Clonal Diversity and Spatial Dissemination of Drug-Resistant *Mycobacterium tuberculosis* Complex among HIV Seropositive Patients in Southwest Nigeria

Abstract

Mycobacterium tuberculosis complex display relatively static genomes and 99.9% nucleotide sequence identity. Studying the evolutionary history of such monomorphic bacteria is a difficult and challenging task. Therefore, this study was undertaken to investigate the clonal diversity and spatial dissemination of drug-resistant *M.tuberculosis* complex among HIV seropositive clients in Southwest Nigeria. A total of two-hundred and seventeen (217) consented HIV positive (case subjects) and fifty (50) non-HIV positive controls were recruited for this study. Two Sputum samples were collected from each participant in two consecutive days. The samples were divided into two parts: one part was decontaminated using NAOH-NALC method and aseptically cultured for Mycobacteria on Lowenstein-Jensen (LJ) medium with and without pyruvate. Mycobacterial isolates were characterized using conventional and molecular methods. Anti-TB drug sensitivity test was done using 1% proportion conventional method on LJ. MTBC isolates were sequenced using Genetic Analyzer 3130xl sequencer. Genetic relatedness and clonal diversity (phylogenetic analysis) of MTBC isolates were done using Bio- Edit software and MEGA 6. Out of 217 HIV positive participants, 105(48.4%) were male, while 112(51.6%) were female. Twenty-six (26(52.0%)) of the control subjects were male, while 24(48.0%) were females. The overall prevalence of TB among PLHIV was found to be 19.1%. The prevalence of NTM among PLHIV was found to be 2.8% with *M.intracellulare* being the predominant organism isolated. Participants' age was found to be significantly associated with TB ($P<0.05$), however there was no significant association between TB and sex of the participants ($P<0.05$). The prevalence of culture confirmed drug-resistant TB (DR-TB) among PLHIV was found to be 11.9%, with Rifampicin having the highest monoresistance rate of 5.5%. The overall prevalence of MDR-TB among PLHIV was found to be 9.2%. The phylogenetic analysis of *M.tuberculosis* strains revealed same clone of all isolates. All PLHIV should be screened for drug-resistant TB especially MDR-TB as the prevalence of primary MDR-TB is high among them. Further study like 3R gene-based studies of adaptation and evolution is needed to facilitate further epidemiological studies of these bacteria.

Keywords: ***Mycobacterium tuberculosis*, Multi-Drug resistant, Clonal diversity, HIV seropositive patients.**

Introduction

Nigeria, with population of around 200 million was ranked 6th in the list of countries with the highest number of TB patients in the world according to a 2021 report by the World Health Organization (WHO, 2022). TB kills around 125,000 Nigerians every year. According to WHO estimates, the number of MDR-TB cases vary between 3.6-15% in high TB burden countries including Nigeria (WHO Africa, 2023).

Primarily, TB treatment relies on two important antibiotics, isoniazid (INH) and rifampicin (RIF). These two antibiotics are first line defense against TB and are given in combination with

ethambutol (EMB) and streptomycin (STR). Due to the evolution of resistant strains, TB patient cannot be treated with two first-line antibiotics, and thus, second-line drugs like fluoroquinolone may be prescribed (Dagne *et al.,* 2021). TB control is difficult due to the fact that it takes six months or even more for complete treatment. Due to prolong treatment duration, some patients are unable to take the drugs for specified time and they become resistant to first line drugs. This treatment for long duration is necessary to kill the slowly growing bacilli (Conradie *et al*., 2020).

Moreover, one of the major causes of failure to effective solutions are human immunodeficiency virus (HIV) infection, epidemics of TB and delay in diagnosis and proper treatment (Naidoo et al., 2019). In 2022, the estimated number of incident cases of TB were about 10.6 million, deaths from TB among HIV-negative patients were1.13 million and deaths from HIV-positive TB were about 0.17 million (WHO, 2023).

Rapid detection of RIF resistance is of particular importance because it is one of the important surrogate markers for MDR resistance (Nandlal *et al*., 2022). Mutations in rpoB gene have been associated with resistant to RIF in Mycobacterium tuberculosis isolates. The main target of RIF is rpoB gene encoding the beta subunit of RNA polymerase. Studies have shown that about 98% of RIF resistant isolates have mutations in rpoB gene. More than 90% of the mutations have been found in 81 bp (codons 507 to 533) core region of RNA polymerase beta subunit (rpoB) gene. Therefore, to identify these mutations, it will be of great help to use assays that use microarrays and the line probe assay are required rather than expensive methods like sequencing (Nguyen et al., 2019). As the mutations types for RIF resistant Mycobacterium tuberculosis isolates varies from country to country therefore, the resistance mutations distribution at the level of each country should be determined before the introduction of routine molecular tests for diagnostics purposes (WHO, 2021; Nguyen *et al.,* 2019).

In isoniazid resistance in the other hand, more complex genetic system containing several genes are involved (Munir *et a*l., 2019). In Mycobacterium tuberculosis isolates, mutation in katG gene encoding catalase-peroxidase enzyme is most frequently associated INH resistance as demonstrated by extensive studies. INH, one of the important first line drugs is in inactive form and catalytic conversion is required to convert it into its active form. This function is accomplished by an enzyme catalase-peroxidase in Mycobacterium tuberculosis isolates (Purkan *et al*., 2020).

This study was therefore necessary as a documentation of representative baseline data of the burden and pattern of MDR-TB among PLHIV in Ibadan and exploration of preventable risk factors that could be used to design, implement and evaluate better preventive and therapeutic interventions.

Materials and Methods

Study Area

The University College Hospital (UCH) is located in Ibadan, the capital city of Oyo State in southwestern Nigeria. Ibadan is the largest city in the country with about 4,004,000 population and is known for its rich history and cultural significance. The geographical coordinates of Ibadan are approximately 7.3776° N latitude and 3.9470° E longitude. It has over 1,000 bed spaces and 163 examination couches

Study Population

The target population included individuals attending the University College Hospital in Ibadan, Nigeria, who are living with HIV, as well as people without HIV infection who served as the control group. Informed consent was obtained from each subject before their inclusion in the study. A total of two-hundred and seventeen (217) consented HIV positive (case subjects) and fifty (50) non-HIV positive controls were recruited for this study.

Eligibility Criteria

Inclusion Criteria

HIV Positive subjects. HIV positive subjects who are not on anti-TB drugs

Exclusion Criteria

Non-HIV positive subjects. HIV positive subjects who are on anti-TB drugs

Sample Size Estimation

The Leslie and Kish formula for descriptive studies was used to calculate the sample size (Kish, 2017):

$N = Z^2 pq/d^2$

Where N = sample size

Z = the standard deviation which corresponds to the 95% confidence interval which is 1.96

P = prevalence of Mycobacterium tuberculosis infection of 17% (0.17) among hospital-based subjects in Ibadan. This was obtained from a retrospective study done at St. Mary's hospital, Ibadan between years 2016 and 2018 (Adetunji, 2020).

Q = 1-P

D = level of precision, which is 0.05

$N = (1.96)^2 \times 0.17(1-0.17) / 0.05^2$

$N = 3.8416 \times 0.17(0.83) / 0.0025$

$N = 3.8416 \times 0.1411 / 0.0025$

$N = 0.5421 / 0.0025$

$N = 216.84 \approx 217$ (Approximately)

Adjusting sample for 10% attrition rate (Walter,S.D.,)

N = 241

Thus, a total of 217 people living with HIV will be recruited for this study.

A total of 50(Fifty) blood and sputum samples were also collected from non-HIV subjects (apparently healthy individuals) which serves as controls

Sample Collection

Sputum samples from a deep cough were collected among known HIV positive patients on ART, attending HIV clinic at UCH, Ibadan. Each subject was asked to spit into standard screw-capped leak-proof sputum container with specific clinic identification number, with each person producing and submitting 2 sputum specimens within two consecutive days. The first sputum specimen was obtained on the first contact with the centre (spot specimen) while the second specimen was an early-morning specimen produced at home after cleaning the mouth with water. The two sputum specimens were processed at the same time.

Sample Assay

The samples were divided into two parts: one part was decontaminated using NAOH-NALC method and aseptically cultured for Mycobacteria on Lowenstein-Jensen (LJ) medium with and without pyruvate. Mycobacterial isolates were characterized using conventional and molecular methods. Antibiotic susceptibility test was performed using 1% proportion conventional method on LJ, as described by Akiko (2021), NIMR (2023) and FMOH (2021). The *Mycobacterium tuberculosis* isolates were tested against Isoniazid (Fisher), Rifampicin (Sigma), streptomycin (Sigma) and ethambutol (Sigma), using Hain Line Probe Assay (MTBDR*plus* and MTBDR*sl*). Pure drug crystals of Isoniazid, Rifampicin, and Ethambutol were produced by Sigma scientific laboratories and Fisher Laboratory, USA as indicated in front of each drug above. MTBC isolates were sequenced using Genetic Analyzer 3130xl sequencer. Genetic relatedness and clonal diversity (phylogenetic analysis) of MTBC isolates were done using Bio- Edit software and MEGA 6.

Data analysis:

Analyses of all obtained data were performed using STATA/IC version 23.0. The x^2 test was used to calculate *p* value when appropriate. *P* values <0.05 was considered statistically significant.

Results

Table1 showed that 105(48.4%) were male, while 112(51.6%) were females. Moreover, 26(52.0%) of the control subjects were male, while 24(48.0%) were females. The mean age with SD was 42.95 ± 8.286 years with a range of 28-65 years, while the control group had a mean age with SD of 42.24 ± 4.745 years with a range of 35-50 years.

Table 2 showed the relationship between Gender and Phenotypic DST. There was no significant association between phenotypic anti-TB drug resistance, and gender (*P*>0.05). However, males had higher resistance rate in Isoniazid and Ethambutol when compared to females, while females had higher resistance rate in other drugs compared to males.

Table 3 showed that there was no significant association between age and anti-TB drug resistance. However, higher Rifampicin resistance rate was 37.5% was found among age group < 40 years

compared to 18.2% for age group > 40years, though not statistically significant (*P*>0.05). Similarly, higher resistance rate of 18.8%, 12.5%, 16.2% and 6.2% were recorded for Isoniazid, Ethambutol, Levofloxacin and Etionamide respectively, when compared with 5% rate for Isoniazid, 2% for Ethambutol, 1% for Levofloxacin, and 2% for Etionamide in age group > 40years.

Table 4 that there was no significant association between genotypic anti-TB drug resistance, and gender (P>0.05). However, Females had higher Rifampicin resistance rate of 22.5% compared to 9.5% rate for males. Similarly, females had higher flouroquinolones resistance rate of 9.1% compared to 4.8% recorded for males. But Males had higher Isoniazid resistance rate of 9.5%, while females had 9.1%.

Table 5 showed age and LPA (Genotypic) DST. There was no significant association between genotypic anti-TB drug resistance and age group (P>0.05). However, higher resistance rate of 20.0% and 13.3% for Rifampicin and Isoniazid respectively were found in age group <40 years compared to 14.3% and 3.6% found in age group >40 years.

Table 6 showed that there was no significant association between GeneXpert Rifampicin resistance and sex of the subjects (P>0.05). However, slightly higher Rifampicin resistance rate of 1.9% was found in males when compared with 1.8% for females.

Table 7 showed relationship between age and GeneXpert Rifampicin resistance. There was no significant association between GeneXpert Rifampicin resistance and age of the subjects (*P*>0.05). However, slightly higher Rifampicin resistance rate of 2.2% was found in age group <40 years compared to 1.6% for age group >40 years.

Table 8 shows the NCBI Blast sequence identity of the isolates' edited sequences. All the isolates had query cover ranging from 99% to 100% with percentage identity from 99.86% to 100%. Isolates included *M.tuberculosis, M.bovis, M.intracellulare, M.kansasi,* and *M.avium* sub sp *paratuberculosis.*

Figure 1 shows the Phylogenetic Analysis of MTBC isolates. A total of two *Mycobacterium tuberculosis* complex (MTBC) were Isolated including *M.tuberculosis* and *M.bovis, and* 3 NTM viz: *M.intracellulare, M.kansasi,* and *M.avium* sub sp *paratuberculosis.* All the *Mycobacteria* species had same clone.

Table 1: Relationship between demographic variables and the study groups

Respondents' Socio-demographic Characteristics	**Cases n(%)**	**Control n(%)**	**Total n(%)**	**Pearson chi-square**	**p-value**
Sex				**0.212**	**0.754**
Male	105(48.4)	26(52.0)	131(49.1)		
Female	112(51.6)	24(48.0)	136(50.9)		
Age Group (years)				**10.154**	**0.017***
<40	90(41.5)	17(34.0)	107(40.1)		
40-49	83(38.2)	30(60.0)	113(42.3)		
50-59	34(15.7)	3(6.0)	37(13.9)		

≥60	10(4.6)	0(0.0)	10(3.7)		
Age Group (years)				**0.945**	**0.424**
<40	90(41.5)	17(34.0)	107(40.1)		
≥40	127(58.5)	33(66.0)	160(59.9)		
Mean (±SD)	42.95 ± 8.286 years	42.24 ± 4.745 years	42.82 ± 7.745 years	0.583	0.560
Range	28 to 65 years	35 to 50 years	28 to 65 years		
Total	**217**	**50**	**267**		

*P<0.05

Table 2: Relationship between Gender and Phenotypic DST

	Sex			
Phenotypic DST	**Male n(%)**	**Female n(%)**	**Pearson chi-square**	**p-value**
RIF			**0.556**	**0.520**
Sensitive	20(80.0)	17(70.8)		
Resistant	5(20.0)	7(29.2)		
INH			**0.504**	**0.702**
Sensitive	20(80.0)	21(87.5)		
Resistant	5(20.0)	3(12.5)		
ETB			**1.180**	**0.349**
Sensitive	24(96.0)	21(87.5)		
Resistant	1(4.0)	3(12.5)		
LEV			**0.001**	**1.000**
Sensitive	24(96.0)	23(95.8)		

Resistant	1(4.0)	1(4.2)		
ETH			**0.313**	**1.000**
Sensitive	23(92.0)	23(95.8)		
Resistant	2(8.0)	1(4.2)		
Total	**25(100.0)**	**24(100.0)**		

**P>0.05*

Table 3: Relationship between Age and Phenotypic DST

	Age Group (Years)			
Phenotypic DST	**<40 years (n=90)**	**≥40 years (n=127)**	**Pearson chi-square**	**p-value**
RIF			**2.175**	**0.169**
Sensitive	10(62.5)	27(81.8)		
Resistant	6(37.5)	6(18.2)		
INH			**0.102**	**1.000**
Sensitive	13(81.2)	28(84.8)		
Resistant	3(18.8)	5(15.2)		
ETB			**0.596**	**0.588**
Sensitive	14(87.5)	31(93.9)		
Resistant	2(12.5)	2(6.1)		
LEV			**0.285**	**1.000**
Sensitive	15(93.8)	32(97.0)		

Resistant	1(6.2)	1(3.0)		
ETH			**0.001**	**1.000**
Sensitive	15(93.8)	31(93.9)		
Resistant	1(6.2)	2(6.1)		
Total	**16(100.0)**	**33(100.0)**		

*$P<0.05$ (i.e. Significant)

Table 4: Relationship between Gender and Line Probe Assay (LPA)

Phenotypic DST	**Sex**			
	Male n(%)	**Female n(%)**	**Pearson chi-square**	**p-value**
RIF			**0.556**	**0.520**
Sensitive	20(80.0)	17(70.8)		
Resistant	5(20.0)	7(29.2)		
INH			**0.504**	**0.702**
Sensitive	20(80.0)	21(87.5)		
Resistant	5(20.0)	3(12.5)		
ETB			**1.180**	**0.349**
Sensitive	24(96.0)	21(87.5)		
Resistant	1(4.0)	3(12.5)		

LEV			**0.001**	**1.000**
Sensitive	24(96.0)	23(95.8)		
Resistant	1(4.0)	1(4.2)		
ETH			**0.313**	**1.000**
Sensitive	23(92.0)	23(95.8)		
Resistant	2(8.0)	1(4.2)		
Total	**25(100.0)**	**24(100.0)**		

*P<0.05 (i.e. Significant)

Table 5: Relationship between Age and Line Probe Assay (LPA)

Age Group (Years)	**<40 years (n=15)**	**≥40 years (n=28)**	**Pearson chi-square**	**p-value**
RIF			**0.234**	**0.680**
Sensitive	12(80.0)	24(85.7)		
Resistant	3(20.0)	4(14.3)		
INH			**0.190**	**1.000**
Sensitive	14(93.3)	25(89.3)		
Resistant	1(6.7)	3(10.7)		
FLQ			**1.434**	**0.275**
Sensitive	13(86.7)	27(96.4)		
Resistant	2(13.3)	1(3.6)		
Total	**15(100.0)**	**28(100.0)**		

*P<0.05 (i.e. Significant)

Table 6: Relationship between Gender and GeneXpert Rifampicin Resistance

Sex	**N**	**Rifampicin Resistance n (%)**	**Pearson chi-square**	**p-value**
Male	105	2(1.9)	0.067	0.987
Female	112	2(1.8)		

*P<0.05

Table 7: Relationship between Age and GeneXpert Rifampicin Resistance

Age Group (years)	N	Rifampicin Resistance n (%)	Pearson chi-square	p-value
<40	105	2(2.2)	1.259	0.987
≥40	112	2(1.6)		

*P<0.05

Table 8: HIV Patients' demographic characteristics and Haematological Parameters (Cases)

Haematological parameters	Male (n=105)	Female (n=112)	t-test	p-value
WBC (X10^6)	8.22 ± 6.07	8.40 ± 5.17	0.230	0.818
RBC (X10^9)	4.35 ± 0.84	4.51 ± 1.39	1.042	0.299
Hb Conc	10.75 ± 2.24	11.07 ± 2.18	1.096	0.274
HCT/PCV	34.09 ± 7.13	33.95 ± 6.90	0.152	0.879
Platelet	263.83±129.88	263.99±101.03	0.010	0.992
Neutrophils	13.20±9.13	13.44±10.56	0.183	0.855
Lymphocytes	35.55±17.31	34.16±16.23	0.611	0.542
Monocytes	51.93±18.47	52.33±18.64	0.159	0.874
	Age Group (years)			
	<40 years (n=90)	**≥40 years (n=127)**	**t-test**	**p-value**
WBC (X10^6)	9.20±6.87	7.68±4.44	1.969	0.050*
RBC (X10^9)	4.51±1.18	4.38±1.14	0.793	0.429
Hb Conc	11.04±2.25	10.83±2.19	0.684	0.495
HCT/PCV	34.71±7.98	33.52±6.19	1.234	0.219
Platelet	268.93±105.91	260.35±122.31	0.538	0.591
Neutrophils	11.80±8.05	14.40±10.89	1.922	0.056
Lymphocytes	33.48±17.91	35.79±15.86	1.000	0.318
Monocytes	54.57±18.31	50.42±18.55	1.631	0.104

*P<0.05 (i.e. Significant)

Table 9: HIV Patients' demographic characteristics and Biochemical Parameters (Cases)

Biochemical parameters	Male (n=105)	Female (n=112)	t-test	p-value
Serum Sodium	135.39±4.05	134.72±12.06	0.543	0.588
Potassium	3.81±0.56	3.85±0.41	0.607	0.544
Chloride	98.62±7.01	99.41±7.63	0.793	0.428
Bicarbonate	26.08±2.03	26.14±2.42	0.226	0.822
Urea	25.70±8.46	26.91±8.05	1.071	0.285
Creatinine	0.79±0.94	0.68±0.14	1.152	0.251
ALT	26.24±16.52	29.34±23.15	1.131	0.260
AST	42.98±49.76	44.35±50.12	0.201	0.841
ALP	321.97±84.59	309.92±92.02	1.003	0.317

Total_Bilirubin	5.59±4.88	5.03±3.87	0.945	0.346
Direct_Bilirubin	2.61±2.44	2.37±1.92	0.820	0.413
Total_Cholesterol	150.48±38.70	148.48±37.03	0.390	0.697
HDL	50.66±14.66	49.90±15.09	0.376	0.707
LDL	43.31±7.91	44.11±7.42	0.770	0.442
Triglyceride	123.56±20.63	125.26±22.33	0.580	0.562
	Age Group (years)			
	<40 years (n=90)	**≥40 years (n=127)**	**t-test**	**p-value**
Serum Sodium	135.55±3.46	134.68±11.53	0.700	0.485
Potassium	3.95±0.55	3.75±0.42	3.034	0.003*
Chloride	99.69±7.77	98.64±7.00	0.943	0.347
Bicarbonate	25.94±2.21	26.23±2.25	0.925	0.356
Urea	26.85±8.45	25.95±8.13	0.781	0.436
Creatinine	0.69±0.14	0.77±0.86	0.854	0.394
ALT	29.33±24.63	26.78±16.44	0.913	0.362
AST	41.12±41.85	45.51±54.88	0.639	0.524
ALP	314.15±81.06	316.89±93.72	0.224	0.823
Total_Bilirubin	5.20±3.26	5.37±5.05	0.279	0.780
Direct_Bilirubin	2.44±1.60	2.52±2.52	0.252	0.801
Total_Cholesterol	145.37±31.47	152.34±41.55	1.342	0.181
HDL	50.65±15.84	50.00±14.18	0.318	0.751
LDL	42.82±6.89	44.36±8.12	1.461	0.145
Triglyceride	121.71±16.29	126.37±24.40	1.581	0.115

*P<0.05 (i.e. Significant)

Table 10: HIV Patients' demographic characteristics and CD4 Count (Cases)

Parameter	**Male (n=105)**	**Female (n=112)**	**t-test**	**p-value**
CD4 Count	443.46±211.31	495.77±319.53	1.413	0.159
Viral load	25820.45±87409.99	63081.73±213905.55	1.660	0.098
	Age Group (years)			
Parameter	**<40 years (n=90)**	**≥40 years (n=127)**	**t-test**	**p-value**
CD4 Count	456.07±259.77	480.65±282.95	0.652	0.515
Viral load	72601.43±236448.45	25529.01±82433.77	2.074	0.039*

*P<0.05 (i.e. Significant)

Table 11: Culture results and CD4 Counts (Cases only)

	Culture results			
Parameters	**Positive (n=49)**	**Negative (n=168)**	**t-test**	**p-value**
CD4 Counts	419.74±298.87	485.25±264.40	1.481	0.140
Viral load	41083.76±124516.26	46209.58±176518.59	0.190	0.850

*P <0.05 (i.e. Significant)

Table 12: NCBI Blast showing the sequence identity of the isolates edited sequences

sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
S3186	Mycobacterium tuberculosis	2686	2686	99%	0	99.86%	PP096769
S1846	Mycobacterium tuberculosis	2682	2682	99%	0	99.93%	PP096770
S3060	Mycobacterium tuberculosis	2695	2695	99%	0	99.86%	PP096771
S3532	Mycobacterium tuberculosis	2684	2684	99%	0	99.93%	PP096772
S1961	Mycobacterium tuberculosis	2678	2678	99%	0	100.00%	PP096773
S1847	Mycobacterium tuberculosis	2675	2675	99%	0	99.86%	PP096774
S1541	Mycobacterium tuberculosis	2697	2697	99%	0	99.86%	PP096775
S1819	Mycobacterium tuberculosis	2680	2680	99%	0	100.00%	PP096776
S1966	Mycobacterium tuberculosis	2676	2676	99%	0	99.86%	PP096777
S849	Mycobacterium tuberculosis	2678	2678	99%	0	99.86%	PP096778
S1009	Mycobacterium tuberculosis	2682	2682	99%	0	99.86%	PP096779
S115	Mycobacterium tuberculosis	2682	2682	99%	0	99.93%	PP096780
S19	Mycobacterium tuberculosis	2702	2702	99%	0	100.00%	PP096781
S49	Mycobacterium tuberculosis	2693	2693	99%	0	99.93%	PP096782
S109	Mycobacterium tuberculosis	2684	2684	99%	0	100.00%	PP096783
S151	Mycobacterium tuberculosis	2699	2699	99%	0	99.86%	PP096784
S29	Mycobacterium tuberculosis	2695	2695	99%	0	100.00%	PP096785

S153	Mycobacterium tuberculosis	2689	2689	99%	0	99.86%	PP096786
S155	Mycobacterium tuberculosis	2697	2697	99%	0	99.93%	PP096787
S114	Mycobacterium tuberculosis	2682	2682	99%	0	99%	PP096788
S105	Mycobacterium tuberculosis	2682	2682	99%	0	100%	PP096789
S48	Mycobacterium tuberculosis	2695	2695	99%	0	100%	PP096790
S108	Mycobacterium tuberculosis	2684	2684	99%	0	100%	PP096791
S47	Mycobacterium tuberculosis	2678	2678	99%	0	100%	PP096792
S107	Mycobacterium tuberculosis	2675	2675	99%	0	100%	PP096793
S111	Mycobacterium tuberculosis	2697	2697	99%	0	100%	PP096794
S30	Mycobacterium tuberculosis	2680	2680	99%	0	100%	PP096795
S112	Mycobacterium tuberculosis	2676	2676	99%	0	100%	PP096796
S44	Mycobacterium tuberculosis	2678	2678	99%	0	100%	PP096797
S23	Mycobacterium tuberculosis	2682	2682	99%	0	100%	PP096798
S06	Mycobacterium tuberculosis	2682	2682	99%	0	100%	PP096799
S152	Mycobacterium tuberculosis	2702	2702	99%	0	100%	PP096800
S103	Mycobacterium tuberculosis	2693	2693	99%	0	100%	PP096801
S10	Mycobacterium tuberculosis	2684	2684	99%	0	100%	PP096802
S71	Mycobacterium tuberculosis	2699	2699	99%	0	100%	PP096803
S110	Mycobacterium tuberculosis	2695	2695	99%	0	100%	PP096804
S102	Mycobacterium tuberculosis	2689	2689	99%	0	100%	PP096805
S104	Mycobacterium tuberculosis	2697	2697	99%	0	100%	PP096806
S008	Mycobacterium tuberculosis	2695	2695	99%	0	100%	PP096807
S25	Mycobacterium tuberculosis	2695	2695	99%	0	100.00%	PP096808

S116	Mycobacterium tuberculosis	2676	2676	99%	0	99.93%	PP096809
S51	Mycobacterium tuberculosis	2680	2680	99%	0	100.00%	PP096810
S106	Mycobacterium tuberculosis	2699	2699	100%	0	99.93%	PP096811
S01	Mycobacterium tuberculosis	2706	2706	99%	0	100.00%	PP096812
S03	Mycobacterium tuberculosis	2699	2699	99%	0	99.93%	PP096813
S08	Mycobacterium tuberculosis	2700	2700	99%	0	100.00%	PP096814
S100	Mycobacterium tuberculosis	2710	2710	100%	0	100.00%	PP096815
S27	Mycobacterium tuberculosis	2700	2700	100%	0	100.00%	PP096816
S12	Mycobacterium tuberculosis	2691	2691	99%	0	100.00%	PP096817
S20	Mycobacterium tuberculosis	2693	2693	99%	0	100%	PP096818

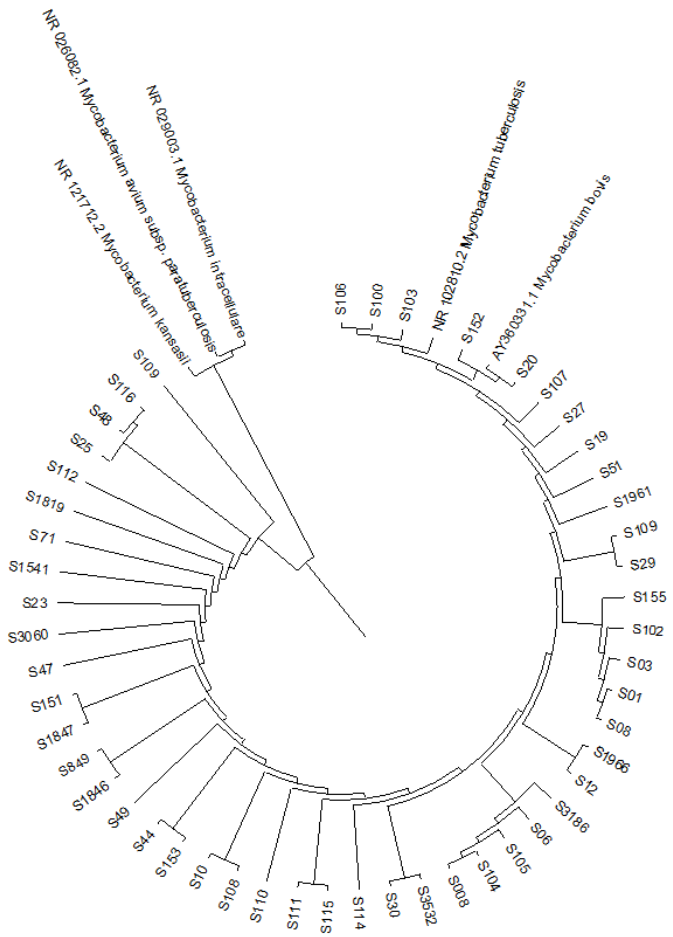

Figure 1: Phylogenetic Analysis of MTBC isolates

Discussions

TB remains a global public health problem and one of the top ten leading causes of death, worldwide, with developing countries bearing the highest burden(WHO,2020).Unfortunately, the problem of TB in Nigeria has been complicated by emergence and spread of drug resistant TB and a high burden of HIV/AIDS (NTBLCP,2017; WHO, 2021).

Accurate and early diagnosis of TB is key to reducing the mortality associated with the disease (Ulu et al.,2024). GeneXpert and Culture media are techniques for many microbiologists to detect *M.tuberculosis*. In this study, culture had significantly higher M.tuberculosis positivity rate of 16.1% compared to 12.9% for geneXpert. This is lower than culture positivity rate of 31.3% and GeneXpert positivity rate of 30% reported by Ulu et al. (2024) in a similar study conducted in Bingham University, Nasarawa State, Nigeria. However, the difference in the findings could be due to quality of specimens used for the study and the paucibacillary nature of PLHIV. Although GeneXpert is a rapid technique compared to other diagnostic tools for MTB identification due to false-negative results, the culture media technique is still considered the gold standard in detecting M.tuberculosis (Nadeem *et al.*, 2022).

This study provides important information on the patterns of MDR-TB and associated determinant predictors among pulmonary TB co-infected with HIV. HIV and MDR-TB are particularly deadly combination. Even with early diagnosis and treatment initiation, people living with HIV with MDR-TB are more likely to die (USAID, 2023). The link between MDR-TB and HIV has been important since the earliest reports of spread of MDR-TB among immunocompromised patients (WHO, 2021). HIV is a powerful risk factor for all forms of TB, drug-susceptible and drug-resistant (WHO, 2021). The 9.2% prevalence of MDR-TB obtained in this study is in agreement with the studies conducted in Zambia (9.8%) but slightly higher than 7.1% rate reported by Aynias et al (2023) in a study titled "Characteristics of TB/HIV co-infection and patterns of Multidrug-Resistance Tuberculosis in the Northwest Amhara, Ethiopia", Saudi Arabia (5%), and Tanzania (5.7%). In this study, the overall prevalence of resistance to one or more first-line anti-TB drugs among HIV patients was found to be 11.9%. This is lower than the study conducted in Zambia (23.5%) (Monde *et al*.,2023) and China (Lev *et al*.(2017). Although, drug resistant TB is primarily resulted from chromosomal alterations due to mutations or deletions, there are several associated factors that have a significant impact on the increasing emergency and transmission of drug-resistant TB strains.

Rifampicin monoresistance obtained in this study was found to be 5.5%. This is in contrast to the study of Ayanias *et al.(*2023) that reported no Rifampicin monoresistance in their study. The possible reasons for the proportion difference in drug-resistant patterns of isolates among different studies of several countries could be due to variations on the study settings, sample size, study period, diagnostic methodological techniques (culture vs susceptibility techniques), geographical location and time of sputum samples collection, laboratory setup and resources for the isolation of drug-resistant strains, TB control and prevention practice, and mutational heterogeneity in the target genes associated with anti-TB drug-resistance. Our assumption was supported by previous investigations (Mesfin et al., 2020, Shibabaw *et al*., 2020, Toru *et al*., 2022).

Human disease caused by M.*bovis* has been confirmed in African countries. HIV infection may be an important risk factor for M.*bovis* disease, and M.*bovis* has been associated with mortality among PLHIV. Non-tuberculous mycobacteria (NTM) is a common opportunistic infection in PLHIV. As obtained in this study, the occurrence of NTM in PLHIV has been reported by (Lee *et al*.,2022). Although the prevalence is low in this study, however, NTM occurred more in males (3.8%) than in females' clients (1.8%). Similarly, NTM occurred more in the age group >40 years (3.9%). These data indicated the persistence of NTM disease even in the modern *cART era*. Lee *et al,* (2022) reported that the distribution of NTM reflects geographical diversity, wherein species vary according to region and country.

Conclusion

The prevalence of multi drug resistant-tuberculosis among HIV clients was high, with Rifampicin monoresistance having the highest resistance rate among the drugs tested. Phenotypic drug resistance testing detected more anti-TB drug resistance than genotypic methods (GeneXpert and LPA). Same genotype may be driving the epidemiology of MDR-TB among People living with HIV across Southwest, Nigeria. All People living with HIV should be screened for drug-resistant TB especially MDR-TB as the prevalence of primary MDR-TB is high among them. Further study like 3R gene-based studies of adaptation and evolution is needed to facilitate further epidemiological studies of these bacteria.

Disclaimer (Artificial intelligence)

Option 1:

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Details of the AI usage are given below:

1.

2.

3.

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Conflict of interest

All authors declared that they have no financial or personal relationship(s) which may influenced them inappropriately in writing this article.

Ethical approval and consent

The study proposal was examined, approved, and permission for work was granted by the ethical committee of University College Hospital/University of Ibadan. All subjects were enlightened on the nature and purpose of the research, after which written consent was obtained from each participants.

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