



OPEN Temporal and regional dynamics of *Mycobacterium tuberculosis* in Oman

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Tuberculosis (TB) remains endemic in Oman, with incidence exceeding 5 per 10⁵ population since 2016 and a rising proportion of cases among expatriates (>40% of the population). This study investigated the spatiotemporal diversity and structure of *Mycobacterium tuberculosis* (MTB) in Oman between 2008 and 2022. A total of 735 MTB isolates from nationals and expatriates across provinces were genotyped by spoligotyping and 17-locus MIRU-VNTR; 96 underwent whole-genome sequencing (WGS). Genetic diversity (expected heterozygosity, h) and differentiation (F_{ST}) were estimated, and phylogenetic analyses performed. Four major lineages and seven sub-lineages were identified, with similar distributions between nationals and expatriates but temporal variation in spoligotype clades. MIRU-VNTR showed high allelic diversity, with most dominant alleles (76.5%) stable over time, though 32.5% fluctuated. F_{ST} values (<0.05) indicated minimal temporal or local differentiation, except for slightly higher values between Dhofar and other regions. WGS revealed close genetic relatedness between Omani and expatriate isolates and strains from expatriates' countries of origin, suggesting recent cross-border transmission. MTB in Oman exhibits high genetic diversity with a stable temporal structure. Shared lineages between nationals and expatriates underscore the role of migration, supporting the need for integrated genomic surveillance.

Keywords *Mycobacterium tuberculosis*, Oman, TB, MIRU-VNTR, Genetic diversity

Tuberculosis (TB) is an ancient human disease and remains a global health issue, with its burden ranging from > 500 to < 5 cases per 10⁵ people. In 2021, there were an estimated 10.6 million TB cases and 1.6 million deaths¹. *Mycobacterium tuberculosis* (MTB), the causative agent, is believed to have co-evolved with humans over centuries². Molecular studies have shown that MTB is structured into distinct lineages, each with varying geographic specificity, revealing the potential origins of a strain's ancestry³. This geographic connection is particularly important in low-incidence countries such as The Gulf Cooperation Council (GCC) countries, where many TB cases are imported, reflecting how human migration influenced MTB's genetic diversification⁴.

The five GCC countries are well positioned to pursue TB eradication as three of them (Oman, UAE and Saudi Arabia) belong to the low incidence category of < 10 per 10⁵ population^{5,6}. Resurgence, however, was observed in recent years in Qatar (34 cases per 10⁵ population) and Kuwait (19 cases per 10⁵ population), and in Oman the incidence hovers below 10 cases per 10⁵ population exceeding the national control target of less than 1 case per 10⁵ population^{7,8}. There is a trend of increasing prevalence of drug-resistant TB among patients with comorbid

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conditions such as renal failure and HIV, emphasizing the vulnerability of immunocompromised individuals⁹. The above reflects hidden obstacles to the prospect of TB elimination in the region¹⁰. The persistence of TB transmission, in the GCC countries, has been attributed to the common demographic feature of a large proportion of expatriates, from high TB burden countries; comprising > 40% of the total population. The majority of them are often latently infected with TB (LTB), representing a potential source of transmission^{7,11}.

The above hypothesis is supported by the observation of studies that revealed a similar prevalence among non-nationals in different GCC countries^{9,12}. Additional supportive data includes the highly diverse MTB lineages in the GCC countries, and shared genotypes between nationals and expatriates (clusters)^{8,13,14}. These findings indicate a high influx of MTB strains and potential transmission between groups. Similar studies in other regions suggest that human migration drives the diversification of local MTB strains into distinct lineages. Consequently, changes in the expatriate population may lead to shifts in the distribution of MTB genotypes over time.

The proportion of expatriates in Oman increased steadily between 2008 and 2018, driven by economic growth and labour demand (Fig. 1). Large groups of Asian workers primary, Indian, Bangladeshi, Pakistani, and Sri Lankan, often live overcrowded, occupational-based residential clusters¹⁵, representing high risk for TB transmission. The influx of workers from high TB burden countries drives sustained importation of diverse MTB strains, potentially reintroducing transmission chains. Expatriates may carry distinct genetic lineages, including drug-resistant strains uncommon in Oman.

Our previous WGS analysis of isolates from Omanis and expatriates provided evidence for cross-transmission between the two groups, though at a limited rate¹⁴. This study expands on those findings, conducting a more comprehensive genetic analysis to assess the impact of expatriates on spatiotemporal dynamics on MTB population structure. Such insights are essential for TB control in low-incidence settings like Oman, where a significant share of cases is imported¹².

Materials and methods

MTB isolates

The present study examined 735 MTB isolates collected from Omanis ($n=406$) and expatriates ($n=329$), between 2008 and 2018, from patients in different provinces, in Oman, and processed in the Central Public Health Laboratories (CPHL), Ministry of Health of Oman, which serves as the national center for diagnosis of TB.

During the study period Oman population expanded from 2,867,428 in 2008 to 4,601,706 in 2018 (NCSI, 2024). The proportion of expatriates saw a steady increase between 2008 and 2014 (from 31.4% to 43.7%) ($P < 0.001$, Spearman's rho 0.897). However, it remained stable between 2014 and 2018, hovering around 42–45% (Fig. 1).

Ethical approval for the study was granted by the Medical Research and Ethics Committee (MREC) of The College of Medicine and Health Sciences, Sultan Qaboos University, Oman, under the reference SQU-EC/075/18.

MTB isolates used in the study were obtained from the CPHL. Consent for obtaining and processing the samples was obtained from all subjects and/or their legal guardian(s). The study was conducted in accordance with the ethical oversight and regulatory framework governing the use of anonymized diagnostic samples for research.

All laboratory works were conducted in Oman, adhering to relevant guidelines and regulations.

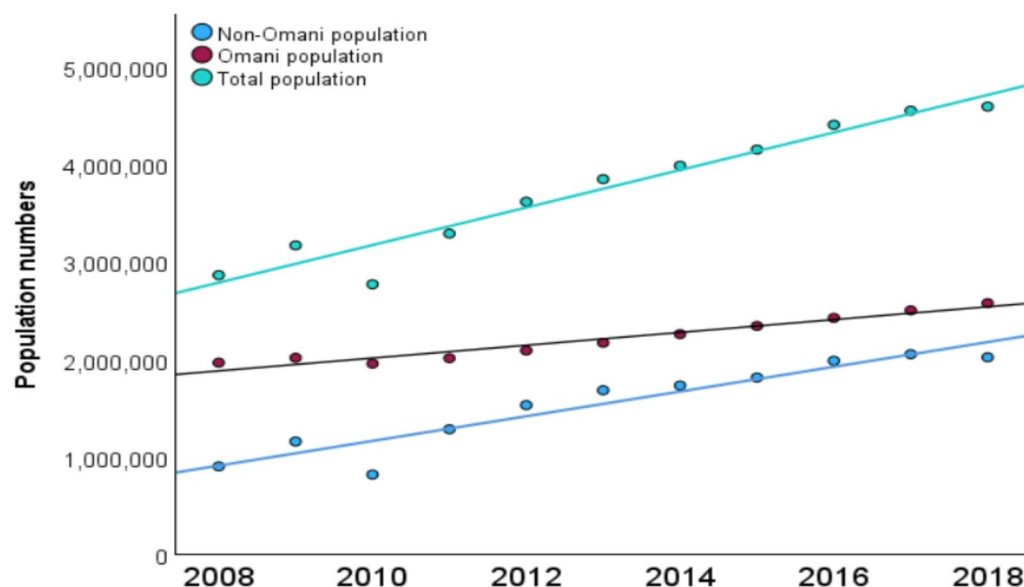


Fig. 1. Increase in total, Omani population, and non-Omani population in Oman between 2008 and 2018. Source NCSI (2024).

Spoligotyping and MIRU-VNTR genotyping

The MTB isolates of both populations were examined for Spacer Oligonucleotide Typing (Spoligotyping) (the classical 43-spacer format) was performed as previously described⁸.

We queried all spoligotype patterns against the SITVIT2 database to determine their corresponding Shared International Types (SITs)¹⁷.

In addition, 17 of the standard 24 MIRU-VNTR¹⁸ were analysed. All 24 MIRU-VNTR loci were initially targeted, but only 17 consistently amplified across all isolates; the remaining seven showed weak or sporadic amplification and were excluded from analysis. PCR fragments for each VNTR loci were generated individually, using a fluorescently labelled primer. The size of each PCR product was determined using Applied Biosystems 3130xL Genetic Analyzer, and GeneMapper V-6.0 software (Life Technologies) was used for allele calling. Alleles sizes of each locus were defined in reference to the size of the tandem repeat defined in standard allele-calling table <http://www.miru-vntrplus.org>.

Whole genome sequencing

MTB lineages in Oman

To explore the hypothesis of gene flow via expatriates from high TB burden countries, we analyzed whole genome sequencing (WGS) of a sub-set of 96 randomly selected MTB isolates from Omanis ($n = 23$) and expatriates ($n = 73$) obtained between 2018 and 2022. Library preparation, sequencing, and analysis were performed by Novogene (UK) Company Limited. Library preparation was performed using the NEBNext DNA Library Prep Kit (New England BioLabs, MA, USA). Index codes were added to each sample. The genomic DNA is randomly fragmented to a size of 350 bp. DNA fragments were end polished, A-tailed, ligated with adapters, size selected, and further PCR enriched. Then, PCR products were purified (AMPure XP system), followed by size distribution determination by Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA), and quantification using real-time PCR. The library was then sequenced on a NovaSeq 6000 S4 flow cell with PE150 strategy. The quality of the raw data was assessed with FastQC software version 0.11.8 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and cleaning was performed with Trimmomatic (version 0.39) (Bolger et al., 2014) using the options ILLUMINACLIP with the proper adapter set, and SLIDINGWINDOW:4:20, TRAILING:20, and LEADING:20. Alignment of the cleaned reads to the downloaded reference genome (GCF_000195955.2) was performed with BWA (version 0.7.17)¹⁹. SAMtools (version 1.13)²⁰ was used to convert the alignments from SAM to BAM files. SAMtools was also used to calculate the coverage of each sample using the option `-coverage`. And finally, bcftools (version 1.19)²¹ was employed to perform the variant calling in all samples using the option `--call`. Variant calls in VCF format were filtered using VCFtools (version 0.01.16)²² to retain only high-quality biallelic SNPs. Variant subsampling was performed using the `vcf random sample` function from VCFtools (Danecek et al., 2011). When necessary, format changes were made using the software PGDspider (version 2.1.1.5)²³. TB lineages were assigned using TB-Profiler (version 2.8.12) software²⁴.

MTB lineages in related countries

To examine the relatedness of MTB lineages in Oman with those in the country of origin of expatriates, we created a database of publicly available isolates representative of MTB diversity in Bangladesh, Tanzania, the Philippines, India, Egypt, Ethiopia, and Pakistan. We downloaded the raw FASTQs from the Sequence Read Archive (SRA) (Supplementary Table 2) using the software `fastq-dump`²⁵. Quality control, assembly, and SNP calling were performed as mentioned before.

WGS genomes generated in this study and isolates downloaded from SRA were used to generate a multisample VCF file containing SNP data was converted into FASTA format using PGDspider v2.1.1.5²³, with the “VCF to FASTA” option. The resulting FASTA was imported into MEGA X²⁶ and the alignment was performed using the ClustalW algorithm with default parameters. Positions with missing data or SNPs present in only a small subset of samples were excluded after alignment in MEGA. The final alignment was exported to nexus format and imported into SplitsTree4 software (version 4.15.1)²⁵ to generate a Median Network based on the alignment of positions using the uncorrected p-distance and 1000 replicates with SplitsTree4. The robustness of the network splits was evaluated using 1,000 bootstrap replicates, where the alignments were resampled with replacement to estimate support for each split within the network. We applied a ≤ 12 single nucleotide polymorphism (SNP) difference threshold to infer potential recent transmission, consistent with established cut-offs used in previous *M. tuberculosis* genomic epidemiology studies²⁷.

Data analysis

Descriptive analysis

Non-parametric correlation was used to assess the correlation between population numbers and time. Wald Chi Square was used to assess the variation in the distribution of spoligotype clades over the years between 2008 and 2018.

Generalized linear binomial regression with a logit link was used to examine temporal variation in MIRU allele proportions between 2008 and 2018, with “year” included as a continuous explanatory variable. The analysis presents as odds ratios (ORs) with 95% confidence intervals (CI) for each year.

Population genetic analysis

The allelic diversity (h) for each MIRU-VNTR was calculated as $h = n / (n-1) \times [1 - \sum p^2]$, where n is the number of isolates analysed and p is the frequency of an allele at a locus.

We constructed a ‘multi-locus genetic (MLG) profile’ encompassing the entire allelic profile of all MIRUs of MTB isolates, in order to demonstrate genetic relationships between MTB populations from different geographical region.

The genetic differentiation of *MTB* infecting Omanis and expatriates was then estimated using Wright’s fixation index (F_{ST}). To examine whether allele frequencies differ between *MTB* isolates in different provinces and between *MTB* collected from Omanis and expatriates, F_{ST} indices were calculated using Weir and Cockerham’s method²⁸, estimator of Wright’s F-statistics using the computer package GENEPOP v.4.7.5 web version <https://genepop.curtin.edu.au/>^{29,30}. A permutation test ($n = 10,000$) was applied (permuting alleles over populations) to test whether FST indices were significantly different from zero. Low pairwise F_{ST} typically below 0.05, is generally indicative of low genetic differentiation and high gene flow between populations³¹. Principal coordinate analysis (PCoA) was carried out to examine the genetic similarities between *MTB* isolates in different provinces using GenALEx Version 6.51b2³². The two-dimensional PCoA plot shows the relationships between the multi-locus variants found in the examined populations.

Results
Demographic characteristics of TB cases

We examined *MTB* isolates collected from expatriates ($n=329$, 45%) and nationals ($n=406$, 55%) in Oman between 2008 and 2018. The majority of patients were male with a mean age of 40.4yrs, The majority of patients from whom *MTB* isolates were obtained from patients in three provinces, Muscat ($n=301$, 41%), Batinah ($n=159$, 22%) and Dhofar ($n=95$, 13%) (Table 1).

Diversity and structure of MTB

Spoligotypes diversity

The examined *MTB* isolates ($n = 735$) comprised 4 main lineages, including lineage 1 (Indo-Oceanic), lineage 3 (East-African-Indian, EAI), lineage 4 (Euro-American), and lineage 2 (East-Asian, Beijing). Spoligotypes were queried against the SITVIT2 database (Couvin et al., 2019) to determine corresponding Shared International Types (SITs). The distribution of SIT-defined sub-lineages varied significantly across the study years (Wald Chi-Square = 21.043, DF = 9, $P=0.012$).

The most prevalent SIT families were SIT26 (CAS1-Delhi) and SIT11 (EAI3-IND), which fluctuated in prevalence from 17.9% in 2015 to 40.0% in 2013, and from 22.2% in 2010 to 41.7% in 2016, respectively (Fig. 2). Other detected SIT families included SIT1 (Beijing), SIT42 (LAM9), SIT50 (Haarlem3), and SIT53 (T1), representing less frequent but globally recognized spoligotype families. No significant change in the distribution of SITs was observed with respect to patient nationality (Wald Chi-Square = 3.268, DF = 1, $P=0.071$). The predominance of SIT26 (CAS1-Delhi) and SIT11 (EAI3-IND) corresponds to strain families commonly circulating in South and Southeast Asia, regions that represent major expatriate source populations in Oman.

MIRU-VNTR diversity

A total of 735 *MTB* isolates, collected between 2008 and 2018, from Omanis ($n = 406$) and expatriates ($n = 329$), in different provinces in Oman were successfully typed for 17 MIRU-VNTR loci. Supplementary Table 1 shows the distribution of alleles of the 17 loci. High allelic diversity of *MTB* was noted in all years ($h>0.5$), among isolates obtained from both Omanis (average $h=0.5885$) and expatriates (average $h=0.5847$).

Binomial regression models were used to assess temporal trends in the common 40 alleles (prevalence of $\geq 10\%$) of the 17 MIRUs between 2008 and 2018. Overall, 13 (32.5%) alleles exhibited significant fluctuation over time, with 5 alleles (e.g., MTUB39-388, OR = 1.125, 95% CI = 1.068–1.185) increased prevalence, whereas 8 alleles (e.g., MIRU20-584, OR = 0.676, 95% CI = 0.634–0.720) decreased. However, the majority of the remaining alleles (27, 67.5%) showed no temporal fluctuation (Fig. 3). These findings suggest a variable fluctuation in the prevalence of MIRU loci over the observed period.

Parameter	
Mean age (SD)	40.4 (26.6)
Gender	
Male	469 (64%)
Female	265 (36%)
Nationality	
Omanis	406 (55%)
Expatriates	329 (45%)
Province	
Muscat	301 (41%)
Batinah	159 (22%)
Dhofar	95 (13%)
Others *	131 (18%)
Missing data	49 (7%)

Table 1. Demographic characteristics of TB patients included in the study. *Sharqiyah, Buraimi, Dakhliyah, Dhahira, Musandam and Wousta.

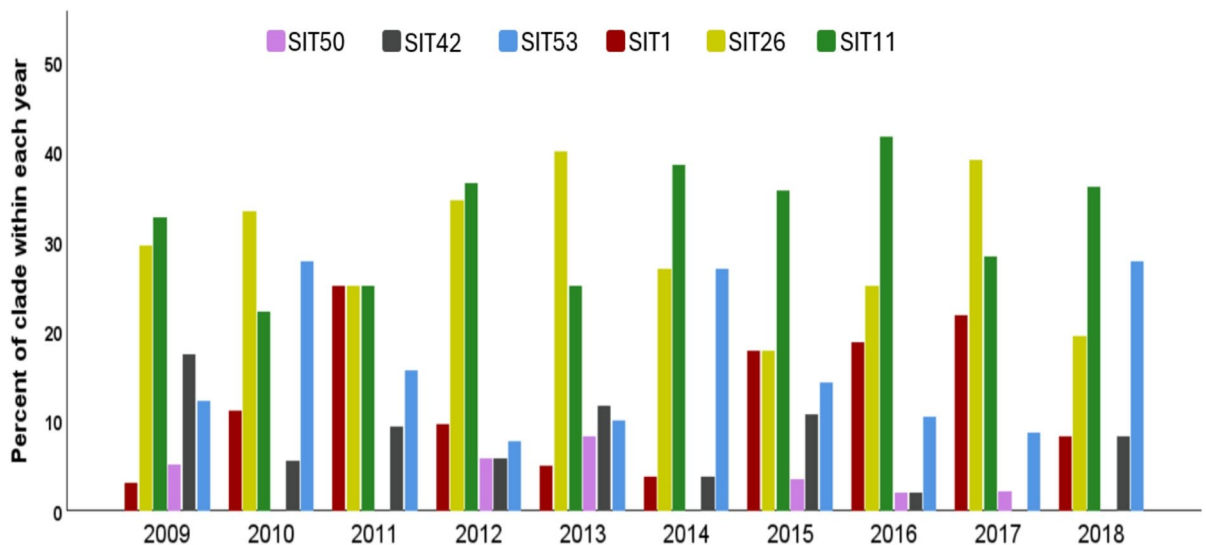


Fig. 2. Temporal *MTB* lineages distribution in Oman, 20,019 – 2018. Figure shows the proportion of spoligotypes categories across each year. No clade data were available for 2008. CAS: The 735 isolates were classified into four major phylogenetic lineages (Lineage 1 – Indo-Oceanic, Lineage 3 – East-African Indian [EAI], Lineage 4 – Euro-American, and Lineage 2 – East-Asian [Beijing]) and assigned to Shared International Types (SITs) using the SITVIT2 database. The most prevalent SIT families were SIT26 (CAS1-Delhi) and SIT11 (EAI3-IND), with fluctuating yearly prevalence. Other less common SIT families included SIT1 (Beijing), SIT42 (LAM9), SIT50 (Haarlem3), and SIT53 (T1). No significant variation in SIT distribution was observed by patient nationality.

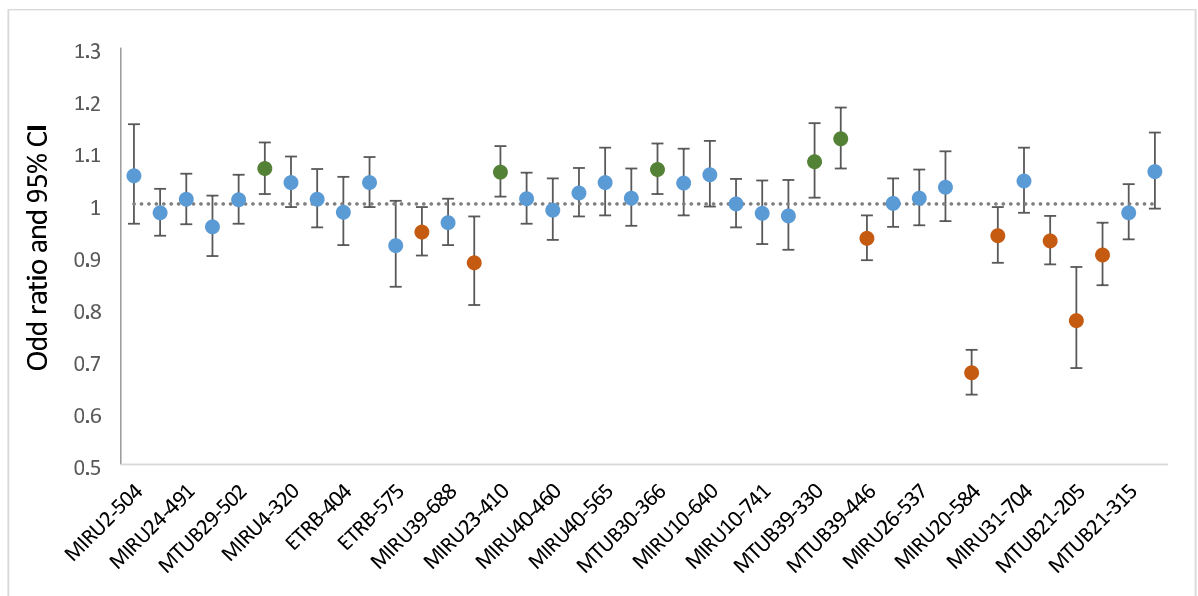


Fig. 3. Effect of time on the MIRU loci proportions. Forest plot of odds ratios (ORs) with 95% confidence intervals for individual MIRU alleles, derived from binomial regression models assessing temporal trends in allele proportions from 2008 to 2018, with “year” being a continuous explanatory variable. Each point represents the estimated OR for a specific allele, with vertical lines showing the corresponding 95% confidence interval (CI). ORs < 1 (orange) indicate decreasing frequency over time, ORs > 1 (green) suggest increasing trends, and ORs ≈ 1 (blue) indicate temporal stability.

Temporal and spatial dynamics of *MTB*

MIRU-VNTR analysis

To investigate temporospatial dynamics of *MTB* in Oman, F_{ST} was estimated using MLG data from the 17 MIRU-VNTR to assess whether the *MTB* populations, across different years, provinces and between Omanis

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
2008	0.0000	0.0081	0.0181	ND	0.0107	0.0120	0.0337	0.0254	0.0183	0.0275	0.0339
2009		0.0000	0.0186	ND	0.0126	0.0102	0.0324	0.0343	0.0245	0.0346	0.0423
2010			0.0000	ND	0.0181	0.0149	0.0338	0.0330	0.0281	0.0256	0.0330
2011				ND	ND	ND	ND	ND	ND	ND	ND
2012					0.0000	0.0045	0.0340	0.0287	0.0229	0.0330	0.0402
2013						0.0000	0.0304	0.0341	0.0228	0.0323	0.0392
2014							0.0000	0.0128	0.0112	0.0125	0.0129
2015								0.0000	0.0057	0.0071	0.0069
2016									0.0000	0.0050	0.0074
2017										0.0000	0.0040
2018											0.0000

Table 2. Pairwise F_{ST} estimates for Temporal genetic variation of MTB populations in Oman (2008–2018).

Year	He	He Omanis	He Expatriates	F_{ST}
2008	0.595	0.5821	0.6065	0.0084
2009	0.583	0.5821	0.5702	-0.0036
2010	0.554	0.5932	0.5275	-0.0139
2011	ND	ND	ND	ND
2012	0.576	0.5205	0.5712	-0.0026
2013	0.580	0.5792	0.5820	0.0134
2014	0.637	0.5707	0.6432	-0.0056
2015	0.625	0.6303	0.6180	0.005
2016	0.586	0.6256	0.5910	-0.0208
2017	0.579	0.5917	0.5511	-0.0113
2018	0.604	0.6097	0.5867	0.025
Overall	0.5919	0.5885	0.5847	

Table 3. Observed genetic diversity (He) and differentiation (F_{ST}) of MTB isolates infecting Omanis and expatriates, in Oman, between 2008 and 2018. ND= Not done.

	AL-Dakhliya	AL-Dhahira	Batinah	Dhofar	Muscat	Sharqiya
AL-Dakhliya	0.0000					
AL-Dhahira	0.0045					
Batinah	0.0031	0.0044				
Dhofar	0.0016	-0.0066	0.0149			
Muscat	0.0013	0.0007	0.0004	0.0074		
Sharqiya	0.003	0.012	0.0028	0.0187	0.0021	0.0000

Table 4. Pairwise F_{ST} estimates for regional genetic differentiation of MTB populations in different provinces in Oman.

and expatriates were genetically distinct or homogeneously mixed.

A low pairwise F_{ST} (<0.05) was obtained between MTB in different years, 2008–2018, indicative of low genetic differentiation over time. The very low or negative F_{ST} value (e.g. -0.0061, -0.0114, -0.0019), indicative of minimal genetic changes over the study period (Table 2). Similarly, very low or negative F_{ST} (-0.0002 to 0.0236) was seen between MTB isolated from Omanis and expatriates (Table 3), indicative of minimal variation and gene flow between the two groups.

Moreover, no spatial variation was observed between provinces, F_{ST} ranged from -0.0066 to 0.0187, indicative of homogeneous MTB population in different provinces. The highest F_{ST} values were noted between Dhofar (southern Oman, near the Yemen border), and other provinces, such as Dhofar and Sharqiya ($F_{ST} = 0.0187$), Dhofar and AL-Dhahira ($F_{ST} = 0.0149$), Dhofar and Muscat ($F_{ST} = 0.0074$), suggesting slight divergence of MTB in Dhofar (Table 4).

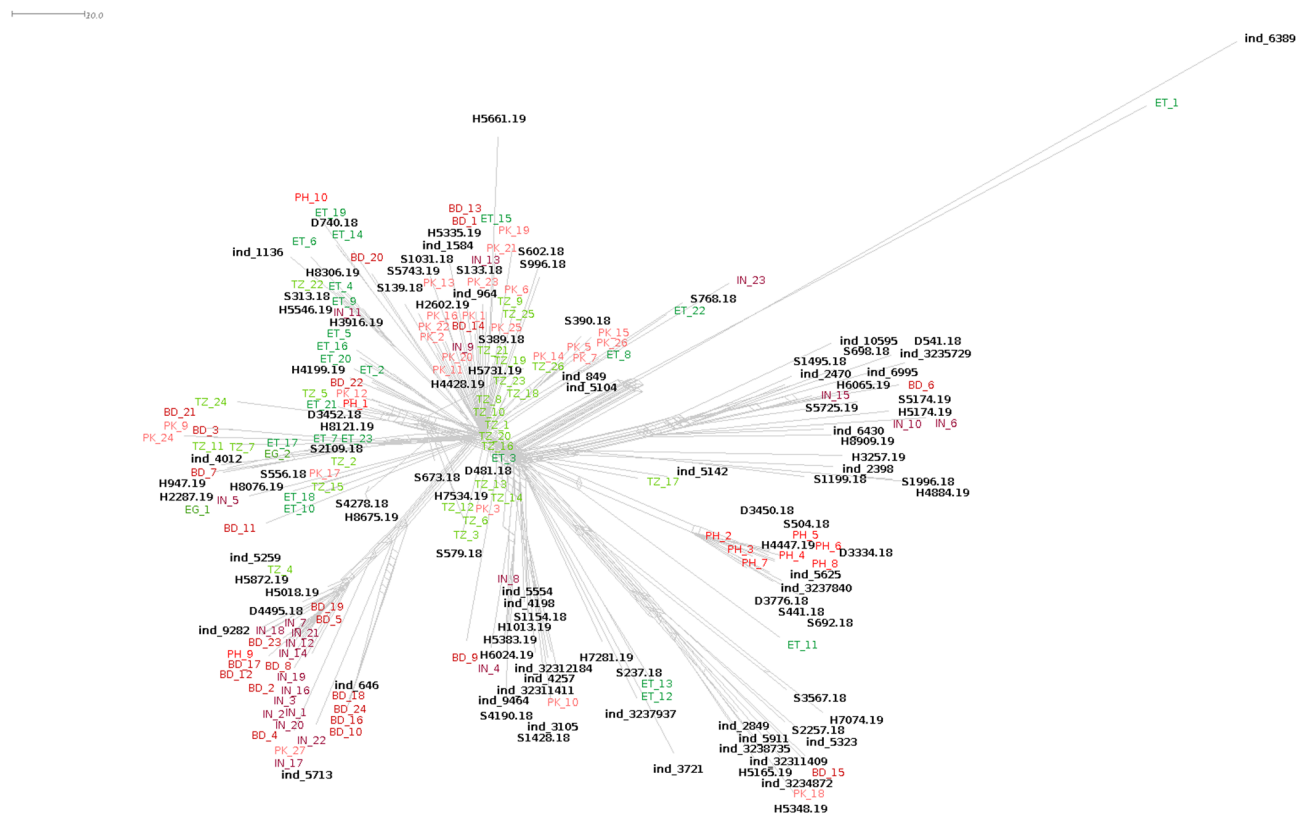


Fig. 4. SplitsTree network, estimated with the Median Network method (Huson DH and Bryant D 2006), illustrates the relationships of MTB stains in Oman and countries of origin for expatriates. Samples from Oman are in bold black. Red colors correspond to Asian origins; green colors correspond to African origins. IN denotes India, PK denotes Pakistan, BD indicates Bangladesh, PH denotes Philippines, EG indicates Egypt, TZ designated Tanzania and ET represents Ethiopia. Samples labeled “ind” were produced specifically for this project. To reduce clutter, some labels were omitted for countries with multiple samples in the same n.

Comparative WGS analysis of MTB lineages

WGS analysis

To explore the impact of gene flow via expatriates from high TB burden countries, we examined genome sequence of 96 MTB strains collected between 2018 and 2022 in Oman together with publicly available MTB sequences from home countries of expatriates. The number of reads per sample ranged from 295,857 to 42,760,643 sequences, with a mean of 5,486,388 and a median of 2,766,511. Sample coverage ranged from 94.3% to 99.6%, with a mean of 99.05% and a median of 99.50%. Mean depth ranged from 14.03 to 835.63, with a mean of 93.71 and a median of 163.32. A total of 216,465 variants were identified remaining 154,581 after the INDELS removal. From those 10% (15421) were randomly selected for perform the phylogenetic analysis. The selected variants were imported into MEGA X²⁶ to generate a multiple sequence alignment using the ClustalW algorithm. Uninformative SNPs and positions containing excessive missing data were removed from the alignment. The resulting dataset, comprising 11,094 high-quality SNPs, was subsequently imported into SplitsTree4 software for phylogenetic network reconstruction.

The phylogenetic analysis (Fig. 4) shows relationships based on SNP distances of MTB isolates collected in Oman (from Omanis $n = 23$ and expatriates 73) and from expatriate countries (e.g., India, Pakistan, Bangladesh, Tanzania, Philippines, Ethiopia, Egypt).

Most isolates form a dense central cluster, suggesting a high degree of genetic similarity (differing by ≤ 12 SNPs), with a mix of isolates from Oman and multiple expatriate countries, pointing to shared strains or recent introductions. However, some isolates (e.g., ET_1, IND_6389, H5661.19) appear on long branches, indicating they are genetically distinct from the central population. These may represent more divergent lineages or recently imported strains.

Isolates from some countries (e.g., India, Pakistan, Bangladesh) are spread across the tree, indicating genetic diversity within those populations, while others (e.g., Tanzania, Philippines) appear in more tight-knit clusters, suggesting either clonal expansion or localized strain evolution.

Discussion

MTB in Oman exhibits a high and stable level of genetic diversity revealed by spoligotyping and MIRU-VNTR ($h = 0.591$), with comparable diversity among both nationals ($h = 0.5885$ for both). The examined MTB isolates

comprised four lineages and seven sub-lineages MTB, with fluctuating prevalence, the common clades, over time. The dominance of SIT26 (CAS1-Delhi) and SIT11 (EA13-IND) lineages mirrors findings from neighboring Gulf countries and from South and Southeast Asia, reflecting the genetic continuity of MTB strains imported through expatriates from high TB burden countries⁸. These findings underscore the influence of population mobility on the genetic landscape of MTB in Oman and highlight the value of integrating SIT-based classification for typing MTB strain for global comparability of molecular epidemiological data.

Across 17 MIRUs, multiple alleles were detected, some showing temporal changes, while most remained stable, consistent with low pairwise years F_{ST} values between years, indicating a largely homogeneous MTB population. This genetic homogeneity was reflected in similar pairwise F_{ST} values between provinces, although slightly elevated differentiation was observed in Dhofar, the southernmost region. Phylogenetic analysis revealed shared clades between Omani and expatriate isolates, suggesting cross-strain circulation and gene flow across demographic groups.

Increasing the resolution from spoligotyping to MIRU-VNTR and WGS reinforced the finding of a genetically diverse but stable MTB population. While isolates initially grouped into seven spoligo clades, each contained unique multi-locus MIRU profiles (Supplementary Table 1), indicating infections stem from multiple unrelated sources rather than clonal expansion or localized outbreaks. These findings align with prior studies¹⁴ and reflect the continual influx of individuals from TB-endemic regions and reactivation of latent TB among expatriates¹². Similarly high levels of MTB diversity have been reported in other Gulf countries, including Saudi Arabia³³, the UAE³⁴, and Kuwait³⁵, characterized by large expatriate populations from high TB-burden countries. The demographic flux across these countries likely contributes to the observed diversity.

Although overall spatial and temporal differentiation was low (Tables 2 and 3) slightly higher F_{ST} values between Dhofar and other provinces suggest some degree of genetic differentiation in the south, likely influenced by geographic distance, border proximity, and population movement. Despite this, no clear regional sub-structuring was found, with pairwise F_{ST} values across provinces ranging from 0 to 0.0187 (Table 4). This low differentiation suggests high gene flow and large effective population sizes³⁶, consistent with the lack of genetic distinction between MTB infecting Omanis and expatriates (Table 3).

With regard to the relatively low differentiation of MTB in Dhofar exhibited modestly elevated F_{ST} values, suggesting tendency to regional genetic differentiation. This may be attributed to geographic isolation, proximity to international borders, or unique migration patterns. Supporting this, SIT1(Beijing) and SIT26 (CAS1-Delhi) lineages, commonly found in South and Southeast Asia, were overrepresented in Dhofar⁸. The Beijing lineage, in particular, is associated with increased virulence and multidrug resistance^{37,38}. These findings raise the possibility of lineage-specific importation patterns linked to particular expatriate groups or regional epidemiology³⁹. Further longitudinal WGS studies integrating demographic and migration histories are needed to track these trends and determine their epidemiological significance.

However, temporal changes in common alleles of some MIRU-VNTR (Fig. 3) likely reflect a combination of genetic drift, selection pressures (e.g., treatment), or the introduction of new strains via migration or outbreaks. Loci that exhibit changing allele frequencies may serve as useful markers for tracking recent transmission dynamics, while stable loci are useful for lineage identification. These patterns emphasize the value of longitudinal molecular surveillance for capturing shifts in TB population structure driven by demographic changes among expatriates. For example, annual fluctuations of 3–5% in the four largest expatriate groups (Indian, Pakistani, Bangladeshi, Egyptian) have been reported¹⁶. As these populations cluster geographically, these demographic shifts likely influence local MTB genetic patterns. However, we did not integrate spatial and temporal components into a combined spatiotemporal model, which may provide additional insights into the transmission dynamics. Future studies integrating spatial and temporal data are warranted to better elucidate how regional and temporal factors jointly influence MTB population structure and transmission dynamics.

Whole-genome phylogenies further support a high level of diversity, with most isolates forming a dense central cluster, indicating strong overall similarity, and lacking distinct regional or nationality-based clustering (Fig. 4). Strains from Omanis and expatriates were interspersed across clades, consistent with frequent cross-population mixing. Additionally, phylogenetic ties between Omani strains and those from expatriates' countries of origin highlight ongoing international transmission dynamics. These findings underscore the influence of labor migration and cross-border mobility on TB epidemiology in Oman and the Gulf region more broadly. Comparable patterns have been observed in France, where historical and modern migration shaped MTB diversity⁴. There, earlier connections to Eastern Europe and North Africa gave way to more persistent links with Algeria, Morocco, and Tunisia, while strains from Asia and sub-Saharan Africa remained distinct indicating limited local transmission from newer migrant communities. Similar dynamics may be unfolding in Oman, emphasizing the need for coordinated regional surveillance and targeted public health strategies.

Although the MTB samples were collected from different provinces across Oman, they may not fully represent the genetic diversity of MTB strains circulating in all governorates. Out of Oman's 11 governorates, the majority of isolates were obtained from patients in just three governorates, Muscat, Batinah, and Dhofar, which are among the most populated and have the highest TB notification rates. As a result, the spatial distribution of MTB genotypes presented in this study may not reflect the full diversity of strains circulating nationwide, particularly in less populated or more remote regions. Furthermore, the limited number of MTB isolates analyzed by WGS constrains the generalizability of these findings. While the results provide valuable insights into the diversity and transmission dynamics of MTB in the sampled regions, they should be interpreted with caution when extrapolating to the entire country. Future studies with broader geographic coverage and larger sample sizes will be necessary to capture a more comprehensive picture of the genetic structure and transmission dynamics of MTB in Oman.

This study reveals that MTB in Oman exhibits high genetic diversity and temporal stability, as demonstrated by spoligotyping, MIRU-VNTR, and whole-genome sequencing. The low genetic differentiation across years

and provinces, along with phylogenetic overlap between strains from Omani nationals and expatriates, points to a largely homogeneous population shaped by diverse sources of infection rather than localized transmission. Regional variation in Dhofar, including a higher prevalence of Beijing and CAS lineages, suggests lineage-specific introductions potentially linked to migration patterns. Temporal shifts in specific MIRU loci further highlight dynamic transmission trends. These findings emphasize the impact of labor migration and regional mobility on TB dynamics in Oman and support the need for sustained molecular surveillance and cross-border public health collaboration. Further longitudinal studies Integrating patient metadata (e.g., demographics, travel history) and WGS may allow monitoring the spread and evolution of drug resistant and/or virulent strains in individual governorates.

Data availability

All relevant data are presented as tables and figures within the main text. The sequence of MTB isolates in Oman have been deposited in SRA under BioProject accession [PRJNA977967] (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA977967>). Supplementary information, including alleles of MIRUs, is available in Supplementary Tables S1, and accession number of sequence obtain from public data bases are given in Supplementary Table 2.

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Author contributions

HB, AJ, ABP conceived of the presented idea. LB, prepared and process MTB isolates. RAH, AZ, FG performed laboratory work. SH, AG, carried data analytical methods. ABP, LP, AB supervised genetic and bioinformatic data analysis and interpretation HB, ABP wrote the manuscript with support from AB and LP. AJ, ABK, AK, SA, FY. helped supervise the project and contributed to the design and implementation of the research. All authors discussed the results and contributed to the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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