BMC Med* 19, 315 (2021). <https://doi.org/10.1186/s12916-021-02189-w>
40. Xu, Y. et al. High-resolution mapping of tuberculosis transmission: Whole genome sequencing and phylogenetic modelling of a cohort from Valencia Region, Spain. *PLoS medicine* 16, e1002961 (2019). <https://doi.org/10.1371/journal.pmed.1002961>
41. Marks, G. B. et al. Community-wide screening for tuberculosis in a high-prevalence setting. 381, 1347–1357 (2019).
42. Bezerra, W. et al. Role of tuberculosis symptoms in transmission risk to cell contacts in prisons. *BMJ Public Health* 3, e003218 (2025). <https://doi.org/10.1136/bmjph-2025-003218>
43. Da Silva, K. E. et al. Comparison of phylogenetic metrics of transmission in symptomatic and asymptomatic tuberculosis. *medRxiv* (2025). <https://doi.org/10.1101/2025.08.27.25334397>

44. Li, M., Cai, L. & Gao, Q. Estimating the contribution to transmission of asymptomatic tuberculosis from population-based genomic epidemiology studies. *Emerg Microbes Infect* 15, 2640282 (2026). <https://doi.org/10.1080/22221751.2026.2640282>
45. McCreesh, N. & White, R. G. An explanation for the low proportion of tuberculosis that results from transmission between household and known social contacts. *Scientific reports* 8, 5382 (2018). <https://doi.org/10.1038/s41598-018-23797-2>
46. Koesoemadinata, R. C. et al. Should neighbours of tuberculosis (TB) cases be prioritised for active case finding in high TB-burden settings? A prospective molecular epidemiological study. *BMJ Glob Health* 10 (2025). <https://doi.org/10.1136/bmjgh-2025-019137>
47. Martinez, L. et al. Transmission of Mycobacterium Tuberculosis in Households and the Community: A Systematic Review and Meta-Analysis. *American journal of epidemiology* 185, 1327–1339 (2017). <https://doi.org/10.1093/aje/kwx025>
48. Auld, S. C. et al. Extensively drug-resistant tuberculosis in South Africa: genomic evidence supporting transmission in communities. *The European respiratory journal* 52 (2018). <https://doi.org/10.1183/13993003.00246-2018>
49. Sung, J. et al. Long-term risk of tuberculosis among individuals with Xpert Ultra trace screening results in Uganda: a longitudinal follow-up study. *Lancet Infect Dis* 26, 203–212 (2026). [https://doi.org/10.1016/S1473-3099\(25\)00536-5](https://doi.org/10.1016/S1473-3099(25)00536-5)
50. Dalmat, R. R. et al. Cumulative TB disease burden following sputum Xpert Ultra 'Trace' results in clinical settings: Results from a multi-site observational clinical study. *medRxiv* (2025). <https://doi.org/10.1101/2025.07.14.25331384>
51. Teo, A. K. J. et al. The relationship between a known diagnosis of tuberculosis and symptom reporting: implications for case detection strategies. *The European respiratory journal* 66 (2025). <https://doi.org/10.1183/13993003.02521-2024>
52. Gareta, D. et al. Cohort Profile Update: Africa Centre Demographic Information System (ACDIS) and population-based HIV survey. *International journal of epidemiology* 50, 33–34 (2021). <https://doi.org/10.1093/ije/dyaa264>
53. World Health Organization. Tuberculosis PREVALENCE SURVEYS: a handbook. (Geneva, 2011).
54. Rabin, R. & de Charro, F. EQ-5D: a measure of health status from the EuroQol Group. *Ann Med* 33, 337–343 (2001). <https://doi.org/10.3109/07853890109002087>
55. World Health Organization. Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis, 27–29 October 2020. (Geneva, 2021).
56. Kohl, T. A. et al. MTBseq: a comprehensive pipeline for whole genome sequence analysis of Mycobacterium tuberculosis complex isolates. *PeerJ* 6, e5895 (2018). <https://doi.org/10.7717/peerj.5895>
57. Verboven, L., Phelan, J., Heupink, T. H. & Van Rie, A. TBProfiler for automated calling of the association with drug resistance of variants in Mycobacterium tuberculosis. *PloS one* 17, e0279644 (2022). <https://doi.org/10.1371/journal.pone.0279644>

58. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120 (2014). <https://doi.org/10.1093/bioinformatics/btu170>
59. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760 (2009). <https://doi.org/10.1093/bioinformatics/btp324>
60. Rausch, T. *et al.* DELLY: structural variant discovery by integrated paired-end and split-read analysis. *Bioinformatics* 28, i333–i339 (2012). <https://doi.org/10.1093/bioinformatics/bts378>
61. Minh, B. Q. *et al.* IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol Biol Evol* 37, 1530–1534 (2020). <https://doi.org/10.1093/molbev/msaa015>
62. Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. & Jermiin, L. S. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 14, 587–589 (2017). <https://doi.org/10.1038/nmeth.4285>
63. Culviner, P. H. *et al.* Evolution of Mycobacterium tuberculosis transcription regulation is associated with increased transmission and drug resistance. *Cell* 188, 6620–6635 e6614 (2025). <https://doi.org/10.1016/j.cell.2025.09.005>

Figures

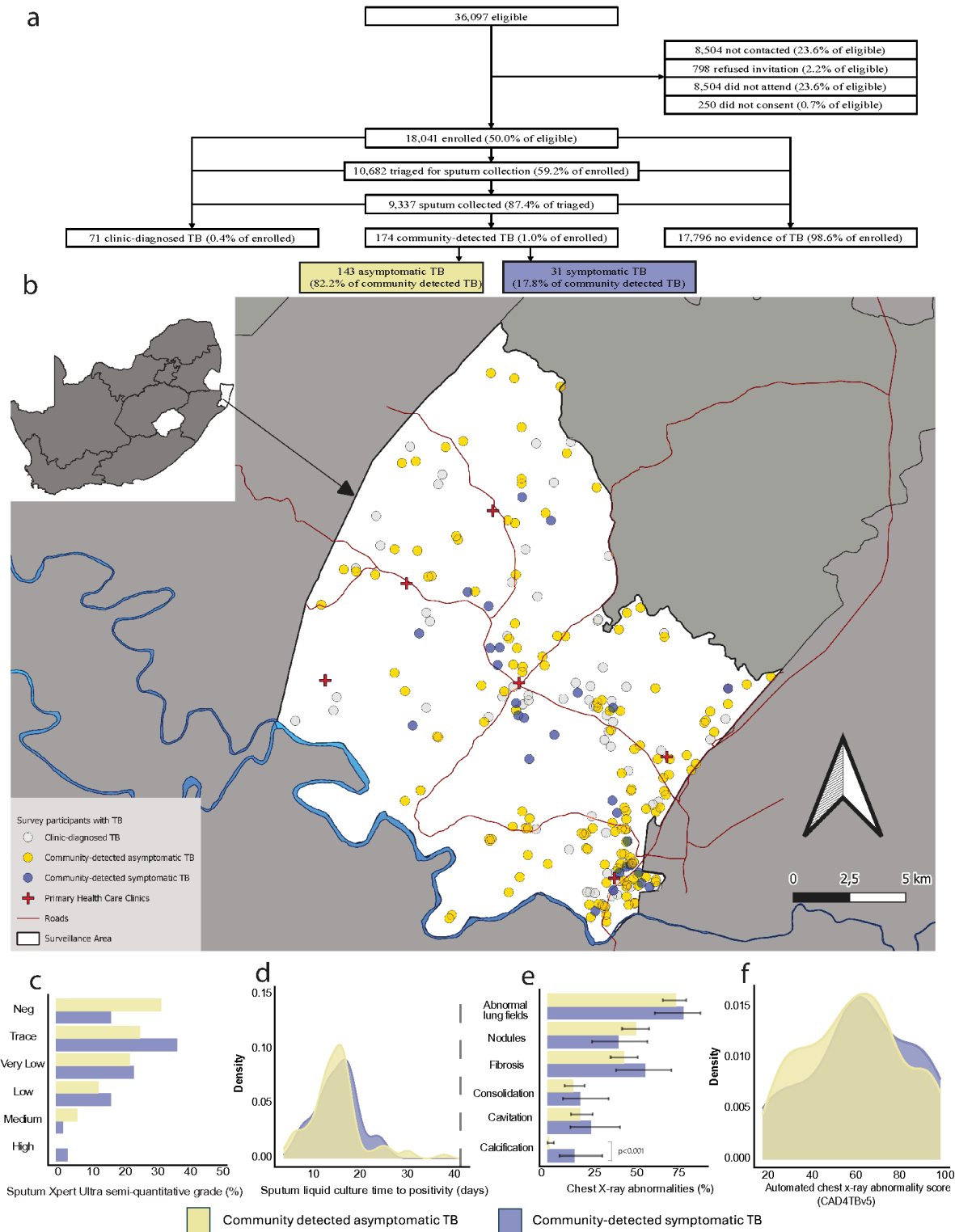


Figure 1

Asymptomatic community-detected tuberculosis is widespread and has microbiological and radiological features consistent with infectiousness. (a) Flow diagram of participation in population-based TB screening activities between May 2018 and March 2020. (b) Spatial distribution of individuals with clinic-diagnosed TB (grey), asymptomatic community-detected TB (yellow) and symptomatic community-detected TB (blue) across the AHRI demographic surveillance area in Northern KwaZulu-

Natal, South Africa; GPS coordinates are randomly displaced to preserve confidentiality. (c) Sputum Xpert MTB/RIF Ultra semi-quantitative grades for asymptomatic (yellow) and symptomatic (blue) community-detected TB. (d) sputum Mycobacterial Growth Indicator Tube (MGIT) liquid culture time-to-positivity for asymptomatic and symptomatic community-detected TB, limited to culture positive samples. (e) Chest radiograph features and (f) Computer-aided TB diagnostic scores (CAD4TBv5) for asymptomatic and symptomatic community-detected TB, limited to individuals with digital chest x-ray results. **For all data in c-f** pairwise comparisons were performed between asymptomatic and symptomatic TB, only significant p values are shown.

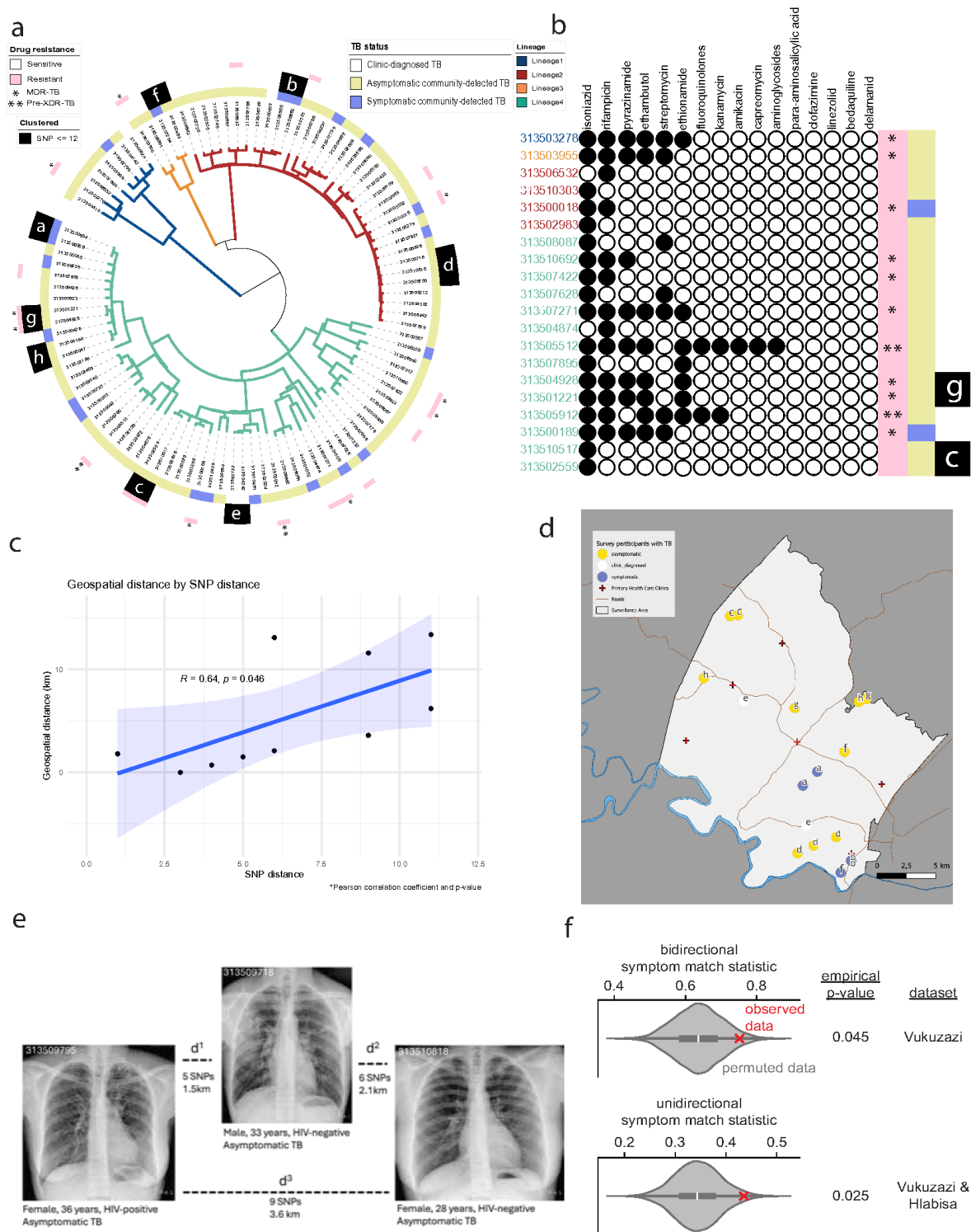


Figure 2

Genomic diversity, drug resistance, and transmission clustering of *Mycobacterium tuberculosis* isolates from asymptomatic tuberculosis. (a) Maximum-likelihood phylogenetic tree of 102 single-strain *Mycobacterium tuberculosis* isolates recovered from community-detected and clinic-diagnosed TB, annotated by lineage (branch color, 1 blue, 2 red, 3 orange, 4 green), symptom status (asymptomatic yellow, symptomatic blue), genomic transmission clusters (based on a ≤ 12 -SNP relatedness threshold,

black) and genotypic drug resistance (resistant strains pink). (b) Drug-resistance identified by genotypic testing (black circles denote drug-resistance; white denote drug-sensitivity). (c) Relationship between single nucleotide polymorphism (SNP) and geographic distance for clustered isolates. (d) Spatial mapping of genomic transmission clusters (a–h). (e) Demographic, clinical and chest x-ray images of individuals in the largest genomic cluster, cluster d; dotted lines denote SNP and geographic distances between each individual. (f) Nearest-neighbour symptom-status association analysis showing that in observed data closely related *Mycobacterium tuberculosis* strains more frequently match in symptom status (red X) than for randomly permuted symptom labels (grey distribution) within the Vukuzazi dataset (top) and when additional clinic-diagnosed TB isolates are included (bottom).

Supplementary Files

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