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Genomic characterization of an extensively drug-resistant *Mycobacterium tuberculosis* clinical isolate

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Abstract:

Background: Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, constitutes a predominant infectious disease with high mortality on a global scale, especially in Nigeria. The emergence of extensively drug-resistant (XDR) TB poses a significant challenge to the control of this disease, underscoring the necessity for the identification of underlying genetic mutations to facilitate informed treatment decisions and mitigate transmission.
Methodology: Sputum samples from a total of 199 patients with validated baseline GeneXpert MTB/rifampicin-resistant positive from the northcentral States of Nigeria including Nasarawa, Benue, Kogi, Niger and Abuja in the month of June 2024 were transported to the Zankli TB Reference Laboratory, and processed using NALC-NaOH method, followed by centrifugation. The processed samples were inoculated onto prepared Lowenstein Jensen (LJ) media (solid culture) and Mycobacterial Growth Indicator Tube (MGIT) for liquid culture. Ziehl Nelsen (ZN) stain was performed on the isolates of the positive cultures to ascertain the presence of Acid-Fast Bacilli (AFB) and the Bio-line SD AgMPT64 test was performed to confirm the presence of *M. tuberculosis.* Drug susceptibility testing (DST) was conducted on all positive cultures using the standard proportion method. Long read sequencing was performed on the 2 XDR isolates using the Oxford Nanopore Technology with 50x coverage. The raw reads of the sequenced data were binned into a high-quality metagenome-assembled genome (MAG) via maxbin (V2.2.7), and the quality of the binned metagenome-assembled genome was assessed via checkM (V1.2.3). Resistance genes were discerned through the Comprehensive Antibiotic Resistance Database (CARD) resistance gene identifier, and existing virulence factors were analysed via the VF analyser of the Virulence Factors Database (VFDB), while the phylogenetic tree was constructed via the auto-Multi Locus Species Tree (auto-MLST).
Results: Mutations in *katG* (R463L, A234V, A431V), *rpoB* (D516G, H526T, L511R), *gyrA* (D94N), *murA* (C117D) and *embC* (R738Q) were detected in one of the two sequenced XDR isolates (XMTB2) that passed sequencing quality control. The VFDB identified virulence genes such as *narG*, *narH*, *sapM*, and *narX*, associated with the XMTB2 MAG, while phylogenetic analysis revealed a close phylogenetic connection between the XMTB2 MAG and the well-known Beijing lineage (L2).
Conclusion: The results of this study revealed that the XMTB2 MAG was potentially an XDR isolate that was very virulent because of the presence of many virulence factors identified in it and its close relationship with two of the most widespread and virulent lineages of *M. tuberculosis;* the Beijing lineage (L2) and the Euro-American lineage (L4).

Keywords: *Mycobacterium tuberculosis*; XDR TB; mutation; virulence factors; phylogenetic analysis

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Caractérisation génomique d'un isolat cliniquement résistant de *Mycobacterium tuberculosis*

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Résumé:

Contexte: La tuberculose (TB), causée par *Mycobacterium tuberculosis*, constitue une maladie infectieuse prédominante avec une mortalité élevée à l'échelle mondiale, en particulier au Nigéria. L'émergence de la

tuberculose ultrarésistante (XDR) pose un défi important pour la lutte contre cette maladie, soulignant la nécessité d'identifier les mutations génétiques sous-jacentes pour faciliter des décisions thérapeutiques éclairées et atténuer la transmission.
Méthodologie: Des échantillons d'expectorations provenant d'un total de 199 patients avec un test GeneXpert MTB/rifampicine résistant de base validé et provenant des États du centre-nord du Nigéria, notamment Nasarawa, Benue, Kogi, Niger et Abuja, au mois de juin 2024, ont été transportés au laboratoire de référence de la tuberculose de Zankli et traités par la méthode NALC-NaOH, suivie d'une centrifugation. Les échantillons traités ont été inoculés sur des milieux Lowenstein Jensen (LJ) préparés (culture solide) et sur un tube indicateur de croissance mycobactérienne (MGIT) pour la culture liquide. Une coloration de Ziehl-Neelsen (ZN) a été réalisée sur les isolats des cultures positives afin de vérifier la présence de bacilles acido-résistants (BAAR) et le test Bioline SD AgMPT64 a été réalisé pour confirmer la présence de M. tuberculosis. Un test de sensibilité aux médicaments (TSM) a été réalisé sur toutes les cultures positives par la méthode des proportions standard. Un séquençage à lecture longue a été réalisé sur les deux isolats XDR à l'aide de la technologie Oxford Nanopore avec une couverture 50x. Les lectures brutes des données séquencées ont été regroupées dans un génome assemblé par métagénome (MAG) de haute qualité via maxbin (V2.2.7), et la qualité du génome assemblé par métagénome regroupé a été évaluée via checkM (V1.2.3). Les gènes de résistance ont été identifiés grâce à l'identifiant de gènes de résistance de la base de données complète sur la résistance aux antibiotiques (CARD), et les facteurs de virulence existants ont été analysés via l'analyseur VF de la base de données des facteurs de virulence (VFDB), tandis que l'arbre phylogénétique a été construit via l'arbre d'espèces multi-locus (auto-MLST).
Résultats: Des mutations dans *katG* (R463L, A234V, A431V), *rpoB* (D516G, H526T, L511R), *gyrA* (D94N), *murA* (C117D) et *embC* (R738Q) ont été détectées dans l'un des deux isolats XDR séquencés (XMTB2) qui ont passé le contrôle de qualité du séquençage. Français La VFDB a identifié des gènes de virulence tels que *narG*, *narH*, *sapM* et *narX*, associés au MAG XMTB2, tandis que l'analyse phylogénétique a révélé une connexion phylogénétique étroite entre le MAG XMTB2 et la lignée bien connue de Beijing (L2).
Conclusion: Les résultats de cette étude ont révélé que le MAG XMTB2 était potentiellement un isolat XDR très virulent en raison de la présence de nombreux facteurs de virulence identifiés en lui et de sa relation étroite avec deux des lignées les plus répandues et les plus virulentes de *M. tuberculosis*; la lignée de Beijing (L2) et la lignée euro-américaine (L4).

Mots-clés: *Mycobacterium tuberculosis*; XDR TB; mutation; facteurs de virulence, analyse phylogénétique

Introduction:

Tuberculosis (TB), caused by the pathogen *Mycobacterium tuberculosis*, represents a significant challenge in public health, ranking as a predominant cause of global mortality and engendering considerable health complications. TB is classified as a potentially perilous infectious ailment that primarily affects the pulmonary system. The transmission of TB occurs between individuals through respiratory actions such as coughing and sneezing, which disseminate microscopic aerosolized droplets containing the pathogen into the atmosphere. Infections caused by *M. tuberculosis* result in several million fatalities annually, with this elevated mortality rate being associated with inadequate diagnostic practices and the swift emergence of multidrug-resistant strains of TB (MDR-TB) (1).

By the year 2023, TB re-established itself as the infectious disease accountable for the highest mortality rate globally. During that year, the World Health Organization (WHO) projected 1.25 million deaths among an estimated 10.8 million cases of TB (2). MDR-TB is characterized as an infection resulting from *M. tuberculosis* that exhibits resistance to both rifampicin and isoniazid, whereas XDR-TB is delineated as MDR-TB with supplemental resistance to any fluoroquinolone (FLQ) and a minimum of one second-line injectable pharmacological agent (3).

Extensive drug resistance (XDR) is still a major global public health issue, particularly in nations with high TB prevalence, such as Nigeria. Numerous of these incidents are unreported, infecting people who are at high risk. Public health and the global economy have been significantly impacted by the growth of drug-resistant and XDR *M. tuberculosis* complex (MTBC), as many cases remain unreported or undiscovered in low-resource settings, leading to misdiagnosis and further transmission (4). Inadequate and improper adherence to prescribed treatment regimens by patients constitutes a principal factor contributing to the development of resistance to TB pharmacotherapies (5).

The virulence factors of *M. tuberculosis* cannot be identified exclusively with toxins and other by-products of bacterial metabolic activity that are known to adversely impact the host organism. With the investigation of the properties of *M. tuberculosis*, the quantity of identified virulence factors has steadily increased. This remarkable plasticity within the *M. tuberculosis* genome permits us to classify this bacterium as a "highly successful pathogen" (6).

There is a distinct geographic distribution among the human-adapted lineages of the MTBC, with certain "generalist" lineages such as Lineage (L2) and L4) existing worldwide and others such as L5, L6, and L7 being regionally restricted "specialists." This continent is home to all seven lineages that have evolved to adapt to humans, including the three "specialist" lineages that are unique to Africa (7). The aim of this study was to investigate the genetic determinants of resistance

and virulence in clinical XDR *M. tuberculosis*.

Materials and method:

Study design:

This study is a descriptive cross-sectional design to identify *M. tuberculosis* (MTB) and non-tuberculous *Mycobacteria* (NTM) infection among Xpert MTB/RIF Ultra confirmed positive TB cases.

Ethical approval:

Ethical approval for this study was obtained from the Bingham University Teaching Hospital, Health Research and Ethics Committee Jos, Plateau State, Nigeria (NHREC/21/05/2005/01287). This study used existing laboratory records (secondary data) hence there was no risk of physical harm to the patients. The data were identified for confidentiality and privacy of patients.

Study population:

In June 2024, sputum samples from a total of 199 patients with validated baseline GeneXpert *M. tuberculosis*/RIF positive samples were collected from clinics and hospitals in northcentral Nigeria and submitted to the Zankli TB Reference laboratory, Bingham University Karu, Nasarawa State. Participants were selected based on their confirmed Xpert-positive status at baseline.

Sample collection:

In accordance with the National Guidelines for Sputum Sample Collection, sputum samples were obtained from the participants from Nasarawa, Benue, Kogi, Niger, and Abuja in northcentral regions of Nigeria. The samples were then transported by triple sample packaging in a cold chain to Zankli Reference Laboratory for analysis

Decontamination and culture procedure:

The standardized N-Acetyl L-cysteine (NALC)/Sodium Hydroxide (NaOH) protocol was used to process the sputum specimens in a biosafety level 2+ (BSL 2+) laboratory environment. To mitigate the risk of *Mycobacteria* viability loss, NaOH was neutralized by the addition of phosphate buffer after a 15-minute period. The resultant mixture was centrifuged at 3000g for 15 minutes, following which the supernatant was discarded. The sediment obtained was used for concentrated AFB smear preparation, solid culture inoculation and BACTEC MGIT liquid culture (8,9).

The Ziehl Neelsen (ZN) acid fast staining was performed on prepared smear of the processed samples followed by examination under an oil immersion objective, with the AFB smears being classified as scanty (1-9 AFB/100 fields), 1+ (10-99 AFB/100 fields), 2+ (1-10 AFB/field), or 3+ (> 10 AFB/ field).

A volume of 0.5 mL of the processed sample was inoculated onto prepared LJ slants and incubated aerobically. Similarly, 0.5ml was inoculated into the MGIT tubes and loaded onto the BACTEC-MGIT 960 machine. Weekly observations were conducted over an 8-week period to monitor for growth on the solid media, while the BACTEC-MGIT 960 machine flagged all positive growth. ZN staining was performed on the isolates of the positive cultures to ascertain the presence of AFB, and the MTBC TB Ag MPT64 Rapid assay was performed to confirm the presence of *M. tuberculosis*.

BIO-LINE SD Ag MPT64 TB test:

A cumulative volume of 100 µl of broth derived from the BACTEC-MGIT 960 culture was administered to the well of the BIO-LINE SD Ag MPT64 TB rapid kit cassette. The inoculated immunochromatographic cassettes were maintained at ambient temperature for a duration of 20 minutes within a Class II Biosafety cabinet and subsequently evaluated for the presence of both control and test bands. The manifestation of a band in the "C" region validated the integrity of the assay. The additional emergence of a band in the "T" region was interpreted as indicative of the presence of the MPT64 Ag antigen within the test broth culture. The absence of a band in the "C" region was regarded as an indication of an invalid test. The standard reference strain, *M. tuberculosis* complex H37Rv ATCC 27294, was used as positive control.

Drug susceptibility testing (DST):

Drug susceptibility testing (DST) was performed on all positive cultures derived from solid culture utilizing the standard proportion method (10). This methodology encompassed the assessment of the susceptibility of *M. tuberculosis* isolates to first-line anti-TB pharmacological agents (Isoniazid, Rifampicin, and Ethambutol), as well as to the second line fluoroquinolone FLQ (Moxifloxacin) and injectables (Kanamycin, Amikacin and Capreomycin), which facilitated their classification as drug-susceptible TB (DS-TB), MDR-TB, or XDR-TB strains (10).

DNA extraction and next generation sequencing:

Genomic DNA of the two XDR isolates from the DST was extracted using the ZYMO quick-DNA Fungal/Bacterial Miniprep kit according to the manufacturer's instructions. Long-read sequencing was performed using Oxford Nanopore Technology with 50x coverage of the extracted gDNA. Sequencing libraries were generated via SQK-RBK114.96. Sequencing was performed on a GridION (Oxford Nanopore technologies) using an R10.4.1 flow cell with the base calling model r1041_e82_

400 bps_hac_v4.2.0. Reads were randomly subsampled to 50x coverage via Rasusa (V 0.7.1) and assembled via flyer (V 2.9.2-b1786). The assemblies were polished via Medaka (V 1.8.0) and the relevant model (r1041_e82-400 bps_hac_v4.2.0). Annotation was performed via Bakta (V 1.8.1). Taxonomic classification of the raw reads and assemblies was performed via Kraken (V 2.0.9-beta Derrick), and 16S identification was performed via Barnap and Sina. The assemblies were analysed and visualized via Quast and Bandage (V 0.8.1). The raw reads of the sequenced data were binned into a high-quality metagenome-assembled genome (MAG) via maxbin (V 2.2.7), and the quality of the binned MAG was assessed via checkM (V 1.2.3).

Bioinformatic analysis:

All antibiotic resistance genes were identified via the Comprehensive Antibiotic Resistance Database (CARD) resistance gene identifier (11). Existing virulence factors were investigated via the VF analyser tool of the Virulence Factor Database (VFDB) (12). For phylogenetic tree analysis, the auto-locus species tree (auto-MLST), which automates all steps, was constructed via de novo method, which allows the user to choose from which additional species can be added to construct a detailed phylogenetic tree and can also choose which genes to use for MLST.

In this study, 20 simultaneous genomes of *M. tuberculosis* in the FASTA were downloaded from the NCBI database. The multiple sequence files, including the query sequenced genome, were uploaded as inputs to the server. Since all the steps are automated by the server, they were left as defaults, and an email address was submitted for notification about the auto-MLST job that was performed.

Results:

Rifampicin-resistant isolates identified:

The number of GeneXpert MTB/rifampicin-resistant (RR) sputum samples of patients received at the Zankli TB reference laboratory for the month of June 2024, were 199. Among the samples, 151 (76.0%) were positive for MTB isolates by culture, while 48 (24.0%) were negative for MTB isolates on culture, with 22 (11.0%) of the isolates being MDR while only 2 (1.0%) isolates were XDR strains (Table 1).

Table 1: Frequency of MTB isolates by culture among GeneXpert MTB/rifampicin-resistant positive samples

MTB isolates	Number (%)
Culture positive	151 (76.0)
Culture negative	48 (24.0)
MDR isolate	22 (11.0%)
XDR isolate	2 (1.0%)
Total	**199**

MDR = Multidrug-resistant; XDR = Extensively drug-resistant

Drug sensitivity test results of the 2 XDR isolates:

Out of the two XDR isolates from the DST, the first isolate (XMTB1) was resistant to all the tested drugs except moxifloxacin, while the second isolate (XMTB2) was resistant to all the tested drugs except isoniazid, with ethambutol, having no effect on the isolate (Table 2).

Resistance genes detected in the XMTB2 MAG:

The resistance genes in the second *M. tuberculosis* isolate that passed the sequencing quality control (XMTB2 MAG) were identified via the CARD resistance gene identifier (Table 3). The mechanisms involved include antibiotic target alteration, antibiotic inactivation, antibiotic efflux and antibiotic target replacement.

Table 2: Drug sensitivity test results of the two XDR-isolates

S/N	Isoniazid (0.2 µg/ml)	Rifampicin (40 µg/ml)	Ethambutol (2 µg/ml)	Kanamycin (30 µg/ml)	Amikacin (30 µg/ml)	Capreomycin (40 µg/ml)	Moxifloxacin/ Levofloxacin (2 µg/ml)
Isolate 1	R	R	R	R	R	R	S
Isolate 2	S	R	-	R	R	R	R

S/N: Serial number; R= Resistant, S= Sensitive; - = No effect; XDR = Extensively drug resistant

Table 3: List of antibiotic resistance genes in the XMTB2 MAG

Resistance gene	Protein	Drug class	Resistance mechanism
erm(37)	erythromycin ribosome methylase	macrolides, lincosamides, streptogramins	antibiotic target alteration
*bla*C	Beta-lactamase	cephalosporins, cephamycin, carbapenems	antibiotic inactivation
Nickel/cobalt **t**ransporter gene	putative nickel/cobalt transporter	fluoroquinolones, aminoglycosides, isoniazid	antibiotic efflux
e*fp*A	putative efpA	Rifamycin, isoniazid	antibiotic efflux
aac(2')-Ic	aminoglycoside 2'-N-acetyltransferase	Aminoglycosides	antibiotic inactivation
*murA (i*ntrinsic)	UDP-N-acetylglucosamine enolpyruvcfl transferase	Fosfomycin	antibiotic target alteration
embC mutant	Arabinosyltransferase	Ethambutol	antibiotic target alteration
katG mutant	Catalase peroxidase variant model	Isoniazid	antibiotic target alteration
gyrA mutant	Gyrase A variant model	Fluoroquinolones	antibiotic target alteration
rpoB mutant	RNA polymerase	Rifamycin	antibiotic target alteration, antibiotic target replacement
23S rRNA mutant	rRNA gene variant model	Aminoglycoside	antibiotic target alteration

Table 4: Chromosomal mutations in antibiotic resistance genes in the XMTB2 MAG

Resistance gene	SNP	Initial amino acid	Final amino acid
murA (intrinsic)	C117D	Cysteine	Aspartic acid
embC mutant	R738Q	Arginine	Glutamine
katG mutant	R463L	Arginine	Leucine
	A234G	Alanine	Glycine
	A431V	Alanine	Valine
	G300W	Glycine	Tryptophan
gyrA	D94N	Aspartic acid	Asparagine
rpoB	D516G	Aspartic acid	Glycine
	H526T	Histidine	Threonine
	L511R	Leucine	Arginine
23S rRNA	A2145G	Alanine	Glycine

SNP = single nucleotide polymorphism

Chromosomal mutations of antibiotic resistance genes in the XMTB2 MAG:

Chromosomal mutations of antibiotic resistance genes in the second XDR *M. tuberculosis* isolate that passed the sequencing quality control (XMTB2 MAG), were identified via the CARD resistance gene identifier (Table 4).

Virulence factor class and related genes:

Virulence factors, including both the proteinaceous and non-proteinaceous factors of the XMTB2 MAG were also identified (Table 5).

Phylogenetic tree of XMTB2 genome constructed via an auto-multi locus species tree:

The phylogenetic tree of XMTB2 MAG was constructed via autoMLST. The relationships were analysed based on mash distances to the query genome, in which all the strains were found to belong to either the popular and virulent Beijing lineage (L2) or the Euro-American lineage (L4), which indicates that XMTB2 MAG belongs to either of the two lineages (Fig 1)

Table 5: Virulence factor class and related genes

Virulence factor class	Virulence factors	Related genes
Amino acid and purine metabolism	Glutamine synthesis	*glnA1*
	Leucine synthesis	*leuD*
	Lysine synthesis	*lysA*
	Proline synthesis	*proC*
	Purine synthesis	*purC*
	Tryptophan synthesis	*trpD*
Anaerobic respiration	Fused nitrate reductase	*narX*
	Nitrate reductase	*narG*
		narH

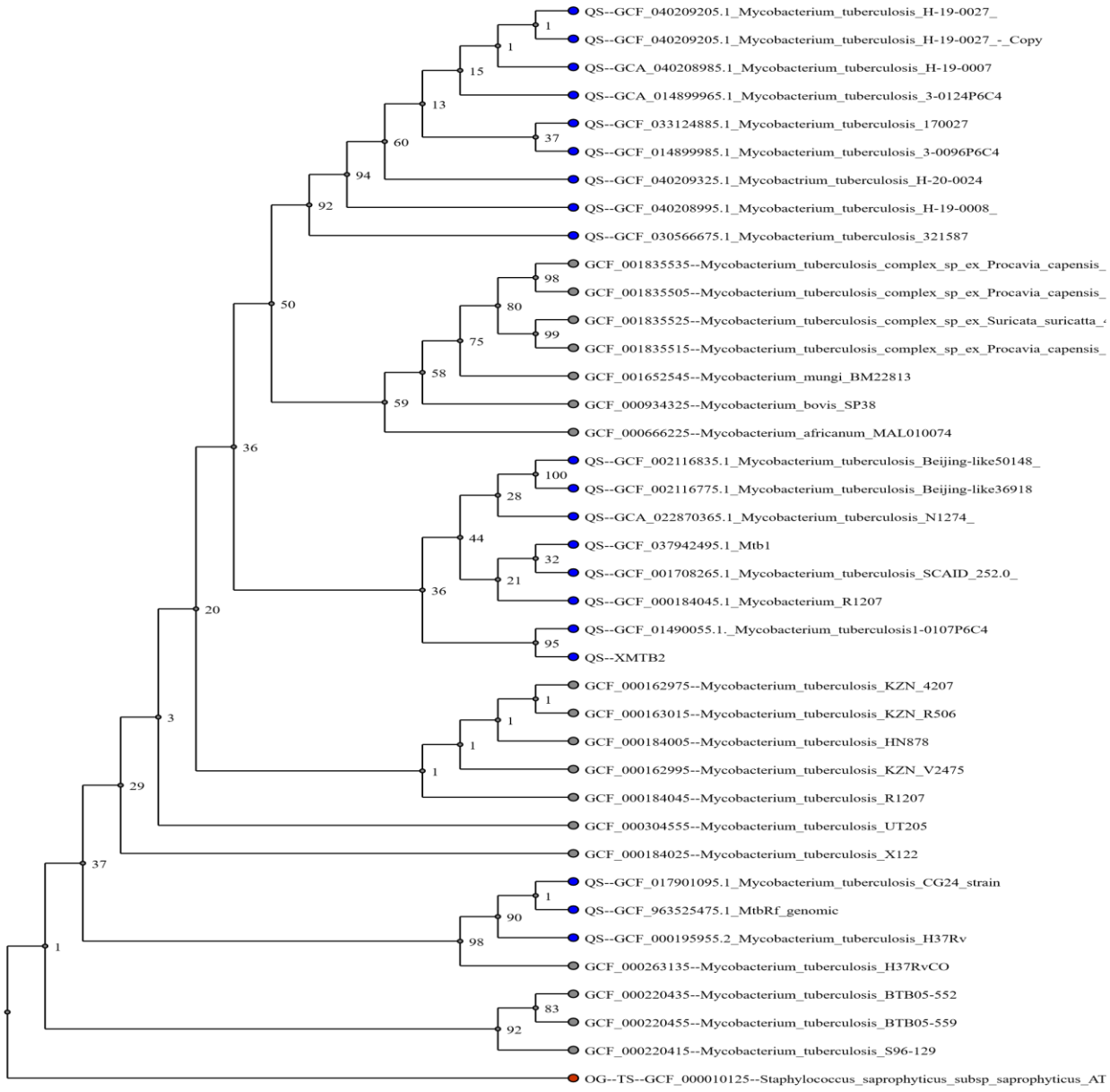

Fig 1: Phylogenetic tree of XMTB2 MAG

Discussion:

Extensively drug-resistant TB (XDR-TB) represents a significant public health concern. The anticipated effectiveness of recently endorsed anti-TB pharmacological agents for the management of DRTB, as well as the relative proportions of primary resistance cases compared with acquired resistance cases within the global context of XDR-TB, has not been unequivocally documented (13). The high number of negative sputum samples (24.0%) among the 199 patients with GeneXpert MTB/rifampicin-resistant TB means these samples proved to be negative by culture despite being GeneXpert positive from various clinics and hospitals. This disparity may be attributed to a probable lack of compliance in GeneXpert testing from most facilities at the local level or poor sample collection and shipment. Only 1% of the isolates were XDR, which signifies that XDR TB is not common in the region. This phenomenon agrees with the study of Trisakul et al., (13), which stated that although phenotypic DSTs are routinely employed in laboratory analyses, the challenge of accruing an adequate number of XDR samples for such testing is exacerbated by the scarcity of cases. Consequently, the procurement of a comprehensive inventory of XDR phenotype strains for the investigation of resistance to critical anti-TB drugs presents a significant challenge.

The DST of the two isolates selected for whole-genome sequencing (WGS) revealed that the first isolate was resistant to both the first- and second-line oral antitubercular drugs tested, with the exception of the second-line fluoroquinolone (moxifloxacin or levofloxacin). Despite showing sensitivity to isoniazid and failing to react to ethambutol, the second isolate was resistant to both the second-line oral fluoroquinolones and the three injectable antituberculous drugs. This discrepancy was resolved by WGS and agrees with the findings of Olawoye et al., (4), who reported that two of their isolates were XDR by WGS despite flagging them as pre-XDR and MDR by the BACTEC MGIT 960 machine.

The evolution of antibiotic resistance genes detected in the XMTB2 MAG may be linked to practices such as incomplete treatment, inappropriate dosing, inappropriate prescribing practices, and poor quality of the antibiotics used in therapy of TB. These genes confer resistance to various antibiotics, which is one of the very important concerns in TB treatment. These genes, such as *erm37* and *blaC*, encode resistance to macrolides, and cephalosporins or carbapenems, respectively. This finding aligns well with the study performed by Sandgren et al*.,* (14) who identified *erm37* as one gene that encodes resistance to macrolides through methylation of RNA on the ribosomes. This association with isoniazid and rifampicin resistance is also enhanced by the mutations in *katG* and *rpoB* genes respectively, confirming that the XMTB2 MAG belongs to a potentially XDR strain.

This finding however contrast with what was described by Hazbón et al*.,* (15) concerning *katG* mutations and designated the R463L mutation as a polymorphism not associated with isoniazid resistance, and Wang et al. (16), who referred to *katG* Arg463Leu, *gyrA* Glu21Gln, *gyrA* Ser95Thr, and *gyrA* Gly668Asp as mutations that are characteristic of specific lineages, and have been documented as natural polymorphisms that serve as valuable indicators for the evolutionary characterization of the genome.

Many distinct chromosomal changes in the XMTB2 MAG illustrate single nucleotide polymorphisms (SNPs) which include variations at one position in a DNA sequence. D516G and H526T are encoded by the *rpoB* gene and change the drug interaction site on the RNA polymerase, hence being highly associated with rifampicin resistance. This finding agrees with the study of Sandgren et al., (14), which established D516G and H526T among the most frequent mutations in the *rpoB* gene that confer resistance to rifampicin. In the *gyrA* gene, the D94N mutation alters the DNA gyrase enzyme, hence contributing to fluoroquinolone resistance (15). Rifampicin resistance has been identified as being linked to a critical region known as the rifampicin resistance determining region (RRDR), specifically within codon positions 507-533, where numerous researchers have documented the existence of both prevalent and novel mutations in the *rpoB* gene, particularly within the hotspot region and in areas outside of the hotspot but still contained within the *rpoB* gene (17).

In contrast to other pathogenic organisms, such as *Vibrio cholerae* or *Corynebacterium diphtheriae*, *M. tuberculosis* does not employ toxins or enzymes, which are conventionally recognized virulence determinants; rather, a multitude of virulence-associated genes serve to fulfil this role (18). Phagosome arrest was one of the virulent factor classes identified in the XMTB2 MAG isolate in our study. The virulence factors obtained were nucleoside diphosphate kinase and tyrosine phosphatase, which were encoded by the *ndK* and *ptpA* genes, respectively. This is in agreement with the study of Wong et al. (19) whom tested *M. tuberculosis* H37Rv (Rv2234) in a THP-1 model and discovered that *ptpA* dephosphorylates the VPS33B protein of macrophages, the key regulator of membrane phagosome fusion to lysosomes.

The *M. tuberculosis* complex is composed of seven discrete phylogenetic lineages

that are endemic to various geographical regions worldwide, thereby substantiating the hypothesis that these strain types exhibit specific adaptations to individuals with diverse genetic backgrounds (20,21). The geographical distribution and dissemination of these lineages are characterized by heterogeneity, with lineage 2 demonstrating particularly significant mobility, as indicated by recent transmission from Asia to Europe and Africa (22). The phylogenetic tree constructed via auto-MLST shows that the first genome observed with query genome GCA_014899965.1 *M. tuberculosis* 3-0124P6C4 was sourced from a human sample, specifically the lung, and it was collected by Hinduja National Hospital, India. The strain belongs to L2, which is popularly known as the Beijing lineage (23,24).

M. tuberculosis R1207 serves as a prototypical member of the R86 cluster within the Beijing lineage of *M. tuberculosis* and was procured as a clinical isolate from the Western Cape region of South Africa. This specific strain is classified as MDR, with resistance to isoniazid and rifampicin but susceptible to ethambutol (24,25). SCAID 252.0 is a clinical sample that was geographically found at Karagandy, Kazakhstan, and was submitted by the Scientific Centre for Anti-infectious Drugs. This particular strain was extracted from the sputum of a patient diagnosed with MDR-TB who was involved in the second phase of a clinical trial of the novel pharmaceutical agent FS-1. The sputum sample was obtained prior to the administration of the initial dosage (during the screening phase). Drug susceptibility assessments on the strain conducted on both solid and liquid media indicated resistance to first-line anti-TB agents as well as to certain second-line anti-TB medications. This strain underwent sequencing to investigate the phenomenon of reversion of sensitivity, which was identified during clinical trials and subsequently verified through experimentation involving guinea pigs (26).

The query strain KZN4207, is a wild-type drug-sensitive strain that belongs to the lineage 4 (4.5). KZN 4207 belongs to F15/LAM4/KZN and was isolated from the sputum of a human sample at KwaZulu Natal Province in South Africa (25,27). The KZN R506 strain is an XDR human sample found in the KwaZulu Natal province of South Africa (24,28). HN878 is a representative of the Beijing lineage and was obtained as a clinical isolate in the USA in 1990. HN878 is considered to be hyper virulent, although it is not drug resistant but rather pan susceptible (24,25).

The XDR-MTB isolate sequenced in our study (XMTB2) is a highly virulent strain that belongs to either the Beijing lineage (L2) or the Euro-American lineage (L4). The occurrence of the Beijing lineage in Nigeria is not surprising, considering that it was discovered in Benin Republic, which shares a western border with Nigeria, and the continuous growth of Nigeria-China economic connections over the past few decades. Another study indicated a degree of association with HIV. The East Asian or Beijing lineage is well known for its increased virulence, rapid illness development, and MDR, which sets it apart from other MTBC lineages (4).

Our study has some limitations. First, the study was limited by the relatively small number of isolates that underwent sequencing, as only two XDR isolates formed the basis of this study. Of these, one relatively failed quality control, thereby limiting the anticipated information from the sequenced genomes. It is likely that a larger sample size of XDR isolates will reveal more information on genomic characteristics and determinants of resistance in XDR *M. tuberculosis*. Secondly, the study did not consider the demographic aspects of the XDR isolate especially the HIV status which could have revealed more information especially with respect to treatment.

Conclusion:

This study investigated the determinants of antibiotic resistance in XDR *M. tuberculosis* isolate, the virulence factors and the phylogenetic relationship with other *M. tuberculosis* strains. Although it was difficult to obtain an XDR isolate, the results of this study proved that the XMTB2 MAG was potentially an XDR isolate that is very virulent because of the presence of many virulence factors identified in it and its close relationship with two of the most widespread and virulent lineages of *M. tuberculosis,* the Beijing lineage (L2) and the Euro-American lineage (L4).

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Contribution of authors:

DI Conducted the laboratory work and analysed data and writing of the first draft of the manuscript. OOA conceptualized, designed and supervised the study. OOA also analysed some data and participated in writing the manuscript. DMA proof read and edited the manuscript. TTE participated in the laboratory work and writing of the manuscript. All authors approved the final copy of the manuscript.

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No conflict of interest is declared

Availability of data and materials:

The dataset of this study is available from the corresponding author on reasonable request. The sequenced data in the study were successfully uploded to the NCBI SRA database with the BioProject accession number PRJNA 1256495

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