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Molecular Characterization and Antibiotic Profiling of *Mycobacterium tuberculosis* Complex Isolates from Slaughtered Cattle at Yola Modern Abattoir, Adamawa State, Nigeria

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Abstract

Bovine tuberculosis (bTB), caused predominantly by members of the *Mycobacterium tuberculosis* complex (MTBC), particularly *Mycobacterium bovis*, remains a major zoonotic and economic challenge in Nigeria. Abattoir-based surveillance provides a critical opportunity to detect and characterize circulating MTBC strains at the livestock–human interface. This study aimed to molecularly characterize MTBC isolates recovered from slaughtered cattle at Yola Modern Abattoir, Adamawa State, Nigeria, using targeted next-generation sequencing (tNGS). Fifteen MTBC isolates previously confirmed by SD Bioline MPT64 antigen testing were subjected to targeted sequencing using the Deeplex® Myc-TB assay. The assay enabled simultaneous species identification, phylogenetic lineage assignment, spoligotyping, and detection of mutations associated with resistance to first- and second-line anti-tuberculosis drugs. Sequencing was performed on the Illumina MiSeq platform, and data were analyzed using the Deeplex automated bioinformatics pipeline. All fifteen isolates were identified as members of the MTBC and were classified as *Mycobacterium bovis* based on hsp65 sequence analysis, SNP-based phylogenetic lineage assignment, and spoligotyping. Composite target coverage breadth ranged from 93.9% to 100%, with high sequencing depth across target regions. Drug-resistance profiling revealed that all isolates harbored mutations in the *pncA* gene conferring resistance to pyrazinamide. Two isolates additionally carried mutations associated with ethionamide resistance. Variants of uncertain or uncharacterized significance were detected in genes associated with fluoroquinolones, linezolid, aminoglycosides, and isoniazid. The exclusive detection of *M. bovis* highlights its dominant role in bovine tuberculosis in northeastern Nigeria. The universal pyrazinamide resistance observed underscores important public health implications for zoonotic tuberculosis management. These findings demonstrate the utility of targeted next-generation sequencing for high-resolution characterization of MTBC in cattle and provide essential data to inform bTB surveillance, control strategies, and One Health interventions in Nigeria.

Keywords: *Mycobacterium tuberculosis* complec (MTBC), Deeplex Myc-TB, Bovine tuberculosis (BTB), *Mycobacterium tuberculosis* Growth Indication Tube

INTRODUCTION

Bovine tuberculosis (BTB) remains an important zoonotic and economic disease of cattle, caused mainly by members of the *Mycobacterium tuberculosis* complex (MTBC), particularly *Mycobacterium bovis*. The disease continues to pose a major challenge in low- and middle-income countries, where close human–animal interactions, consumption of unpasteurized animal products, and occupational exposure increase the risk of interspecies transmission [1,2]. In Nigeria, bovine TB is endemic, with higher occurrence reported in northern regions due to extensive pastoral production systems, transboundary cattle movement, and limited implementation of structured test-and-slaughter control programs [3,4].

Abattoirs serve as critical points for bovine TB surveillance, as infected cattle are often detected during routine post-mortem inspection. However, reliance on gross pathology alone is inadequate, as lesions may be missed or confused with those caused by other pathogens, and the approach does not allow differentiation of MTBC species or strains [2]. This diagnostic gap limits understanding of MTBC diversity and transmission dynamics at the livestock–human interface in Nigeria.

Recent advances in molecular epidemiology have improved MTBC detection and characterization. In particular, targeted next-generation sequencing (tNGS) approaches, such as the Deeplex® Myc-TB assay, enable simultaneous species identification, phylogenetic lineage assignment, and detection of genetic markers relevant to epidemiology and drug resistance directly from clinical or cultured samples [5,6]. The Deeplex assay has demonstrated high sensitivity and resolution compared with conventional genotyping methods, making it a powerful tool for detailed characterization of MTBC isolates in both human and animal tuberculosis studies [7].

Yola Modern Abattoir in Adamawa State is a major slaughter facility receiving cattle from diverse local and transboundary sources in northeastern Nigeria. Molecular characterization of MTBC isolates from slaughtered cattle at this abattoir using targeted next-generation sequencing with the Deeplex assay will provide high-resolution data on circulating MTBC species and lineages. Such information is essential for understanding the epidemiology of bovine tuberculosis in the region, assessing zoonotic transmission risk, and generating evidence to support effective disease control and public health interventions in Nigeria.

MATERIALS AND METHODS

Study Area

A cross-sectional study was carried out at the Yola modern abattoir in Yola, Adamawa State, Nigeria. Adamawa State is in the North-East geopolitical zone of Nigeria, bordered by Borno to the northwest, Gombe to the west, Taraba to the southwest, and **C**ameroon to the east. Its capital is Yola. Yola is the state capital of Adamawa State, located roughly at latitude 9.2089° N and longitude 12.4802° E. It is divided administratively into Yola North and Yola South Local Government Areas, forming the metropolitan region where major facilities such as markets, government infrastructure, and the abattoir are located.

The Yola modern abattoir, is situated between Jimeta and Yola town of Adamawa State (North Eastern Nigeria). The abattoir is owned by the Adamawa state Government, and managed by the Ministry of Livestock and Nomadic Resettlement. The abattoir is the major source of meat for the people of Yola and its environs. It lies between latitude 9^{o} 14 N of the equator and longitude 12^{o} 14 E of the Greenwich-meridian [8].

Sample Preparation and DNA Extraction

Fifteen *Mycobacterium tuberculosis* complex isolates cattle slaughtered at the Yola modern abattoir, were characterized using the Targeted Next-Generation Sequencing by Deeplex® Myc-TB Assay. This method was designed for simultaneous Mycobacterial species identification, genotyping and prediction of drug-resistance strains. The assay integrates a 24-plex PCR system that amplifies selected gene regions known to harbour mutations associated with resistance to both first-line and second-line anti-tuberculosis drugs, followed by high-throughput sequencing on an Illumina platform and automated bioinformatic analysis.

DNA Extraction and Quantification

Fifteen (15) MTBC cultures previously confirmed using SD Bioline MPT 64 antigen kit, were used for DNA extraction. Bacterial colonies were harvested from Lowenstein–Jensen (LJ) slants using sterile loops and suspended in 400 µL of nuclease-free water. Heat-inactivation was performed at 95 °C for 30 min to ensure biosafety, followed by centrifugation at 12,000×g for 5 min. Genomic DNA was extracted from the pellet using the GenoLyse® kit (Hain Lifescience, Nehren, Germany) according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA) and confirmed with the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). Samples with an A260/A280 ratio between 1.8 and 2.0 and concentrations ≥ 0.2 ng/µL were considered suitable for amplification.

Targeted Multiplex PCR Amplification

Each of the fifteen isolate was amplified using the Deeplex® Myc-TB 24-plex primer mix, which targets 18 gene regions implicated in drug resistance, including *rpoB* (rifampicin), *katG* and *inhA/fabG1* (isoniazid), *embB* (ethambutol), *pncA* (pyrazinamide), *gyrA* and *gyrB* (fluoroquinolones), *rrs*, *eis*, and *rpsL* (aminoglycosides and capreomycin), *tlyA* and *atpE* (bedaquiline and linezolid), and *gidB* (streptomycin). The assay also amplifies *hsp65* for species identification and the Direct Repeat (DR) region for spoligotyping and lineage determination.

PCR reactions were prepared in a total volume of 25 µL containing 2.5 µL of 10× Deeplex PCR buffer, 0.5 µL of DNA polymerase mix, 20 µL of primer pool, and 2 µL of template DNA. Amplifications were carried out in a Veriti™ 96-well thermal cycler (Applied Biosystems, USA) under the following conditions: initial denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min; with a final extension at 72 °C for 5 min. Amplified products were confirmed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualised under UV illumination following the Deeplex Myc-TB protocol.

Library Preparation and Sequencing

Amplicons were purified using AMPure XP magnetic beads (Beckman Coulter, USA) to remove primers and unincorporated nucleotides. Purified DNA was quantified with the Qubit™ fluorometer and normalised to 0.2 ng/µL for library preparation. Libraries were constructed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, USA) following the manufacturer's protocol, which includes tagmentation, adapter ligation, and limited-cycle PCR for indexing. Libraries were purified and normalised before pooling in equimolar concentrations. Sequencing was performed on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle; 2 × 300 bp paired-end reads). A no-template control and a *M. tuberculosis* H37Rv reference strain were included as internal controls in each sequencing run to monitor contamination and assay performance (See Deeplex ® Myc-TB user manual RUO (V5-2023).

Bioinformatic Processing and Variant Calling

Raw sequencing reads were demultiplexed and uploaded to the Deeplex® Myc-TB secure web application (https://myc.tb.genoscreen.com/) for automated analysis using the integrated Deeplex pipeline. The pipeline performs read quality filtering, alignment against the *M. tuberculosis* H37Rv reference genome (GenBank accession NC_000962.3), variant calling, and functional annotation of mutations. It also performs *hsp65*-based species identification, spoligotype

determination, and lineage classification based on single-nucleotide polymorphism (SNP) patterns.

Variant detection was set at an allele-frequency threshold of ≥ 3% for minority variants, with a minimum per-site coverage depth of 100×. Mutations were annotated as resistance-associated when they corresponded to variants validated in the GenoScreen mutation database and WHO mutation catalogue for *M. tuberculosis* [9]. Uncharacterised non-synonymous mutations were recorded and subjected to further literature review. The limit of detection (LOD) for minority resistant subpopulations in this assay was approximately 3%, as recommended by the manufacturer.

Quality Control and Data Interpretation

Run quality was evaluated using metrics generated by the Deeplex platform, including mean coverage depth across all targets, uniformity of coverage, percentage of mapped reads, and detection of positive-control markers. Samples with ≥ 95% of target regions covered at ≥ 100× depth were deemed acceptable for analysis. Results were expressed as antibiotic susceptibility profile, indicating the presence or absence of resistance-conferring mutations for each drug, along with the associated lineage and genotypes. A strain was classified as resistant when one or more confirmed mutations conferring resistance to a specific drug were detected; susceptible strains showed no such mutations within the analysed loci. The Deeplex Myc-TB web app generated automatic reports that included sample information, the date, analysis mode, quality summary, experiment set, control results and all mutation details as derived by the software. All results were exported in Fast Q files and PDF formats for data management and inclusion in downstream phylogenetic and statistical analysis.

RESULTS AND DISCUSSION

Results: All the fifteen *Mycobacterium tuberculosis* complex isolates belonged to the *Mycobacterium tuberculosis* complex group by the *hsp65* based identification best match and were all *M.bovis* strains by single nucleotide polymorphism- based phylogenetic lineage and spoligotyping. The composite target coverage breadth, which is the percentage of the total targeted genomic regions that are covered by this sequencing reads, ranged from 93.9% in isolate 044 and 100% in isolates 026 and 030.

The 15 *M. bovis* strains that were sequenced, had average coverage depths which ranged from 10.5x in isolate 044 to 1,829.8x in isolate 004, which is the average number of times each nucleotide position in the *hsp65* gene target was sequenced. The consensus length for *hsp65* gene target which is the number of base pairs, ranged from 387bp in isolate 044 to 400bp in isolates 004,022,026,030,059,072, and 098. The percentage identity, which is the degree of nucleotide sequence matching between the DNA sequence obtained from the MTBC isolate (the query sequence) and a known reference sequence in the Deeplex database, ranged from 99.176% in isolate 008 sub-strain and 100% in isolates 004,008 sub-strain,022,026,030,031,059,071,072,073,081,084,092 and 098. The expect value (E-value) of the BLAST search for sequences related to MTBC, was 0.0 for each of the *M. bovis* strain and the best match analysis showed that, all the *M. bovis* strains, belonged to *Mycobacterium tuberculosis* complex (MTBC) (Table 2).

Table 1: Deeplex Myc-Tb Next Generation Sequencing of *Mycobacterium tuberculosis* complex Isolates

Sample ID	Sequencing result acceptibility	Composite target coverage breadth %	*hsp65*-based identification best match analysis versus hsp65 reference sequences	Single nucleotide polymorphism based phylogenetic strain lineages (SNP)
004_TB	+	99.9	MTBC	*M. bovis*
008_TB	+	96.5	MTBC:86.1/MTBC:13.9	*M. bovis*
022_TB	+	99.9	MTBC	*M. bovis*
026_TB	+	100	MTBC	*M. bovis*
030_TB	+	100	MTBC	*M .bovis*
031_TB	+	99.3	MTBC	*M. bovis*
044_TB	+	93.9	MTBC	*M. bovis*
059_TB	+	99.7	MTBC	*M. bovis*
071_TB	+	99.9	MTBC	*M. bovis*
072_TB	+	99.8	MTBC	*M. bovis*
073_TB	+	99.6	MTBC	*M .bovis*
081_TB	+	98.1	MTBC	*M. bovis*
084_TB	+	99.4	MTBC	*M. bovis*
092_TB	+	99.8	MTBC	*M. bovis*
098_TB	+	99.9	MTBC	*M. bovis*
PC_TB	+	98.8	MTBC	*M. bovis*

TB-Tuberculosis MTBC-*Mycobacterium tuberculosis* complex *M.bovis- Mycobacterium bovis.*

Table 2: Average coverage depth and percentage identity of MTBC isolates

Sample No.	Av coverage depth (x)	Consensus length (bp)	% Identity	E-value	Best match
004	1829.8	400.0	100	0.0	MTBC
008	23.6	396.0	100/99.176	0.0/0.0	MTBC: 86.1/ MTBC:13.9
022	132.6	400	100	0.0	MTBC
026	211.6	400	100	0.0	MTBC
030	669.1	400	100	0.0	MTBC
031	164.7	399	100	0.0	MTBC
044	10.5	387	99.73	0.0	MTBC
059	555.8	400	100	0.0	MTBC
071	194.8	398	100	0.0	MTBC
072	460.5	400	100	0.0	MTBC
073	43.6	399	100	0.0	MTBC
081	39.8	396	100	0.0	MTBC
084	139.6	396	100	0.0	MTBC
092	44.3	395	100	0.0	MTBC
098	359	400	100	0.0	MTBC

MTBC-*Mycobacterium tuberculosis* complex.

Drug Susceptibility Profile of MTBC Isolates from Slaughtered Cattle in Yola, Adamawa, State.

Table 3, shows that out of the fifteen (15) MTBC isolates characterized, had gene variants in pyrazinamide drugs sector, conferring resistance to pyrazinamide seen as red colour on the deeplex map. Two (2) of the 15 isolates, also had gene variants associated with ethionamide resistance shown as yellow and red respectively. All the 15 MTBC isolates, also had gene variants associated with the Fluoroquinolone drug sector, seen as deep blue colour. 5 isolates had gene variants associated with Linezolid and one isolate had gene variants associted with isoniazide seen as blue colour on the Deeplex maps. The samples were placed into seven groups based on their drug susceptibility profiles, as shown in Table 3.

The targeted genes and gene variants seen in the isolates were *pncA*- (cac57gac), *ethA* – (InsA) *rrl*- (a2872t), *rrs* –(g482a and g483c), *gyrA*- (gac122ggc, atc36gtc and gac122ggc), *gyrB*- (gcg403tcg) and *inhA*- (gca191aca). They were associated with pyrazinamide, ethionamide, linezolid fluoroquinolones, aminoglycosides and isoniazide respectively. The drug association shows that all 15 isolates, were resistant to pyrazinamide while two of those isolates were also resistant to ethionamide. Other drug associations were uncharacterized and uncertain.

Table 3: Drug susceptibility Profile of MTBC from Slaughered Cattle in Yola, Adamawa State

Group Number	Sample Group	Detected Gene Target	Detected Gene Variants	Associated Drug	Drug association	Reference
1.	004	*ethA*	InsA,	Ethionamide,	R	WHO, 2021
		pncA	cac57gac,	Pyrazinamide,	R	
		gyrB	gcg403tcg	Fluoroquinolones	VUS	
2.	008	*pncA,*	cac57gac	Pyrazinamide,	R	WHO, 2021
		rrl,	a2872t	linezolid, Aminoglycosides,	UV	
		rrs	g482a,	Aminoglycosides	UV	
		rrs,	g483c,	fluoroquinolones	VUS	
		gyrB	gcg403tcg		VUS	

3.	022,026,030, 044,059,072,084 and 092	*pncA*	cac57gac	Pyrazinamide	R	WHO,2021
		gyrB	gcg403tcg	fluoroquinolones	VUS	
4.	071	*pncA*	cac57gac	Pyrazinamide	R	WHO, 2021
		rrl,	a2365g	Linezolid	UV	
		gyrA	atc36gtc	Fluoroquinolone	UV	
		gyrB	gcg403tcg	fluoroquinolone	VUS	
5.	031,073	*pncA*	cac57gac	Pyrazinamide	R	WHO,2021
		rrl	a2365g	Linezolid	UV	
		gyrB	gcg403tcg	fluoroquinolone	VUS	
6.	081	*pncA*	cac57gac	Pyrazinamide	R	WHO,2021
		ethA	InsA	Ethionamide	R	
		rrl	A2365g	Linezolid	UV	
		gyrB	gcg403tcg	fluoroquinolone	VUS	
7.	098	*pncA*	cac57gac	Pyrazinamide	R	WHO,2021
		inhA	gca191aca	Isoniazide	VUS	
		gyrA	gac122ggc	Fluoroquinolone	UV	
		gyrB	gcg403tcg	fluoroquinolone	VUS	

Resistance, VUS- Variants of uncertain significance, UV- Uncharacterized Variants.

Discussion: In this study, all fifteen (15) isolates, were identified as *M.bovis* strains. No other species of the MTBC was identified. This result agrees with studies which showed that *M. bovis* strains are mostly the cause of bovine tubersulosis [10,11]. A study from Brazil, reported that 17/30(57%) isolates from granulomas in bovine lymp nodes of cattle with clinical TB, were characterized as *M.bovis* by spoligotyping [12], coroborating with the result from this study.

Findings from this study, also coroborrates with two other studies, which have reported prevalence rates of 55.6% and 47% of bovine Tb caused by *M.bovis* identified by spoligotyping and region of difference (RD) molecular assays, in Ethiopia [13,14]. Another study on the molecular epidemiology of *M.bovis* isolated from cattle, showed a prevalence of 86.7% from lung samples, in Cameroon [15]. In Maiduguri abattoir of Bornu State, a study reported that 44(42.9%) were detected as *Mycobacterium bovis,* 3(14.3%) were identified as *Mycobacterium tuberculosis* and 2(42.9%) were identified as *Mycobacterium africanum* by MTBC Genotyping assay showing that, *M.bovis* was the highest in the bovine samples [16].

The high detection of *M.bovis* in this study can also be attributed to the fact that, cattle are the primary host and reservoir of *M.bovis* [10, 17, 18,19].

CONCLUSION

All the *M.bovis* strains, were resistant to pyrazinamide which is one of the anti-tuberculosis drugs used for first line treatment in humans. Two of the *M. bovis* strains which were resistant to pyrazinamide, and some showed resistance to ethionamide, raising concerns for future management of zoonotic tuberculosis. This resistance to both drugs, is not classified as a multi-drug resistance because, multi-drug resistance involves resistance to rifampin and isoniazid based on WHO significance grading for drug resistance [9].

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