Genetic Diversity of *Mycobacterium tuberculosis* complex isolates circulating in North Central Nigeria

Stella S. Makpu[1], Kome Otokunefor[1*] and Tosanwumi Vincent Otokunefor[1]

[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

*kome.otokunefor@uniport.edu.ng

Abstract

With the higher risk of tuberculosis associated with developing countries, it has increasingly become imperative to determine the genetic diversity of strains of the *Mycobacterium tuberculosis* complex (MTBC). This is essential for establishing control programs as it provides information on possible sources and chains of transmission.

This study aimed to assess the genetic diversity of *M. tuberculosis* complex strains isolated from the North Central Nigeria using the 12-locus MIRU-VNTR.

Mycobacterium tuberculosis complex isolates (106) obtained from a previous study between November 2017 and December 2018 were characterized using the 12-locus mycobacterial interspersed repetitive unit variable number tandem repeats (MIRU-VNTR) typing method. Data generated were then analyzed using the MIRU-VNTRplus web application to determine the allelic diversity.

Allelic diversity as determined by the Hunter-Gaston diversity index showed a variation from 0.21 to 0.81 associated with MIRU locus 2 and 26 respectively. In total, 41.7% of the loci were highly discriminant, 41.7% moderately discriminant, and 16.7% were poorly discriminant. MIRU-VNTR typing revealed a high diversity, with a clustering rate of 1.9% and a total of ten different genotypes. UgandaII (Lineage 4) was the most common (14, 21.2%), followed by Bovis (10, 15.1%) and Cameroon (6, 9.1%); UgandaI (Lineage 4) was the least common (1, 1.5%).

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The 12-locus MIRU-VNTR provided an adequate typing tool for assessing the genetic diversity of MTBC in this study as evidenced by the 0.94 HGDI value for total isolates. We report a high diversity of MTBC strains circulating in the North Central region of Nigeria, indicating multiple introductions of this pathogen to this region and a lack of clonal spread.

Keywords: Nigeria, *Mycobacterium tuberculosis*, MIRU-VNTR, high diversity

Introduction

Tuberculosis is responsible for up to 1.8 million deaths worldwide each year and is caused by members of the *Mycobacterium tuberculosis* complex.[1,2] Developing countries are particularly at risk of tuberculosis and by 2021, Nigeria was ranked with the 6th highest tuberculosis burden globally,[3,4] with an estimated 154,000 deaths each year.

Over the years, most studies have focused on the prevalence of strains within the *Mycobacterium tuberculosis* complex (MTBC). More recent studies have focused on the molecular epidemiology of circulating strains of *M. tuberculosis* complex strains in different locales to determine possible sources and chains of transmission, and monitor outbreaks, all of which would serve as the basis for creating control programs.[5,6]

Several studies have explored the genetic diversity of these MTBC in Nigeria. Most of these studies used PCR-based typing methods, particularly the spacer oligonucleotide typing (spoligotyping) and MIRU-VNTR typing methods. Some of these Nigerian studies combined both methods,[3,7-9] while others made use of only spoligotyping. [10-14] These methods differ in their discriminatory power, ease of use and cost. MIRU-VNTR is a more advantageous technique, as it has a higher discriminatory power compared to spoligotyping [9,10] basically because it involves multiple loci as compared to the single loci of spoligotyping.[15] Three variants of MIRU-VNTR exist based on differences in the number of loci analysed; these include the 12-locus, 15-locus and 24-locus MIRU-VNTR. The 12-locus variant, which was the first to be developed, is the least discriminatory and the 24-locus variant is the most discriminatory.[16]

Of all these studies, none has focused on accessing molecular epidemiology of the North Central region of Nigeria using the MIRU-VNTR method. Studies using this method in Nigeria are actually limited. A single previous study covered the North Central region by Pokam and colleagues, made use of spoligotyping and the Large Sequence Polymorphisms (LSPs) typing method.[14] This study was therefore aimed at assessing the genetic diversity of strains of *M. tuberculosis* complex isolated from the North Central region of Nigeria using MIRU-VNTR typing method.

Methods

Ethical Statement

This study was approved by the Federal Ministry of Health, Abuja. The Ethics approval number is as stated: NHREC Approval Number NHREC/01/01/2007-16/03/2018. Informed consent was obtained from all participants in the study and all individual identifiers were removed to create anonymity.

Study Population

Mycobacterium tuberculosis complex test isolates were obtained from sputum specimen as part of a study exploring the prevalence of multidrug-resistant *Mycobacterium tuberculosis* in the North Central area of Nigeria (consisting of Niger, Benue, Kogi, Plateau, Kwara, Nasarawa and the Federal Capital Territory).[17] Sputum samples were collected over a period of 12 months (November 2017 to December 2018) from presumptive tuberculosis patients (based on clinical symptoms of a cough for ≥2 weeks). 696 individuals (18 – 75 years) were randomly selected from directly observed treatment short-course clinics and centres in the study region (100 from each zone apart from the Federal Capital Territory (FCT) where 96 were enrolled). Patients currently on tuberculosis treatment regimen were excluded.

Sample Processing and Culture

Two sputum samples of 5 mL volume were collected from each presumptive tuberculosis patient. Culturing of sputum samples was carried out as previously described.[18] In brief, following processing of samples using the petroff method,[19] culture was carried out on Lowenstein-Jensen agar slants (Hi-Media, India) whereby set up was incubated at 37°C with visual inspection for characteristic growth done for up to 8 weeks.

Genomic DNA extraction

Genomic DNA was extracted from the 106 isolates using the mechanical method as previously described[20] with a slight modification. In brief, first, MTBC colonies were recovered from the Lowenstein-Jensen agar slants and re-suspended in molecular grade water. This suspension was then vortexed to ensure proper mixing and incubated at 95°C for 20 mins. This was followed by sonication at 85°C for 15 mins and centrifugation for 5 mins at 10000 rcf. Finally, the supernatant containing the DNA was transferred to a new tube and stored at -20°C for further processing.

MIRU-VNTR typing

MIRU-VNTR typing was carried out using the previously described 12-locus mycobacterial interspersed repetitive unit variable number tandem repeats (MIRU-VNTR) typing method.[21] In brief, the 12 different loci were amplified in simplex PCR setups with the previously described primers, reaction mixes and amplification conditions.[21] Amplification products were then visualized using 1.5% agarose gel electrophoresis and a 100 bp DNA ladder as the molecular weight standard.

MIRU-VNTR Data Analysis

The results of the MIRU-VNTR amplification were then manually analysed to determine the band sizes and the number of repeats for each locus based on a standard table

(http://www.MIRU-VNTRplus.org) and a unique code was generated for each isolate following the numerical order of MIRU 2-4-10-16-20-23-24-26-27-31-39-40. Isolates with completely identical codes were identified as part of the same cluster and this was used to calculate the clustering rate with the following equation:

clustering rate = (nc – c)/n [Eqn 1]

Where nc is the total number of clustered cases, c is the number of clusters, and n is the total number of isolates.[22]

The resulting data was then analyzed using the MIRU-VNTRplus web application tools (http://www.MIRU-VNTRplus.org) to determine the relatedness between strains using the provided dendrogram program with the UPGMA algorithm.[15] Also, allelic diversity was determined for each locus using the Hunter and Gaston Discriminatory index[23] with the following formula:

$$\text{HGDI} = 1 - \frac{1}{\text{N(N-1)}} \sum_{j-1}^{s} n_j(n_j - 1) \qquad \text{[Eqn 2]}$$

Where N is the total number of isolates in the sample population for a given locus, S is the total number of distinct repeat unit values identified for the locus, and n_j is the number of isolates having the jth value

Each locus was then classified based on allelic diversity as previously described.[24] The loci were classified as very discriminant (Hunter-Gaston discriminatory index (HGI) > 0.6), moderately discriminant (0.3 ≤HGI ≤0.6), or poorly discriminant (HGI < 0.3)

Finally, lineages were assigned using the MIRU-VNTRplus web tool based on the MIRU-VNTR patterns.

Results

Loci amplification

A total of 106 unique isolates were initially analysed (Benue: 14, FCT: 17, Kogi: 5. Kwara: 23, Nasarawa: 17, Niger: 22, Plateau: 8). A 100% MIRU-VNTR locus amplification was absent in

all 106 strains; locus 27 had the highest level of amplification (83%, 88/106) and locus 20 the lowest (18.1%, 19/106) (Figure 1). Only 7.5% (8/106) of isolates had amplification products from all 12 loci. About 3.8% (4/106) of isolates were untypeable using MIRU-VNTR as none of the loci were amplified.

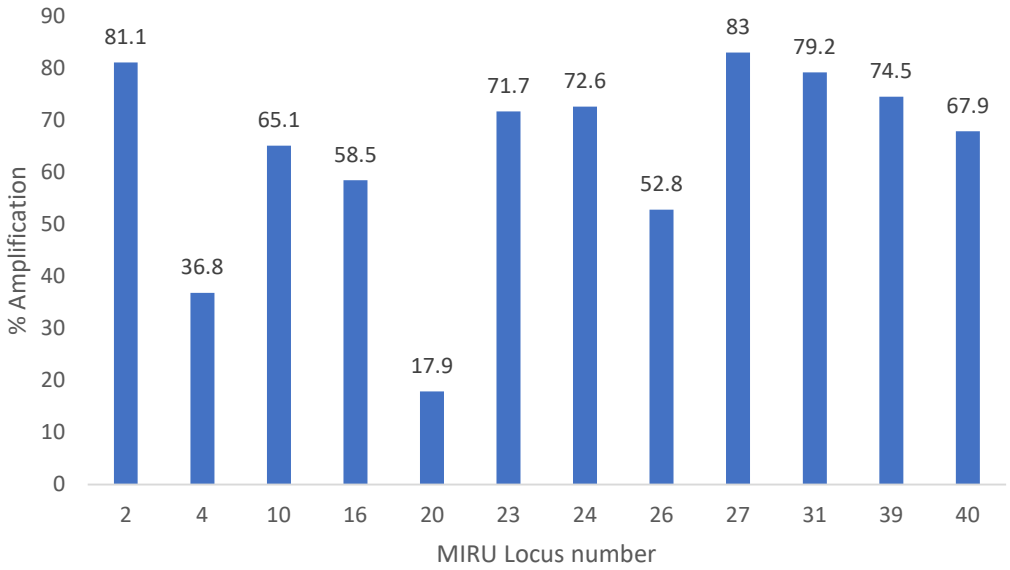

Figure 1: Detection variation of the various MIRU-VNTR loci present in *Mycobacterium tuberculosis* complex isolates obtained from North central Nigeria between November 2017 and December 2018

Allelic diversity of MIRU-VNTR loci

The number of repeats observed for each locus varied from zero to eight among test isolates (Table 1). Among all loci, the highest number of allelic variants observed was six (five loci) and the lowest was three (six loci). Two loci had a high predominance of a specific copy number; 88.4% (76/86) of the isolates carrying locus 2 had two repeats and 84.6% (33/39) of the isolates with locus 4 had three repeats. The allelic diversity varied from 0.21 to 0.81 with MIRU locus 26 having the highest allelic diversity and MIRU locus 2 the least. 41.7% (5/12) of the loci had a high level of discrimination, 41.7% (5/12) were moderately discriminant, and 16.7% (2/12) were poorly discriminant using the Hunter-Gaston discriminatory index.

Table 1: MIRU loci variations and Allelic diversity of MTBC isolates obtained from North central Nigeria between November 2017 and December 2018

	Occurrence per locus (%)

		2 (86)	4 (39)	10 (69)	16 (62)	20 (19)	23 (76)	24 (77)	26 (56)	27 (88)	31 (84)	39 (79)	40 (72)
Repeat Copy Number	0			2 (2.9)		5 (26.3)		43 (55.8)				48 (60.8)	6 (8.3)
	1			4 (5.8)	5 (8.1)	10 (52.6)		20 (26.0)			1 (1.2)	26 (32.9)	19 (26.4)
	2	76 (88.4)	5 (12.8	18 (26.1)	8 (12.9)	4 (21.1)		12 (15.6)	16 (28.6)	68 (77.3)	21 (25)	5 (6.3)	3 (4.2)
	3	6 (7.0)	33 (84.	30 (43.5)	20 (32.3)			2 (2.6)	9 (16.1)	8 (9.1)	52 (61.9)		33 (45.8)
	4	4 (4.7)	1 (2.6)	14 (20.3)	22 (35.5)		7 (9.2)		12 (21.4)	12 (13.6)	3 (3.6)		5 (6.9)
	5				6 (9.7)		47 (61.8)		8 (14.3)				2 (2.7)
	6				1 (1.6)		22 (28.9)		10 (17.9)		6 (7.1)		4 (5.6)
	7			1 (1.4)					1 (1.8)				
	8										1 (1.2)		
No of AV		**3**	**3**	**6**	**6**	**3**	**3**	**4**	**6**	**3**	**6**	**3**	**6**
HGI		**0.21**	**0.27**	**0.71**	**0.75**	**0.64**	**0.47**	**0.60**	**0.81**	**0.38**	**0.55**	**0.53**	**0.71**

AV = Allelic Variants, HGI = Hunter-Gaston Index

A high diversity was noted among the isolates as indicated by the total of 91 different patterns generated from the 106 isolates tested. These comprised 5 clusters (consisting of two or more isolates with identical patterns) and 86 singletons having unique patterns. Of the 6 clusters, only 1 was exploitable which is defined as patterns with information missing at less than 4 loci (233205032303). This cluster comprised of three isolates. Thereby indicating a clustering rate of 1.9% for 12-locus MIRU-VNTR in this study.

Further analysis was then carried out on the 66 exploitable patterns using the MIRU-VNTRplus web application tools (http://www.MIRU-VNTRplus.org). A dendrogram analysis of these patterns at 60% similarity resulted in 14 groups made up of more than 1 isolate and 9 singletons (Figure 2). Groups ranged in sizes from 2 to 9 (Table 2). In all, typing of isolates using the 12 loci MIRU-VNTR showed a very high level of diversity both when applied to total isolates

(0.94) i.e. with respect to the whole North Central region and during state wise comparison with HGDI ranges from 0.83 to 1.

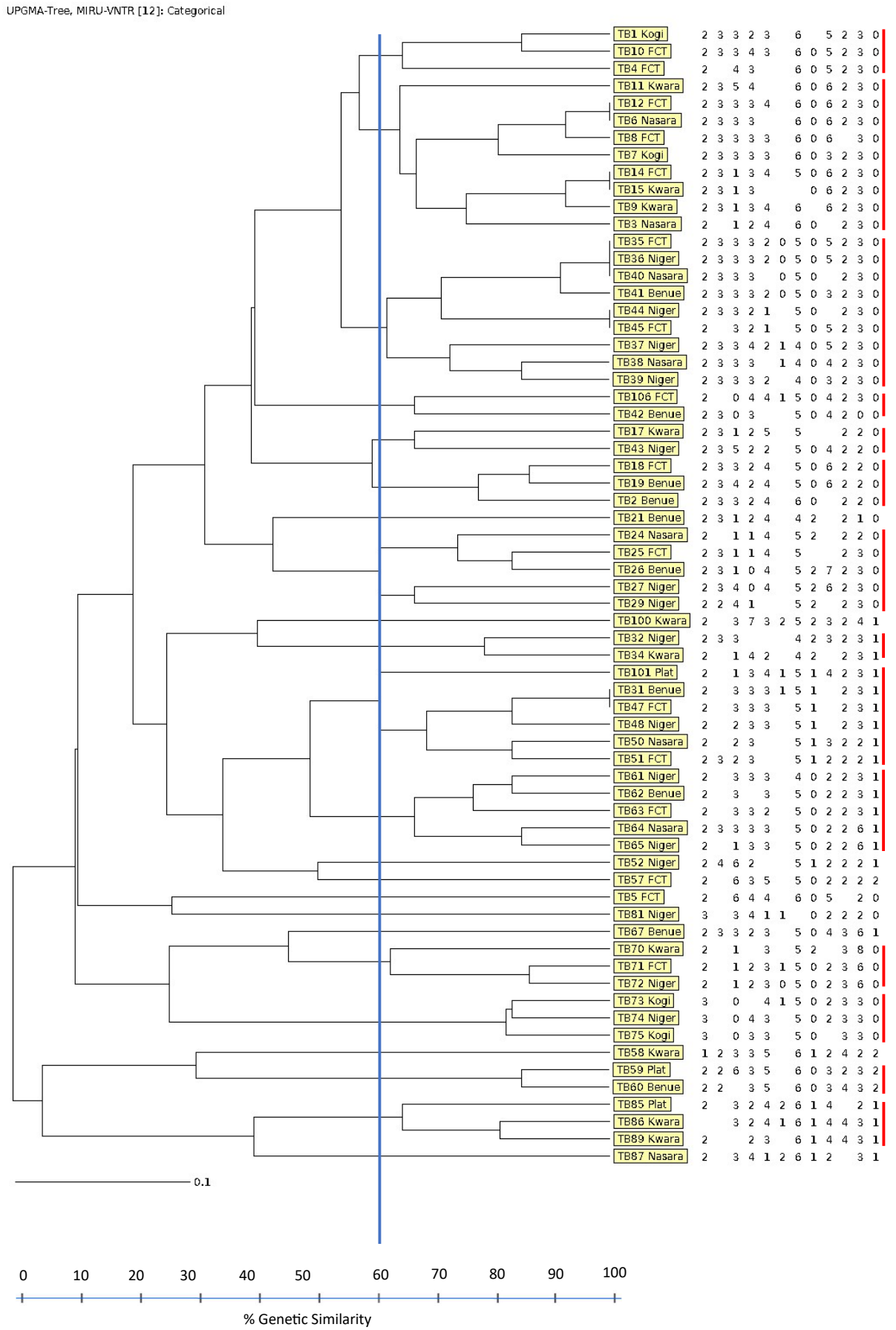
UPGMA-Tree, MIRU-VNTR [12]: Categorical
TB1 Kogi 2 3 3 2 3 6 5 2 3 0
TB10 FCT 2 3 3 4 3 6 0 5 2 3 0
TB4 FCT 2 4 3 6 0 5 2 3 0
TB11 Kwara 2 3 5 4 6 0 6 2 3 0
TB12 FCT 2 3 3 3 4 6 0 6 2 3 0
TB6 Nasara 2 3 3 3 6 0 6 2 3 0
TB8 FCT 2 3 3 3 3 6 0 6 3 0
TB7 Kogi 2 3 3 3 3 6 0 3 2 3 0
TB14 FCT 2 3 1 3 4 5 0 6 2 3 0
TB15 Kwara 2 3 1 3 0 6 2 3 0
TB9 Kwara 2 3 1 3 4 6 6 2 3 0
TB3 Nasara 2 1 2 4 6 0 2 3 0
TB35 FCT 2 3 3 3 2 0 5 0 5 2 3 0
TB36 Niger 2 3 3 3 2 0 5 0 5 2 3 0
TB40 Nasara 2 3 3 3 0 5 0 2 3 0
TB41 Benue 2 3 3 3 2 0 5 0 3 2 3 0
TB44 Niger 2 3 3 2 1 5 0 2 3 0
TB45 FCT 2 3 2 1 5 0 5 2 3 0
TB37 Niger 2 3 3 4 2 1 4 0 5 2 3 0
TB38 Nasara 2 3 3 3 1 4 0 4 2 3 0
TB39 Niger 2 3 3 3 2 4 0 3 2 3 0
TB106 FCT 2 0 4 4 1 5 0 4 2 3 0
TB42 Benue 2 3 0 3 5 0 4 2 0 0
TB17 Kwara 2 3 1 2 5 5 2 2 0
TB43 Niger 2 3 5 2 2 5 0 4 2 2 0
TB18 FCT 2 3 3 2 4 5 0 6 2 2 0
TB19 Benue 2 3 4 2 4 5 0 6 2 2 0
TB2 Benue 2 3 3 2 4 6 0 2 2 0
TB21 Benue 2 3 1 2 4 4 2 2 1 0
TB24 Nasara 2 1 1 4 5 2 2 2 0
TB25 FCT 2 3 1 1 4 5 2 3 0
TB26 Benue 2 3 1 0 4 5 2 7 2 3 0
TB27 Niger 2 3 4 0 4 5 2 6 2 3 0
TB29 Niger 2 2 4 1 5 2 2 3 0
TB100 Kwara 2 3 7 3 2 5 2 3 2 4 1
TB32 Niger 2 3 3 4 2 3 2 3 1
TB34 Kwara 2 1 4 2 4 2 2 3 1
TB101 Plat 2 1 3 4 1 5 1 4 2 3 1
TB31 Benue 2 3 3 3 1 5 1 2 3 1
TB47 FCT 2 3 3 3 5 1 2 3 1
TB48 Niger 2 2 3 3 5 1 2 3 1
TB50 Nasara 2 2 3 5 1 3 2 2 1
TB51 FCT 2 3 2 3 5 1 2 2 2 1
TB61 Niger 2 3 3 3 4 0 2 2 3 1
TB62 Benue 2 3 3 5 0 2 2 3 1
TB63 FCT 2 3 3 2 5 0 2 2 3 1
TB64 Nasara 2 3 3 3 3 5 0 2 2 6 1
TB65 Niger 2 1 3 3 5 0 2 2 6 1
TB52 Niger 2 4 6 2 5 1 2 2 2 1
TB57 FCT 2 6 3 5 5 0 2 2 2 2
TB5 FCT 2 6 4 4 6 0 5 2 0
TB81 Niger 3 3 4 1 1 0 2 2 2 0
TB67 Benue 2 3 3 2 3 5 0 4 3 6 1
TB70 Kwara 2 1 3 5 2 3 8 0
TB71 FCT 2 1 2 3 1 5 0 2 3 6 0
TB72 Niger 2 1 2 3 0 5 0 2 3 6 0
TB73 Kogi 3 0 4 1 5 0 2 3 3 0
TB74 Niger 3 0 4 3 5 0 2 3 3 0
TB75 Kogi 3 0 3 3 5 0 3 3 0
TB58 Kwara 1 2 3 3 5 6 1 2 4 2 2
TB59 Plat 2 2 6 3 5 6 0 3 2 3 2
TB60 Benue 2 2 3 5 6 0 3 4 3 2
TB85 Plat 2 3 2 4 2 6 1 4 2 1
TB86 Kwara 3 2 4 1 6 1 4 4 3 1
TB89 Kwara 2 2 3 6 1 4 4 3 1
TB87 Nasara 2 3 4 1 2 6 1 2 3 1
0.1
0 10 20 30 40 50 60 70 80 90 100
% Genetic Similarity

Figure 2: Dendrogram based on 12 MIRU-VNTR typing data for 66 MTBC isolates obtained from North Central Nigeria between November 2017 and December 2018.

60% similarity cut-off indicated by blue line

Red lines indicate isolates grouped at 60% similarity cut-off

Table 2: Cluster analysis and genetic diversity of MTBC isolates (obtained from North Central Nigeria between November 2017 and December 2018) at 60% similarity coefficient cut-off

Cluster ID	Frequency of Isolates	Source						
		Niger	FCT	Kwara	Kogi	Nasarawa	Plateau	Benue
1.	3		2		1			
2.	9		3	3	1	2		
3.	9	4	2			2		1
4.	2		1					1
5.	2	1		1				
6.	3		1					2
7.	5	2	1			1		1
8.	2	1		1				
9.	6	1	2			1	1	1
10.	5	2	1			1		1
11.	3	1	1	1				
12.	3	1			2			
13.	2						1	1
14.	3			2			1	
Singletons	9	2	2	2		1		2
Total	66	15	16	10	4	8	3	10
HGDI*	0.94	0.92	0.95	0.91	0.83	0.93	1.00	0.97

HGDI = Hunter-Gaston Index

FCT = Federal Capital Territory

Based on the results of the MIRU-VNTR typing ten genotypes were identified (Table 3). UgandaII (Lineage 4) was the most commonly identified (14, 21.2%) while UgandaI (Lineage 4) was the least common (1, 1.5%). Bovis and Cameroon (Lineage 4) were the next most commonly identified with levels of 10 (15.1%) and 6 (9.1%) respectively. No genotype was present in all the sampled North Central Nigerian states. Majority of the genotypes belonged to Lineage 4 (46.9%, 31/66)

Table 3: Occurrence and Distribution of *M. tuberculosis* genotypes in test in test isolates obtained from North Central Nigeria between November 2017 and December 2018

S/N	Species	Genotype (Lineage)	Number (%)	State Distribution
1.	*M. africanum*	West African 1 (5)	7 (9.1)	Benue 2, FCT 2, Kwara 2, Nasarawa 1

2.	*M. bovis*	Bovis (Bovis)	10 (15.1)	Benue 2, Kogi 1, Kwara 2, Nasarawa 2, Niger 3,
3.	*M. tuberculosis*	Uganda II (4)	14 (21.2)	Benue 2, FCT 4, Kwara 4, Nasarawa 2, Niger 1, Plateau 1
4.	*M. tuberculosis*	Cameroon (4)	6 (9.1)	Benue 1, FCT 2, Niger 3
5.	*M. tuberculosis*	S (4)	5 (7.6)	FCT 1, Kogi 2, Nasarawa 1, Niger 1
6.	*M. tuberculosis*	LAM (4)	3 (4.5)	FCT 2, Kwara 1
7.	*M. tuberculosis*	Haarlem (4)	2 (3.0)	FCT 1, Niger 1
8.	*M. tuberculosis*	EAI (1)	2 (3.0)	Nasarawa 1, Niger 1
9.	*M. tuberculosis*	Uganda I (4)	1 (1.5)	Niger 1
10.	*M. tuberculosis*	NEW-1	1 (1.5)	FCT 1
11.	*M. tuberculosis*	Mixed	13 (19.7)	Benue 3, FCT 2, Kogi 1, Kwara 1, Nasarawa 1, Niger 3, Plateau 2
12.		Unassigned	2 (3.0)	FCT 1, Niger 1

Mixed indicates 2 genotypes predicted

State distribution of test isolates is: Benue: 10, FCT: 16, Kogi: 4, Kwara: 10, Nasarawa: 8, Niger: 15, Plateau: 3

Discussion

Typing of MTBC strains is essential for understanding transmission, and invaluable for control. This study reports on the genotyping of 106 MTBC isolates from North Central Nigeria obtained between November 2017 and December 2018 using the 12-locus MIRU-VNTR typing method. Results showed that the allelic diversity as determined by the Hunter-Gaston diversity index for each locus ranged from 0.21 to 0.81 thought 83.4% of loci had values deemed to be highly and moderately discriminant. MIRU-VNTR typing revealed a high diversity, with a clustering rate of 1.9% and a total of ten different genotypes, though in general genotypes belonging to Lineage 4 were more common (46.9%, 31/66).

The 12-locus MIRU-VNTR has been widely used as a typing tool. Despite its lower discriminatory power compared to the 15-locus and 24 locus methods it is still quite relevant as this method has a higher resolution and performs better than spoligotyping in addition to its cost advantage.[25,26] One of the limitations associated with this technique is amplification failure[27] as this leads to reduced typeability. A similar phenomenon was noted in this present study. A recent study by Melo and colleagues[28] carried out in Brazil also noted such a phenomenon and attributed it to possible degradation of the genetic material or mutations in the primer binding region of the specific loci. Also, Ei and colleagues postulated that the phenomenon could be as a result of deletions in the region.[29] The concept of degradation of genetic material was demonstrated in a 2019 study by Adesokan and colleagues[13] where

amplification failure was noted for all loci in a single isolate. Further research could be done to determine which of these reasons caused the phenomenon as observed in our test isolates.

Variations in allelic diversity have always been noted, with some loci known to have more diversity than others. This study's observation that MIRU 02 had the lowest allelic diversity (0.21) was not unexpected as similar results have been reported. One of the original studies assessing the validity of the MIRU-VNTR methodology in typing MTBC[16] reported a 0.1 allelic diversity for MIRU 02 while several of other studies have also reported low levels ranging from 0.015 to 0.388.[16,30,31] Conversely, while the allelic diversity of MIRU 04 was below 0.3 in this study, in the 2006 Supply study,[16] values of 0.38 and 0.55 were reported. Yun and colleagues also reported a much lower value of 0.096 and in a more recent study by Shi and colleagues, MIRU 04 was actually identified as one belonging to the ten most discriminatory loci.[30,31] The low levels of allelic diversity as noted in MIRU 02 and the subsequent clustering of possibly unrelated strains (false clustering) necessitated the development of the newer variants of this technique through the incorporation of more loci. Similar to previous reports,[16,30,31] MIRU 26 in this study was highly discriminatory with an HGI value of 0.81. It is one of the more discriminatory loci as noted by its inclusion in the 15-locus MIRU-VNTR method which only included the best 6 loci from the original 12-locus variant. These differences in diversity indices between studies might represent location-based variations in MTBC genomes. The Shi 2018 report also appears to indicate lineage-based variations, with the Beijing family generally having higher discriminatory indices than non-Beijing family isolates.[31]

Studies on the population structure of MTBC in various regions around the world have often shown a predominance of some lineages in specific regions. For example, the Beijing strain which is one of the most predominant of the strains has been found to dominate in the East and South East Asia, as well as Eastern Europe and South Africa.[22,32-34] It has however been described less often in most of Africa,[5,23,35] though a study from Kenya reported it as one of the more prevalent lineages.[36] The first mention of this Beijing strain in Nigeria was only just noted in 2021 and it was detected by whole genome sequencing.[37] This present study confirms this trend as the Beijing lineage was absent among the test strains.

A recent review on transmission dynamics of MTBC in Africa[38] noted that an average of 7% of TB cases were caused by *Mycobacterium africanum* and this was similar to the 9.1% observed in this present study. In general, strain diversity noted in this study was similar to previous reports from within Nigeria and in nearby African countries.[3,5,8,39] Unlike these reports however, the Cameroon genotype was not the most dominant strain detected in this study. The Cameroon genotype, which was originally erroneously grouped as part of the Latin American Mediterranean (LAM) strains, was more recently recognized as a family of its own found predominantly in Cameroon and West African nations and some parts of the Caribbean. It has since been described in Nigeria as replacing *M. africanum* strains albeit via spoligotyping.[3,40]

UgandaII along with UgandaI were originally thought to be subtypes of *M. africanum* but later reclassified as *M. tuberculosis* variants.[41] These strains have formerly been reported as predominant in East Africa,[42,43] though they have also been detected in West Africa.[44,45] Considering that most of these studies determined lineages by spoligotyping rather than MIRU-VNTR, it would be interesting to note the effect of methodology on classification.

Limitations

Despite its higher resolution than spoligotyping, one major limitation of this study has to do with the limitations associated with the 12-locus MIRU-VNTR as a method specifically in the area of amplification failures which results in poorly discriminant allelic diversity for some of the loci. If the resources are present, the 15-locus and 24 locus MIRU-VNTR variants are preferred. An additional limitation of this study is the absence of epidemiological data making it difficult to determine transmission dynamics.

Conclusion

The 12-locus MIRU-VNTR provided an adequate typing tool for assessing the genetic diversity of MTBC in this study as evidenced by the 0.94 Hunter-Gaston diversity index. This study notes a high diversity of MTBC strains circulating in North Central Nigeria with the 10 genotypes observed indicating multiple introductions of this pathogen to this region. This study confirmed the lack of the Beijing lineage and an unexpected preponderance of the UgandaII lineage using the 12-locus MIRU-VNTR method.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Sources of support

None

Authors' contributions

T.V.O. was the project leader. S.S.M and K.O. were responsible for project and experimental design. S.S.M. carried out most of the laboratory investigations. K.O. managed the analyses of the data, managed the literature searches, and wrote the first draft of the manuscript. All authors read and approved the final manuscript

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