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Genetic Diversity and Drug-Resistance Patterns of *Mycobacterium tuberculosis* from Pulmonary Tuberculosis Patients in the Southwest and Littoral Regions of Cameroon

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Abstract

Background: In Cameroon, data on *Mycobacterium tuberculosis* (MTB) lineage diversity, drug-resistance burden and on-going disease transmission are lacking. Existing-reports are derived from conventional genotyping and phenotyping methods, with limited power to discriminate between MTB sub-lineages. Therefore, we used Whole-Genome Sequencing (WGS) to investigate MTB lineage diversity and drug-resistance patterns in Pulmonary TB patients in the Southwest and Littoral Regions of Cameroon, relevant to Tuberculosis (TB) prevention and control. **Methods:** Sputum samples were collected from

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552 Pulmonary Tuberculosis (PTB) patients and cultured on Lowenstein-Jensen (LJ) medium. DNA was extracted from the MTB isolates and whole genome sequenced. A total of 88 high quality MTB sequence reads were obtained and used to generate a Variant Calling File (VCF), which was analyzed using TB-Profiler pipeline to predict MTB lineage diversity and drug-resistance pattern. **Results:** Three MTB lineages were identified. The most abundant being the L4-Euro-American lineage (98%), followed by L2-East Asian (1%) and L3-East-Asian India (1%). Nine sub-lineages were identified: L4-LAM_10 Cameroon (45%), L4-Haarlem (20%), L4-Euro-American (10%), L4-Mainly T (6%), L4-Uganda II (2%), L4-LAM (1%), L3-Delhi-CAS (1%), L4-Congo type (1%) and the L2-Beijing genotype (1%). Notably, no isolates of the West African strains (L5 or L6) were found. Three isolates were Rifampicin resistant (3.4%); one was isoniazid resistant (1.1%); one was streptomycin resistant (1.1%) and four (4.5%) were resistant to second-line drugs. No Multi-Drug-Resistant (MDR) MTB was identified. **Conclusion:** This study revealed that the MTB Complex population in the Littoral and Southwest Regions of Cameroon is diverse, indicating ongoing transmission. There also appears to be marked regression of *Mycobacterium africanum* in favor of the LAM_10 Cameroon. Further investigation could provide insight into the genetics, epidemiology, and transmission dynamics of the LAM_10 Cameroon genotype.

Keywords

Mycobacterium tuberculosis complex, Whole Genome Sequencing, MTBC Lineage, Genotyping, TB Severity

1. Background

Tuberculosis (TB) is the main cause of morbidity and mortality, due to a single infectious cause, globally, with over 10.0 million new cases and 1.3 million deaths annually, a quarter of all cases coming from Africa [1]. Cameroon is among the top 20 countries with the highest burden, with 46,000 new cases annually and an incidence rate of 150/100,000 per year [1]. TB disease, is due to infection with *Mycobacterium tuberculosis* complex (MTBC) [2], comprising nine different lineages; lineages 1 - 4, and 7 referred to as *M. tuberculosis sensu stricto,* lineage 5 and 6 as *M. africanum* lineages, lineage 8 recently discovered in the Great Lakes region of East Africa [3] and lineage 9 in East Africa [4]. Lineages 2, 3 and 4 have a global distribution (generalists), whereas other lineages are more geographically restricted (specialists), like L7, which is restricted to the Horn of Africa, L5 and L6, are found mostly in West Africa [3] [5] [6]. Lineage 2 (the Beijing genotype) originated in East Asia [7] and has expanded to some parts of the world, including Africa [8]. Lineage 4 (the Euro-American lineage) is globally distributed, though some of its genotypes (such as the Uganda and the Cameroon genotypes predominant in Ugandan and Cameroonian populations, respectively) are geographically restricted. Despite control efforts, there is evidence of ongoing TB transmission

in Cameroon [9]. Early studies reported *Mycobacterium africanum* West, as the leading etiology of pulmonary TB in Cameroon [10]. However, recent studies suggest the predominance of *M. tuberculosis* in place of *M. africanum* [11]-[13]. The L4 LAM_10 Cameroon genotype has a high clustering rate, indicating active transmission and expansion within communities and this is often linked to socio-economic factors, healthcare access, and population density, coinciding with human population growth and movement, particularly in regions with limited tuberculosis control measures. The emergence and spread of MTBC genotypes previously absent in a region could be associated with immigration, clinical and demographic factors, or evolution of MTB isolates, due to evolutionary pressures such as drug resistance, host adaptation (host-pathogen interaction) and environmental factors, suggesting a constant variation in MTBC genotypic population structure [14] [15]. Furthermore, in Cameroon, previous studies on TBMTB were largely based on conventional phenotypic and genotypic methods, such as Restriction Fragment Length Polymorphism (REFLP), Spoligotyping and Mycobacterium Interspese Repetitive Unit-Variable Number (MIRU-VNTR), with subjective interpretations often yielding inconclusive results [16], limited ability for MTBC sublineage resolution [17] and unable to discriminate between MTBC and Non-Tuberculous Mycobacteria (NTM) genotypes. To expand these findings, Whole Genome Sequencing (WGS) for higher phylogenetic resolution was used to map the genetic diversity of MTB and their drug-resistance patterns, in pulmonary TB patients from the Southwest and Littoral Regions of Cameroon.

2. Materials and Methods

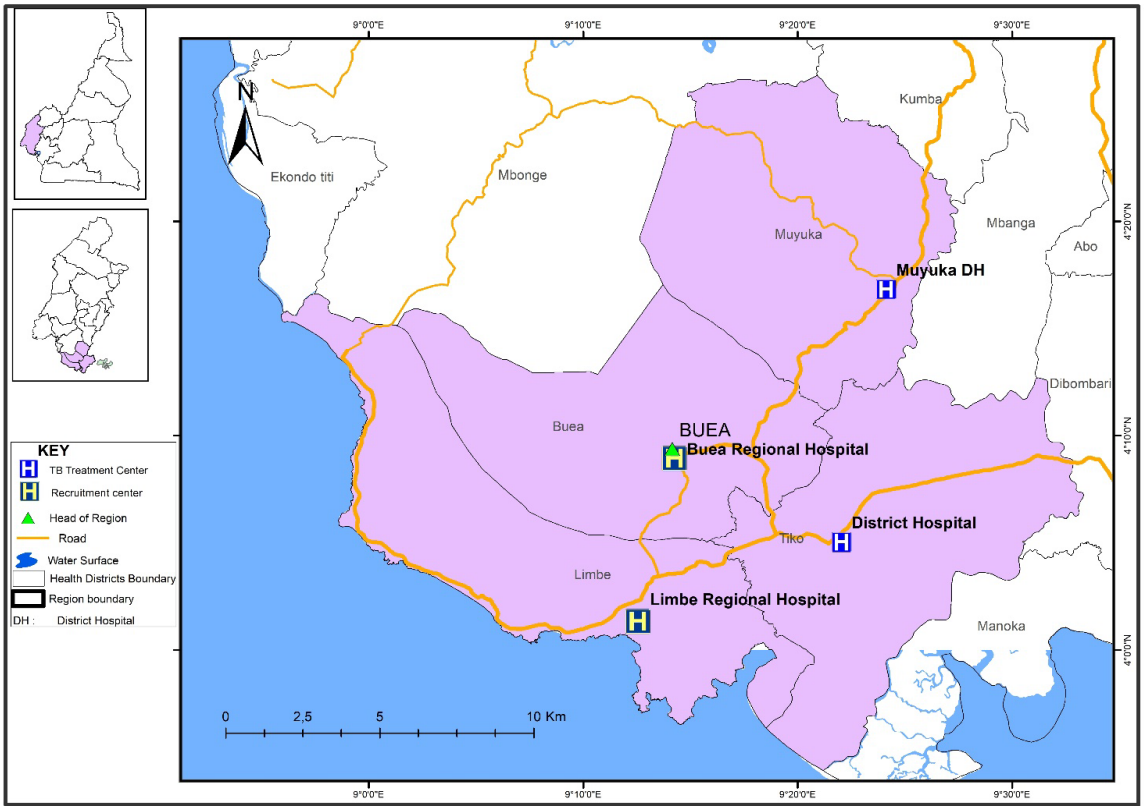
2.1. Study Design

This study adopted a prospective cross-sectional design, and sample collection occurred from February 2020 to February 2023 involving newly diagnosed bacteriologically confirmed pulmonary TB patients, seeking TB diagnosis and treatment in TB diagnostic and treatment centres in the Southwest and Littoral Regions of Cameroon.

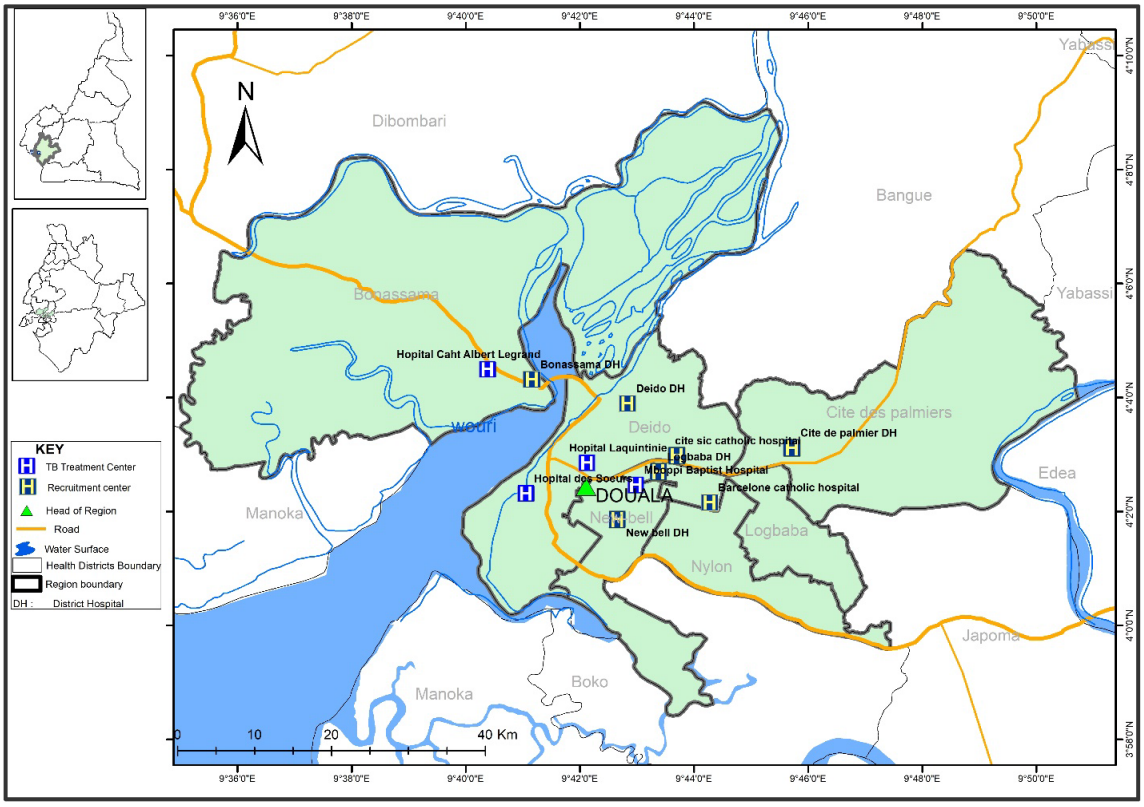
2.2. Description of Study Sites and Study Population

Nine TB diagnostic and treatment centres were selected purposively for participant recruitment based on proximity and accessibility and also high turn-out of individuals seeking TB diagnosis and care. In the Southwest, the Buea and Limbe Regional hospitals were selected (**Figure 1(A)**) and seven centres, were selected in Douala, in the Littoral Region, that is, the Deido, Cité des Palmiers, New-Bell, Logbaba the Bonassama, Cite Sic Catholic hospitals, and the Barcelone Catholic Hospital (**Figure 1(B)**). The city of Douala, is the headquarters of the Littoral Region of Cameroon and it is also economic capital. It is situated at approximately 4.0511˚ N latitude and 9.7679˚ E longitude. The city is densely populated with an estimated population size of 4.3 million people (https://www.city-facts.com/douala-cm/population). Buea is the headquarters of the Southwest Region, situated at

approximately 4.16667˚N latitude and 9.23333˚E longitude and has a population of approximately 300,000 people (https://www.city-facts.com/buea/population). Limbe often referred to as the "Town of Friendship", is a charming seaside city located in the Southwest Region of Cameroon. It is nestled along the Atlantic Ocean, at the foot of Mount Cameroon. It is located at nearly 4.017˚ N latitude and 9.217˚ E longitude and has an estimated population of 200,000 people (https://www.city-facts.com/limbe/population). The study population consisted of individuals aged 18 years and above, of both sexes, newly diagnosed with smear-positive Pulmonary TB, who consented to participate in the study and were willing to provide sputum samples. Relapse cases, TB-positive cases who had already started treatment were excluded from the study. Participants, were recruited before their enrolment into the Directly Observed Treatment (DOT) program in these centres. The DOT program is a key component of Cameroon's National Tuberculosis Control Program (NTP). It is in line with the World Health Organization's DOT strategy, which aims to ensure effective TB treatment and reduce transmission. The DOT program in Cameroon comprises healthcare workers and/or volunteers trained to directly observe patients as they take their TB medication. This approach helps improve adherence to treatment regimens and prevents drug-resistant TB strains from developing. Case Report Forms (CRF) for consenting participants were filled out to capture their clinical, socio-economic and demographic data prior to sample collection.



(A)



(B)

Figure 1. (A) A Map indicating the two participant recruitment centres, in the Southwest Region of Cameroon; (B) A Map indicating the seven participant recruitment centres in the Littoral Region of Cameroon. (Source: Frank Nietcho)

2.3. Assessment of HIV and Anemia Status of Study Participants

Prior to sample collection, the HIV status of the participants was obtained from their medical records or determined by performing the HIV rapid diagnostic test using Determine HIV1/2 RTD (Alere Medical Co., Ltd.). Anemia was determined by clinical examination and assessment of hemoglobin levels. As point-of-care testing, clinical examination of pallor, for paleness of skin and mucous membrane of the lower palpebral conjunctiva of the eye and hemoglobin levels were assessed using a portable hemoglobin machine and test strips (Mission diagnostics), following the manufacturer's instructions. Anemia was determined if hemoglobin levels were beneath the normal range: <13 g/dL for men and <12 g/dL for women.

2.4. Sputum Collection, Processing and Culture

Sputum samples were collected from 554 participants prior to treatment initiation (two participants were unable to provide samples). Approximately 5 mL of fresh sputum was collected in 50 mL Falcon tubes (Corning, USA) and transported in a cool chain to the research laboratory for processing and culture. All procedures were performed at appropriate biological safety standards for pathogenic bacteria. Sputum samples were processed using equal volumes of sputum to MycoPrep re-

agent containing sodium hydroxide and N-acetyl L-cysteine solution (Becton, Dickinson), and were cultured on Lowenstein Jensen slopes as previously described [18]. Tubes were checked weekly for mycobacterium growth. Smears were also prepared prior to culture using the Ziehl-Neelsen staining procedure to count Acid Fast Bacilli (AFB), observed at 100x objective, and positive slides were graded as: 1+, 2+, 3+ or rare bacilli, based on microscopic examination [19].

2.5. MTB DNA Extraction

Two loops of culture positive colonies, from LJ slopes were added into 2 mL Eppendorf tubes, with 500 µL sterile 1x Tris EDTA buffer. Isolates were heat-killed by incubating the tubes in a water bath at 80˚C for 20 minutes. DNA was extracted using the cetyl trimethylammonium bromide-sodium chloride (CTAB/NaCl) protocol as previously described [20]. The tubes containing DNA were allowed to dry at room temperature before adding 35 µL of elution buffer. DNA samples were run on 1% agarose gel to check the quality and the concentration of DNA was measured by Q fluorimetry (INVOTROGEN, Thermo Fisher Scientific). Samples were stored at −20˚C. A total of 125 high-quality DNAs, with concentration of ≥ 12.5 ng/µL, were selected out of the 202 extracted DNA samples and shipped for sequencing.

2.6. DNA Library Preparation and Sequencing

A total of 125 MTB DNA samples were sequenced (at the Beijing Genomic Institute in Poland), and all samples met the standards for sequencing following quality control assessment. 17 µL DNA per sample was used to construct WGS PCR libraries of ≤ 800 bp and whole-genome sequenced using the DNA nanoball sequencer (DNBSeq-500), following the manufacturer's recommendations, through the steps of DNA fragmentation and size selection [21] end repair, "A" tailing and adapter sequence ligation [22], rolling circle replication by PCR reaction [22], DNA nanoball patterned array for fluorescent detection of hybridization and ligation reactions [23], and imaging for detection of DNA nanoball fluorescence [23]. The raw sequence data of 150 bp reads (short reads), were generated as standard paired-end fastq files. Adapters were trimmed, and low-quality sequences and contaminations were filtered using the SOAPnuke software filter parameters proprietary to BGI: "–n 0.01, –l 20, –q 0.3—adaMR 0.25—ada_trim—polyX 50—minReadLen 150" [24].

2.7. *In Silico* Genotyping of MTB Lineages and Drug-Resistance Prediction

The quality of the raw reads was checked using FastQC toolkit followed by trimming of adapters and low-quality bases. Sequences with low % GC content (<63%) were excluded as contaminants. Sequences with Phred scores of ≥ 30 and a % GC content > 63% were aggregated into a single file using the MultiQC tool. Reads were then mapped onto *M. tuberculosis* H37Rv reference genome (NC_000962.3)

using the Burrows-Wheeler Alignment Tool (BWA). Alignment files with good quality sequences, were sorted, indexed, and visualized using the Sequence Alignment Map tool (SAMtool). Variants: Single Nucleotide Variants (SNVs) and Insertion/Deletions (In/Dels) were called and filtered using binary variant call format tool (BCFtools). Variant filtering was carried out by removing variants with <5 reads and low base quality, with a Phred quality score > 20. The variants were annotated using the Single Nucleotide Polymorphism Effect tool (SNPEff tool) to generate the annotated Variant Calling File (VCF) or script 1. MTB main lineages and sub-lineages were identified, and drug-resistance profiling was performed using TB-profiler tool (version 6.3) from the annotated VCF file. Figure 2 illustrates the design of workflow from sample collection to sequencing and bioinformatics analysis.

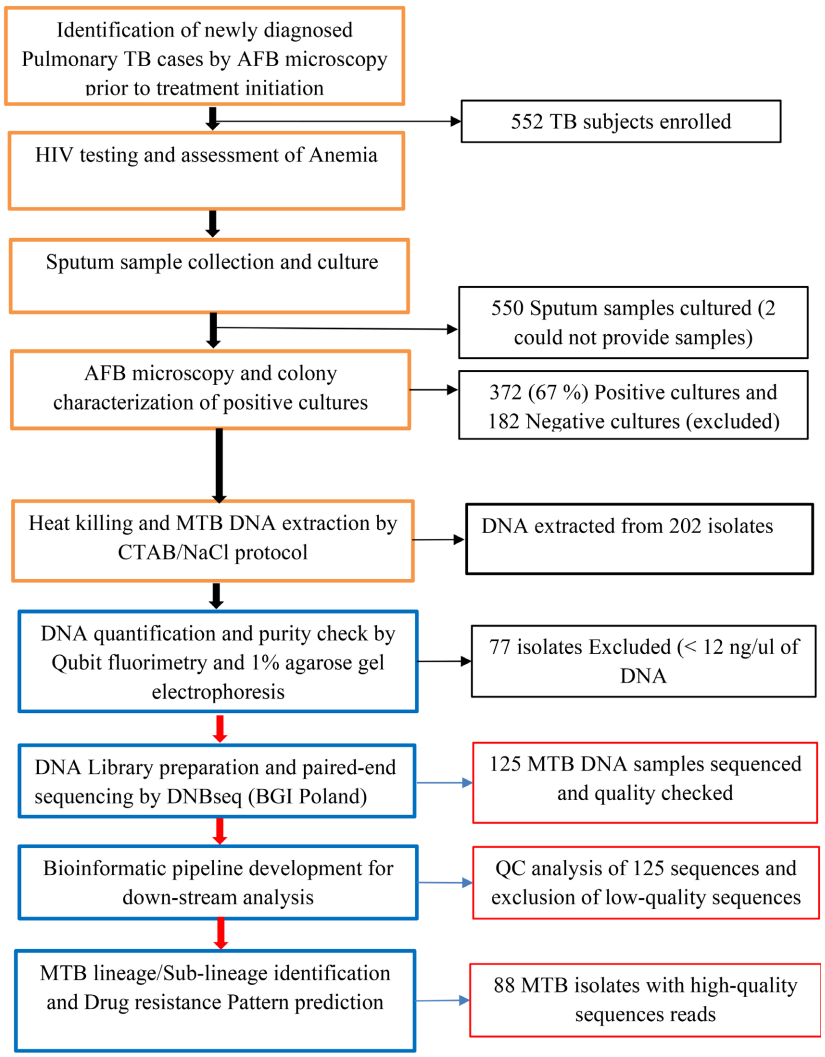


Figure 2. Flowchart summarizing the design of the study and the quality control measures applied from sample collection, WGS to Bioinformatics analysis.

2.8. Classification of TB Disease Severity

To capture the severity of TB pulmonary disease, we assessed both clinical and

microbiological measures of disease. To determine the clinical TB disease severity, we used the TBscore II (also known as the modified TB Bandim score), developed by Frauke in 2014 [25]. This is a clinical algorithm or model used to predict the clinical outcome or prognosis of TB. The model is constructed using five clinical parameters: cough, dyspnea, chest pain, anemia, Body Mass Index (BMI) < 18 kg/m^2, BMI <16 kg/m^2, Mid-Upper Arm Circumference (MUAC) < 220 mm, and MUAC < 200 mm [25]. Each parameter accounts for 1 point on a total score of 8. The TB severity classes for TBscore II are: Class I, score < 2 (mild disease), Class II, score 2 - 3 (moderate disease), Class III, score 4 - 7 (severe disease) and Class IV, score > 7 (very severe disease). **Table 3** summarizes the 8 clinical parameters for the TB disease classification based on the TBScore II algorithm (Modified BANDIM score) and **Figure 3** shows the proportions of the TB disease severity categories or classes. Most of the TB patients, 88/125 (70%), had moderate TB disease, 24/125 (19%) had mild TB, whereas 13/125 (10%) had severe TB.

3. Results

3.1. Demographics of Study Population

The data presented are for 125 out of the 552 participants, with available MTB WGS data. Of these participants, 84 (67.2%) were male and 41 (32.8%) were female. The mean ± SD age of the participants was 34.77 ± 12.43 years, the mean ± SD weight was 60.1 ± 9.5 kg and the mean ± SD Body Mass Index (BMI) was 20.9 ± 3.3. Cameroon is divided into five main bio-ecological zones, according to the vegetation, climate, and ethno-linguistic and cultural characteristics, into: the Mono-modal humid rain forest, the Grass-fields, the Sudano-Sahelian, the Bi-modal rainforest and the Quinea Savannah. 45 (28%) of the participants were from the Mono-modal humid rain forest, 55 (44%) from the Grass-fields, 22 (17.6%) from the Sudano-Sahelian region, 9 (7.2%) from the Bi-modal humid rain forest. Three (2.4%) participants were from Nigeria and one (0.8%) participant was from Benin. **Table 1** is a summary statistic, of the demographics, for the 125 study participants. **Table 2** is a description of the participants' clinical parameters, medical history, socio-economic and environmental risk factors. Majority of the participants were laborers, 53/125 (42%), with a low monthly income of XAF < 30,000 (USD 49.2). The most prevalent clinical symptom of pulmonary TB was cough, which all 125 (100%) participants reported suffering from. This was followed by chest pain, 65/125, (52%), haemoptysis 23/125 (18.4%), dyspnoea, 22/125 (17.6%) and anemia, 16 (12.8%). The HIV positivity rate was 20/125 (16%), whereas 102/125 (81.6%) were HIV negative and 3/125 (2.4%) were not tested. The most prevalent co-morbidity after HIV was diabetes 10/125 (8%), followed by hypertension, 5/125 (4%), kidney disease, 3 (2.4%), then cardiovascular disease, 2/125 (1.6%) and then hepatitis, 1 (0.8%). For risk indicators, 26/125 (20.8%) reported having contact with a TB patient. Other risk factors were cigarette smoking, 33/125 (26%), alcohol consumption, 74/125 (59.2%) and then 8/125 (6.4%) reported having been to prison at least once.

Table 1. Descriptive statistics of participant demographics.

Gender	Frequency (n)	Percentage (%)
Male	84	67.2
Female	41	32.8
Age Category (years)		
18 - 35	81	64.8
36 - 50	28	
51 - 65	13	10.4
>65	03	2.4
Occupation		
Student	18	14.4
Government Employed (Public)	07	5.6
House wife	04	3.2
Laborer	58	46.4
Others (Private or self-employed)	38	30.4
Income (XAF)		
Low < 30,000	41	33.0
Middle low (30,000 - 60,000)	50	40.0
Middle (60,000 - 100,000)	22	18.0
Upper middle (100,000 - 200,000)	9	7.0
High (> 200,000)	3	2.0
Education		
No Formal Education	6	5.0
Primary	69	55.0
High School	37	30.0
Diploma and above	13	10.0
Bio-ecological Region		
Bi-modal humid forest	9	7.2
Mono-modal humid forest	35	28.0
Grass field	55	44.0
Sudano-Sahelian	22	17.6
Nigerian (Yoruba/Akwaibom)	3	2.4
Benin republic	1	0.8

Table 2. Clinical history and socio-environmental risk factors.

Clinical Parameters	Yes n (%)	No n (%)	Don't know n (%)
Anemia	16 (12.8)	109 (87.2)	-
Dyspnoea	22 (17.6)	103 (82.4)	-
Chest pain	65 (52.0)	60 (48.0)	-

Continued

Cough	125 (100)	-	-
Hemoptysis	23 (28.5)	102 (81.6)	-
TB Severity Class			
Mild	24 (19.0)	-	-
Moderate	88 (70.0)	-	-
Severe	13 (10.0)	-	-
Medication History			
Diabetes	10 (8.0)	110 (88.0)	5 (4.0)
Hypertension	5 (4.0)	115 (92.0)	5 (4.0)
Cardiovascular disease	2 (1.6)	119 (95.2)	4 (3.2)
Kidney disease	3 (2.4)	116 (92.8)	6 (4.8)
Hepatitis	1 (0.8)	118 (94.4)	6 (4.8)
HIV Status	20 (16)	102 (81.6)	3 (2.4)
Other infections	30 (24)	82 (65.6)	60 (48)
TB Contact	26 (20.8)	99 (79.2)	-
Hospital admission	44 (35.2)	81 (64.8)	-
Other medication	69 (55.2)	56 (44.8)	-
Risk Factors			
Firewood smoke	55 (44.0)	70 (56.0)	-
Cigarette smoking	33 (26.0)	92 (74.0)	-
Prison	8 (6.4	117 (93.6)	-
House window	121 (96.8)	4 (3.2)	-
Alcohol	74 (59.2)	51 (40.8)	-
Meat consumption	**Monthly**	**Weekly**	**Rarely**
	15 (12.0)	38 (30.4)	72 (57.6)
Room size	**1 - 2 rooms**	**3 - 4 rooms**	**> 4 rooms**
	66 (53.0)	37 (30.0)	24 (17.0)
Family size	**1 - 5 persons**	**6 - 10 Persons**	**> 10 persons**
	81 (65.0)	41 (33.0)	3 (2.0)

3.2. Classification of TB Disease Severity

To capture the severity of TB pulmonary disease in the cohort, we assessed both clinical and microbiological measures of disease. To determine the clinical TB disease severity, we used the TBscore II (also known as the modified TB Bandim score), developed as previously described. Table 3 summarizes the clinical parameters for the TBscore II Algorithm to classify TB disease severity. Most of the participants, 88/125 (70%), had moderate TB disease, 24/125 (19%) had mild TB, whereas 13/125 (10%) had severe TB as indicated in Figure 3.

Sputum smear examination for Acid-Fast Bacilli (AFB) is crucial for initial diagnosis and in the follow-up of smear-positive pulmonary TB patients to monitor their treatment and assess their treatment outcome. Figure 4 indicates the AFB

burden of the 125 pulmonary TB participants. Our study revealed that the majority 43/125 (34,4%) of participants had an AFB smear grade of 3+, 39/125 (31.2%) had a smear grade of 2+, 29/125 (23.2%) had a smear grade of 1+, and 8/125 (6.4%) had rare AFB. Seven (5.6%) did not have AFB results.

Table 3. Clinical parameters for TBscore II Algorithm.

TBscore II Clinical Parameters	n (%)
Anemia	16 (12.8)
Dyspnoea	22 (17.6)
Chest pain	65 (52.0)
Cough	125 (100)
BMI < 16 kg/m^2	03 (2.4)
BMI < 18 kg/m^2	12 (9.6)
MUAC < 200	03 (2.4)
MUAC < 220	10 (12.5)

BMI: Body Mass Index; **MUAC:** Mid-Upper Arm Circumference.

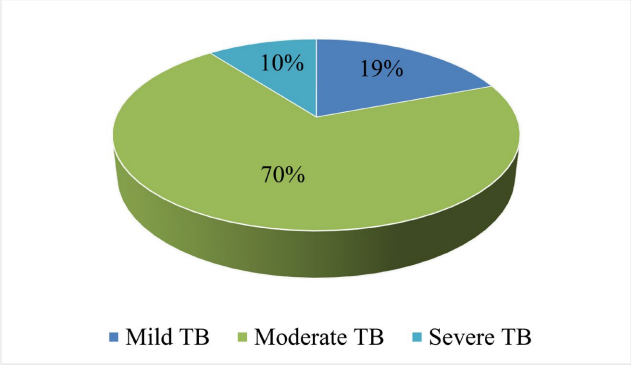


Figure 3. Distribution of TB severity classes.

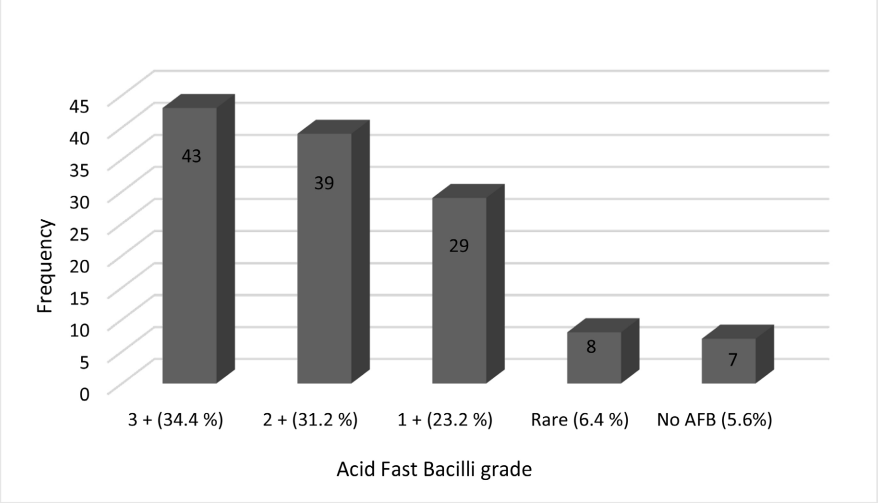


Figure 4. Classification of participants according to Acid Fast Bacillus (AFB) grade.

3.3. Genetic Diversity of MTBC from Whole Genome Sequencing

We used WGS to map the genetic diversity of MTBC in Southwest and Littoral Cameroon. After excluding isolates with poor sequence quality (low depth and poor coverage) or contamination, 88 isolates with high-quality sequence reads were identified as MTBC. One sample was identified as mixed lineage, consisting of two sub-lineages L4-LAM_10 Cameroon (L4.6.2.2) and L5-Mycobacterium africanum West (L5.1.1), leaving 87 high-quality sequenced MTBC isolates. Lineage 4 (Euro-American lineage) was the most abundant, 85/87 (98%), followed by lineage 2 (East Asian lineage) 1/87 (1%), and lineage 3 (East Africa India) 1/87 (1%). Lineages 5 and 6 were not identified. Three of the 87 isolates appeared as mixed sub-lineages. One of the mixed-sub-lineages consisted of three sub-lineages: L4-LAM_10 Cameroon (L4.6.2.2), the L3-Haarlem (L4.1.2.1) and the L4-Euro-American (4.1.3) sub-lineages. The second mixed sub-lineage consisted of two sub-lineages: the L4-LAM_10 Cameroon (L4.6.2.2) and the L4-Euro-American (4.1.3). The third mixed sub-lineage had two Euro-American (L4.6.4 and L4.1.3) sub-lineages. Although these mixed lineages had a high percent coverage target (pct) mapping of ≥ 99%, they were not represented since further investigation was needed to confirm whether they were real cases of mixed infections or cases of cross-contamination. Nine MTBC sub-lineages were identified as represented in Figure 5. Seven of them belonged to lineage 4, of which the LAM_10 Cameroon genotype was the most abundant sub-lineage 42/84 (50%). This was followed by Haarlem 20/83 (24%) and Euro-American 10/83 (11%). The two other sub-lineages were L2-Beijing ancestral 3 l/83 (1%), and the L3-Delhi/CAS 1/83 (1%) belonging to lineages 2 and 3, respectively.

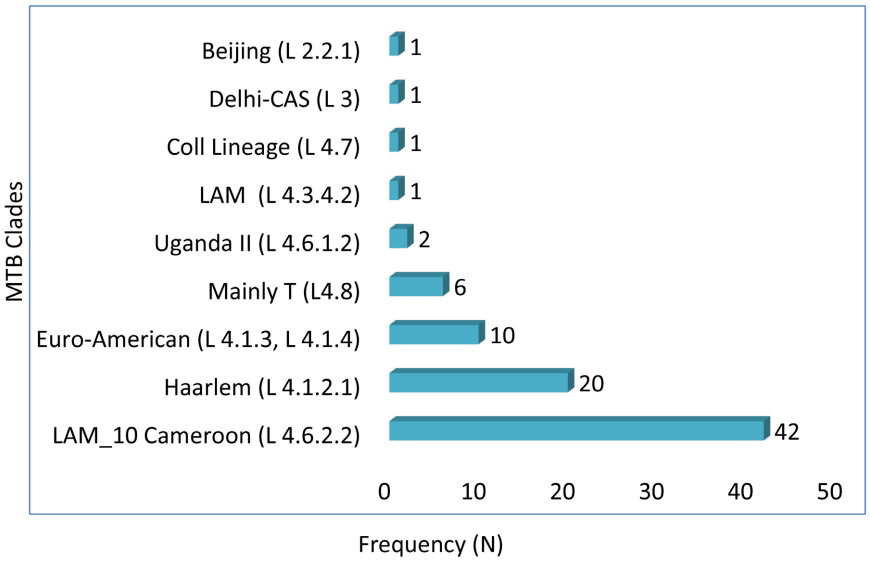


Figure 5. Distribution of MTB sub-lineages and their Coll designations in parentheses in Southwest and Littoral Cameroon.

Figure 6 illustrates the distribution of MTB clades according to AFB glade. The

LAM-10 Cameroon genotype is associated with higher AFB grades (2+ and 3+), and was the predominant strain in severe TB cases as shown in Figure 7. High AFB grade and greater disease severity could aid transmission.

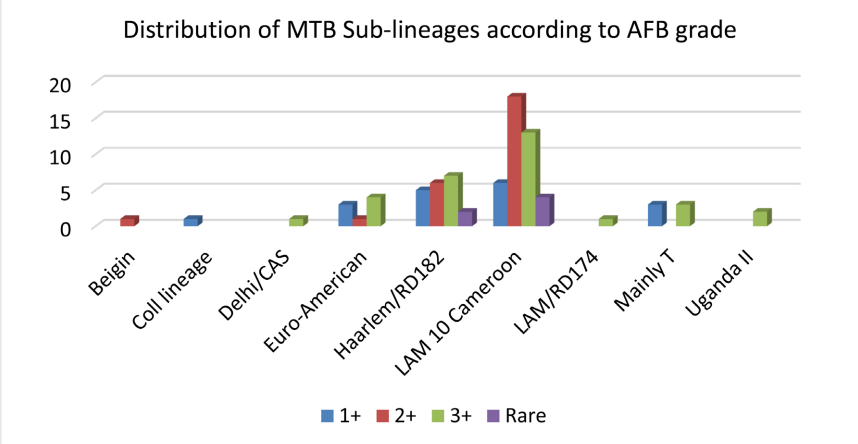


Figure 6. Distribution of MTB sub-lineages according to AFB category.

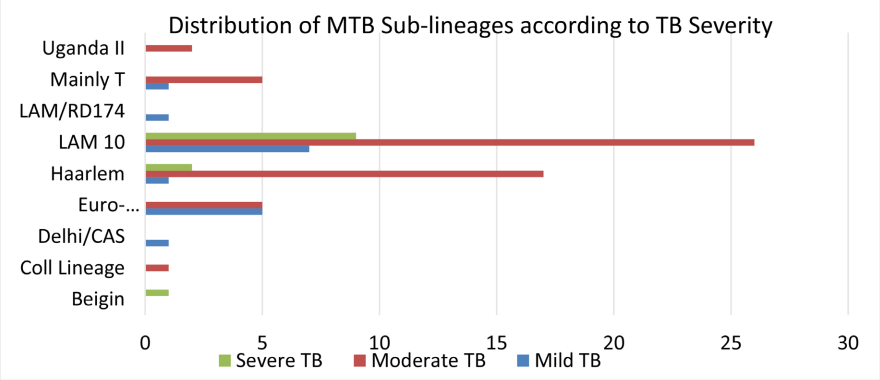


Figure 7. Distribution of MTB Clades according to TB severity.

3.4. Association between Co-Morbidities, Mycobacterium Strain and TB Disease Severity

A multivariate regression analysis was performed using the data analysis software R-version 4.4.1, to determine the association between co-morbidities, mycobacterium strain (LAM-10 Cameroon genotype), and TB disease severity class. The analyses indicate that an increase in AFB category (1+ to 3+) increases the likelihood of developing severe TB (OR = 39, p = 0.022). HIV positivity and Dyspnea, were significantly associated with severe TB; p = 0.014 and 0.021 respectively. Cigarette smoking was also found to be a significant risk factor for developing severe TB disease (OR = 34.6; p = 0.022). Analyses showed no significant association between mycobacterium strain and sex or age. However, an increase in age by a year increases the likelihood of having TB due to LAM_10 Cameroon genotype (OR = 1.10, p = 0.009). There was a significant association between LAM-10 genotype and moderate TB infection (OR = 7.94, p = 0.03) compared to severe TB

disease. There was no significant association between LAM_10 strain, co-morbidities (including HIV), drug-resistant type and ethnicity. The lack of significant association could be attributed to low statistical power, resulting from the small sample size.

3.5. Prediction of MTBC Drug-Resistance Patterns

To identify polymorphisms, Single Nucleotide Polymorphisms (SNPs) and insertions/deletions (In/Dels), associated with drug-resistance, variant calling was performed and mapped to the *Mycobacterium tuberculosis* reference genome, H37Rv. This was done, using TB-Profiler (version 6.3) with a curated TB drug-resistance database of SNP panels for 21 first and second-line anti-TB drugs recommended by WHO for treatment of TB. TB profiler predicted nine drug-resistant variants (10. 2%) and 79/88 (89.8%) of the isolates were drug sensitive. The distributions of the total number of drug-resistant variants according to MTB sub-lineages and drug types are summarized in Tables 4-6. Of the nine resistant isolates, 4/88 (5.7%) showed resistance to the first-line drug Rifampicin, 3/87 (3.4%) to Isoniazid, 1/87 (1.1%) to Pyrazinamide 1/87 (1.1%), and 4/87 (4.5%) were resistant to Streptomycin. The distribution of drug resistance variants according to MTB sub-lineages is shown in Table 4 and description of MTB sub-lineages by drug resistance and drug susceptibility is summarized in Table 7. One of the three Rifampicin-resistant isolates belonged to the Euro-American sub-lineage (4.1.3) and this isolate was poly-resistant, as it harbored resistant genes *rrs*1402C>A, for aminoglycoside, amikacin, capreomycin and kanamycin. This poly-resistant isolate harbored the double mutations rpoB_His445Asn and PanD_Val138Ala in the *rpoB* gene, conferring resistance to Rifampicin. The two remaining Rifampicin mono-resistant isolates belonged to the LAM_10 Cameroon genotype (4.6.2.2). This Rifampicin mono-resistant LAM 10 isolate had the mutations *rpoB*_His445Asp and *rpoB*_Pro454Ser in the *rpoB* gene. The isoniazid-resistant isolate belonged to the Haarlem sub-lineage (4.1.2.1) and harbored resistance to ethionamide, due to the presence of the mutation *fab*G1_15C>T in the *katG* gene of the promoter region, conferring resistance to both drugs. The Pyrazinamide-resistant isolate had the *panD*Val138Ala mutation. Of the four streptomycin-resistant isolates, one isolate was of the Beijing clade (2.2.1), having the mutation *rpsL*_Lys43Arg in *rpsL* gene, one Euro-American sub-lineage (4.1) with the mutation *gid*_351delG and two Haarlem sub-lineages (4.1.2.1), with mutations *gid*_115delC, and *gid*_386delG, all in the SM/*gid* gene. Table 6 summarizes the distribution of drug resistance mutation types according to MTB sub-lineages. The mutations Ser441Ala, His445Asp and Pro454Ser in the *rpoB* gene conferring resistance to Rifampicin, the 386 delG, 115 delG and 315 delG in the *gidB* gene conferring resistance to streptomycin, the rare mutation *panD*Val138Ala confers resistance to pyrazinamide and the 1402 C>A mutation in *rrs* gene confers resistance to the second-line anti-TB drugs: aminoglycosides, amikacin, capreomycin and kanamycin, were all new mutations not previously reported in Cameroon. No ethambutol-resistance mutation was

found. Table 7 describes the gene mutation types and the cellular component encoded.

Table 4. Distribution of drug-resistant variants according to MTB sub-lineages.

Lineage	Clade	Number of drug-resistant variants (%)
L 2	Beijing	1 (11.0)
L 3	Deli CAS	0 (0.0)
L 4	Congo type	0 (0.0)
	Euro-American	2 (22.0)
	Haarlem	3 (33)
	LAM	0 (0.0)
	LAM 10 Cameroon	2 (22.0)
	Mainly T	0 (0.0)
	Uganda II	0 (0.0)
Mixed	Mixed lineages	1 (11.0)
	Total	**9**

Table 5. Distribution of MTB sub-lineages by resistance and susceptibility status.

Lineage	MTB Sub-Lineages (clade)	Drug-Resistant Types				
		HR-TB n (%)	Other Drug n (%)	RR-TB n (%)	Sensitive n (%)	Total n (%)
L 2	Beijing	-	1 (1.1)	-		1 (1.1)
L 3	Deli CAS	-	-	-	1 (1.1)	1 (1.1)
L 4	Congo type	-	-	-	1 (1.1)	1 (1.1)
	Euro-American	-	1 (1.1)	1 (1.1)	9 (10.2)	11 (12.5)
	Haarlem	1 (1.1%)	2 (2.3)	-	17 (19.3)	20 (22.7)
	LAM	-	-			1 (1.1)
	LAM 10 Cameroon	-	-	2 (2.3)	40 (45.5)	42 (47.7)
	Mainly T	-	-		3 (5.7)	5 (5.7)
	Uganda II	-	-	-	2 (2.3)	2 (2.3)
Mixed	* Mixed lineage	-	1 (1.1)	-	3 (3.4)	4 (4.5)
	Total	**1 (1.1)**	**5 (5.7)**	**3 (3.4)**	**79 (89.8)**	**87 (100)**

* HR-TB: Isoniazid mono-resistant, RR-TB: Rifampicin mono-resistant.

Table 6. Summary of drug resistance status and gene mutation of MTB sub-lineages.

Clade	Number of Isolates	Gene Name	Mutation Type	Drug Name	Drug-Resistance
Beijing genotype	1	*rpsL*	Lys43Arg	Streptomycin	Mono-resistant
Euro-American	1	*gid*	351delG	Streptomycin	Mono-resistant
	1	*rpoB*	His445Asn Ser441Ala	Rifampicin	Poly-resistant

Continued

		Rrs	1402C>A	Aminoglycosides Amikacin Capreomycin Kanamycin	
Haarlem	1	*gid*	_386delG	Streptomycin	Mono-resistant
	1	*gid*	115delG	Streptomycin	Mono-resistant
	1	*fabG1-inhA*	15C>T	Isoniazid Ethionamide	Mono-resistant Mono-resistant
LAM_10 Cam	1	*rpoB*	His445Asp	Rifampicin	Mono-resistant
	1	*rpoB*	Pro454Ser	Rifampicin	Mono-resistant
*Mixed lineage	1	*panD*	Val138Ala	Pyrazinamide	Mono-resistant

* HR-TB: Isoniazid mono-resistant, RR-TB: Rifampicin mono-resistant.

Table 7. Description of gene mutation type conferring resistance to first and second-line drugs and the cellular component they encode.

Drug-Resistance Gene	Nucleotide Position	Codon Position	Polymorphism	Amino Acid Change	Gene Mutation Type	Cellular Component Encoded
RIF| *rpoB*	1333	445	CAC/AAC	Histidine/Asparagine	Substitution	*Mycobacterium* DNA gyrase sub-unit B
RIF| *rpoB*	1322	441	AGC/GCG	Serine/Alanine	Substitution	
RIF| *rpoB*	-	454	CCG/GCG	Proline/Serine	Substitution	
INH| *fabG1-inhA*	−15	315 in KatG gene	C/T	Serine/Threonine	Missense/ Promoter	*Mycobacterium* **3-Oxoacyl-thioester reductase**
SM| *rpsL*	128	43	AAG/AGG	Lysine/Arginine	Substitution	*Mycobacterium* ribosomal protein S12
SM| *gid*	351	117	Del 1 bp	Guanine deletion	Frameshift	*Mycobacterium* tRNA **Methyl-transferase**
SM| *gid*	386	-	Del 1 bp	Guanine deletion	Frameshift	
SM| *gid*	115	-	Del 1 bp	Guanine deletion	Frameshift	
ETH| *fabG1-inhA*	−15	315 in KatG gene	C/T	Serine/Threonine	Missense/ Promoter	*Mycobacterium* **3-Oxoacyl-thioester reductase**
AMI| *rrs*	1402	513	C/A	Cytosine/Adenine	Substitution	Mycobacterium 16S rRNA
AMK| *rrs*	1402	513	C/A	Cytosine/Adenine	Substitution	
CAP| *rrs*	1402	513	C/A	Cytosine/Adenine	Substitution	
KAN| *rrs*	1402	513	C/A	Cytosine/Adenine	Substitution	
PZA| *panD*	412	138	GTT/GCT	Valine/Alanine	Substitution	*Mycobacterium* **Aspartate decarboxylase**

4. Discussion

In this study, we report the genetic diversity and drug-resistance patterns of MTB

pulmonary TB isolates using whole genome sequencing. A total of 125 MTB DNA samples were sequenced from which 88 high-quality sequence reads were obtained for analysis. The TB severity was determined following the TBScore II algorithm (modified BANDIM score). Bacteriological analysis (AFB microscopy), showed that majority of participants, 43/125 (34.4%), had an AFB smear grade of 3+. It has been shown that effective sputum smear conversion is hindered by initial high bacillary count (3+) and advancing age of TB patients [26]. Smear microscopy has also been reported to reflect the extent of disease severity, with higher smear grades, associated with extensive lung damage and cavitation. However, reports indicate that close to 2% of patients may be smear negative at treatment completion and this could be due to non-viable bacteria and colonization by non-tuberculous mycobacteria [27]. Early diagnosis is paramount to interrupting TB disease transmission. It is established that AFB smear-positive patients are the primary source of TB transmission to healthy individuals if left untreated. Studies have reported that close to 17% of TB transmission is by AFB smear-negative TB suspects; hence, the risk of disease transmission by AFB-negative cases to healthy individuals cannot be ignored [28].

Africa is home to extensive genetic diversity of humans and pathogens, including mycobacteria, and it is the only continent with all known MTB lineages. Cameroon, a country situated in the West and Central parts of Africa, is endemic for TB and there is still a dearth of information on MTBC population diversity in the country. This can be important for the development of better diagnostic tools and novel vaccine therapies, and to understand the changing structure of the tuberculosis epidemic in Cameroon. Epidemiological studies on MTB in Cameroon have previously been based on phenotypic characterization and low-resolution molecular typing methods. In this study, WGS approach was used to genotype MTBC in newly acquired pulmonary tuberculosis cases, seeking care in TB treatment centers in the Southwest and Littoral regions. The analysis revealed a predominance of MTB lineage 4, accounting for 85/87 (98%) of the isolates, whereas the East African Indian lineage (Lineage 3) and the East Asian lineage (Beijing Ancestral 3) were less prevalent, accounting for 1/87 (1%) each of all isolates. No Indo-Oceanic lineage (lineage 1) strains were identified. This observation is in line with the findings of Negrete-Paz and colleagues, who suggested that lineage 4 and Beijing isolates were significantly associated with pulmonary TB, whereas lineage 1 and the ancient Beijing genotype were significantly associated with extra-pulmonary TB [29]. Thus, we may have missed these lineages in our study, which focused on pulmonary TB. The modern lineages, 2, 3 and 4, are more virulent, genetically more diverse, and have a global geographic distribution [30] compared with the ancient lineages, Lineages 1, 5, 6, 7, 8 and 9, which are less virulent and geographically restricted [31]. Modern lineages are associated with higher inflammatory response, than ancient strains, and this has resulted in a selective advantage of the modern lineages, leading to impaired bacterial control by the host, rapid disease progression, and heightened disease transmission [32]. Studies in

the early 1970s by Huet and colleagues in the West Province of Cameroon found *M. africanum* (L5 and L6) to be the main etiologic agent of TB, with a prevalence rate of 56 %. However, this paradigm has shifted over the last five decades. Studies from early 2000 to the present day show a consistent regression of *M. africanum* and the predominance of *M. tuberculosis* as the etiologic agent of TB in Cameroon, with a drastic drop in the *M. africanum* prevalence from 56% [12] to 6% [16], to almost zero. *M. africanum* genotypes and *M. bovis* were not identified in this study. This finding further confirms the decline of *M. africanum*, and the rarity of *M. bovis* in human TB infection in Cameroon. All the *Mycobacterium* isolates identified in this study were genotypes of *M. tuberculosis*. This is consistent with previous findings, in which *M. tuberculosis* accounted for more than 90% of TB cases [33] [34]. This raises the question of why *M. africanum* is regressing in Cameroon, while it remains high in most Central and West African countries such as Benin [35] [36], Mali [37] and the Gambia [38]. One hypothesis is that the systematic introduction of newborn BCG vaccination in Cameroon may have selected for some genotypes, as with the case of the Beijing family (in East and Central Asia) [39] [40]. Hence, *M. africanum* may have diminished significantly as the cause of TB after the widespread use of BCG vaccination in Cameroon. However, the low sample size may constitute a bias, accounting for the absence of *M. africanum* and *M. bovis* in our study. LAM-10 Cameroon sub-lineage of lineage 4 accounted for 42/83 (42%) of all sub-lineages in our study and is the genotype that is the leading cause of TB in Cameroon. It is hypothesized that the Cameroon family has recently emerged through clonal expansion and is heterogeneously distributed in the population. The LAM_10 Cameroon strain, as suggested by similar studies on the Beijing family, could likely have a selective advantage over other *M. tuberculosis* genotypes in Cameroon, in terms of virulence, pathogenesis, and epidemiologic characteristics. Phylogenetic dating suggests that the Cameroon genotype diverged from a common ancestor approximately 161 years ago to form three main clusters [41]. A limitation of this study is that a transmission cluster analysis was not performed to determine the transmission pattern and cluster rate of the LAM_10 Cameron strain in the study population. The data revealed the L4 sub-lineages Haarlem (20%), the LAM (1%), and the Uganda II (2%) sub-lineages and the Delhi/CAS (1%), a sub-lineage of lineage 3, all of which have been reported previously in Cameroon [16]. Of interest is the Beijing ancestral 3 genotype (L 2.2.1), belonging to lineage 2, not previously reported in Cameroon. The absence of this genotype in previous studies could be explained by the inability of the genotyping methods used to identify lineage 2 (Beijing-type) sub-lineage-specific loci [18] or by the rarity of this genotype in West Africa [42]. We also identified the Coll lineage 4.7, previously described by Malm and colleagues in 2017, as a new Euro-American sub-lineage in Congo Brazzaville and named it the Congo type [43]. The emergence in our study of geographically more restricted isolates like Uganda II, predominant in Uganda, East Africa [44]; the Congo genotype (Coll lineage 4.7) in Congo Brazzaville and the Beijing ancestral 3 genotype

in East and Central Asia [45] [46], suggests cross-border transmission through migration, followed by local spread. This warrants close monitoring and investigation of these strains in the overall transmission dynamics and epidemiology of TB in Cameroon. The introduction of new MTB strains that are more transmissible, more virulent, and more prone to drug-resistance has been associated with migration, especially population movement, to larger cities in search of better health care facilities and employment prospects and can drive the current changing TB situation [47]. In the current context of globalization and population movement, it is a challenge to ascertain and characterize the mycobacterium strains in a given human population and to control the emergence of drug-resistant strains and their dissemination. In addition, this study revealed significant association between TB disease severity and HIV positivity and dyspnea. In high-burden settings, HIV co-infection is the most important risk factor for developing active TB [48]. People living with HIV (PLHIV) are at a much higher risk of developing active TB due to their weakened immune systems. The risk of TB increases as the CD4 cell count decreases, making PLHIV particularly vulnerable. Severe TB primarily affects the lungs, causing inflammation and damage to lung tissues. This can lead to airflow obstruction and reduced lung function, resulting in dyspnea [49]. Studies have shown that the degree of dyspnea is related to the functional capacity of patients with TB sequelae. This means that more severe dyspnea is often associated with greater limitations in physical activity and overall lung function [50]. Upper and higher income earners have lower odds of developing severe TB compared to middle to low-income earners. TB is a disease of poverty; a lack of basic health services, poor nutrition, and inadequate living conditions all contribute to the spread of TB and its impact upon the community. An absence of good-quality health care facilities is common in poor communities. With no health services to diagnose or treat patients, there is a longer delay between disease and cure, perpetuating the spread of TB (StopTB.org). Our study showed that the LAM_10 Cameroon was significantly associated with moderate TB (OR = 7.94, p = 0.03) and this could imply a more severe disease presentation although our data could not establish this association. No direct correlation has been established consistently between the LAM_10 genotype, drug resistance and HIV positivity, as shown in this study. However, the lack of correlation in this study could be attributed to low statistical power, resulting from the small sample size. Although the LAM_10 genotype is associated with Majority of TB cases, TB disease severity classification is typically based on radiographic findings, bacillary load, and clinical symptoms and not just MTB genotype or strain.

We used WGS to infer drug-resistance to first- and second-line anti-TB drugs in 88 MTB clinical isolates from newly acquired TB cases. 79 (89.8%) of the isolates were sensitive, whereas 9 (10.2%) were drug-resistant. This finding is highly suggestive of circulating drug-resistant TB. One isolate of the Haarlem sub-lineage was isoniazid mono-resistant, and three isolates, one Euro-American and two LAM_10 Cameroon, were Rifampicin resistant, and one (mixed isolate) was re-

sistant to Pyrazinamide (PZA). No Multi-Drug-Resistant (MDR) isolates were identified. We found Rifampicin, isoniazid, and streptomycin resistance rates to be 3.4%, 1.1% and 4.5% respectively, which are far less compared to the rates of 27.6%, 54.1% and 25.5% for the same drugs, identified by using Drug Susceptibility test (DST) [51]. The difference could be attributed to sampling bias or inherent variability of the DST compared to WGS approach in this study. The trends in drug resistance in this study are similar to those reported by Noeske and colleagues in 2018 [52] and Thumamo *et al.* 2019 [53], who found Rifampicin resistance rates to be 1.6% and 8.8% respectively in the Littoral Region of Cameroon, using GeneXpert/RIF. These findings suggest a decreasing trend in drug resistance to first-line anti-TB drugs. This could be explained in part by a scale-up in national TB control measures such as improved TB diagnosis, proper adherence to TB treatment regimens owing to the Directly Observed Treatment (DOT) program, effective follow-up of TB patients and contact tracing, and regular surveillance and monitoring of Rifampicin resistance or MDR-TB cases.

Rifampicin resistance is acquired through several different mutations in the *rpoB* gene. We also identified a double Rifampicin resistance mutation, *rpoB*_His445Asn and *rpoB*_Ser441Ala, in a poly-resistant Euro-American sub-lineage 4.1.3 and rpoB_His445Asn and *rpoB*_Pro454Ser in two LAM-10 isolates each. The *rpoB*_His445Asn and rpoB_Ser441Ala are positive mutations primarily occurring within the Rifampicin Resistance-Determining-Regions (RRDRs) and have been reported in several studies [54]. The *rpoB* gene in *MTBC* encodes the beta subunit of RNA polymerase, which is essential for RNA transcription [55]. It is known that more than 95% of Rifampicin-resistant strains harbor a mutation within an 81-bp region of the *rpoB* gene between codons 507 and 533 and this region is called the Rifampicin Resistance-Determining-Region (RRDR) [56]. The highest level of Rifampicin resistance of the *rpoB* gene occurs in codons 531 and 526. The mutations identified in this study are very low-level mutations that occur outside the RRDR [57]. The poly-resistant isolate, in addition to having the *ropB* gene mutation, also harbored the canonical mutation *rrs*1402C>A in the "hot" *rrs* gene, reported to be associated with cross-resistance to the second-line aminoglycoside anti-TB drugs amikacin, capreomycin and kanamycin [58]. However, it is clear from previous studies that mutations in *rrs* gene are not always associated with amikacin resistance; traditional drug susceptibility testing will still be helpful to evaluate such samples [59]. The *rrs* gene encoded the Mycobacterium 16S rRNA, which plays a crucial role in protein synthesis. The isoniazid-resistant isolates we identified in this study acquired the *fabG1 inhA* promoter-region mutation, known to confer resistance to isoniazid and ethionamide. Isoniazid resistance is linked to several gene mutations, such as *katG* (catalase-peroxidase gene), *kasA* (*β*-ketoacyl-ACP-synthase gene), *ndh* (NADH-dehydrogenase), *inhA* region (NADH-dependent ACP-reductase *InhA* gene) and *ahpC* region (alkyl-hydroxyperoxide-reductase gene). *katG* and *inhA* gene mutations are most clinically relevant and determine resistance in most of the clinical isolates [60]. The *fabG1 inhA* gene

codes for the *Mycobacterium* 3-oxoacyl-thioester reductase, an enzyme which has a crucial role in mycobacterium mycolic fatty acid biosynthesis [61] essential in bacterial cell wall synthesis, and isoniazid is known to interfere with this process by targeting mycolic acid [62].

Of the 4/88 (4.5%) isolates that were resistant to streptomycin, we found the mutation *rpsL*_Lys43Arg in *rpsL* gene of a Beijing strain (2.2.1). We also identified the *gid*_351delG in the SM/*gid* gene of a Euro-American (4.1), *gid*_115delC and *gid*_386delG in the SM/*gid* genes of 2 Harlem genotypes. Mutations in the *rpsL* gene (especially *rpsL*_Lys43Arg) account for approximately 80% of streptomycin resistance [63] [64]. However, these reports are at variance with our findings since we identified only 1/88 (1.1%) of the isolates harbored the *rpsL* gene mutation. Rather, we identified a mutation in the *SM*/*gid* gene in three isolates. Negrete-Paz and colleagues reported the *gid*_351delG mutation associated with resistance to streptomycin in extra-pulmonary TB patients. The *gid* gene in mycobacteria encodes a tRNA methyltransferase, which is responsible for adding a methyl group to the 2'-O position of the guanosine at the 18th nucleotide of tRNAs. This modification is essential for proper tRNA function and stability.

One of the isolates identified as a mixed, harbored the rare mutation *panD*Val138Ala in the *panD* gene conferring resistance to pyrazinamide. Zhang and colleagues reported the panDVal138Ala mutation in *M. canetti and M. tuberculosis* as a potential new mutation conferring resistance to PZA, besides the well-known *pncA* and *rpsA* mutations [65]. This finding is similar to that of Özgür *et al.*, *in* 2022 [66], who found one Mtb strain out of 11 with the *panD* mutation. The *panD* gene in Mycobacterium, encodes aspartate decarboxylase, a 139 amino acid (15 kD) enzyme involved in several essential processes, such as the formation of β-alanine from *l-aspartate*, which is a precursor for pantothenate (vitamin B5) and coenzyme A (CoA) biosynthesis. CoA is crucial for energy metabolism, where carbohydrates, fats, and proteins are utilized. The exact activity of Pyrazinamide is not known; however Zhang and colleagues speculated that Pyrazinamide might inhibit pantothenate and CoA synthesis, thereby interfering with diverse metabolic functions such as energy production and fatty acid metabolism in *M. tuberculosis*. *PanD* mutation in *M. tuberculosis* has been shown to cause higher attenuation of virulence in mice than the BCG vaccine, indicating that the *panD* may be critical for survival and persistence of the bacilli in vivo [67]. The absence of the *panD* gene in humans and its significant role in the cellular metabolism of Mtb make it a potential drug target candidate. The mutations Ser441Ala, His445Asp and Pro454Ser in the *rpoB* gene, the 386 delG, 115 delG and 315 delG in the *gid* gene, the *panD*Val138Ala mutation in the *panD* gene and the 1402 C>A mutation in *rrs* gene, were not previously reported in Cameroon.

5. Conclusion

We recruited pulmonary tuberculosis participants and used WGS approach to map the MTBC population structure in the Southwest and Littoral Regions of

Cameroon. Findings revealed a diverse population of MTBC, predominated by the Euro-American lineage (L4). The absence of *M. africanum* (L5/L6) isolates is additional evidence of its continued previously reported regression in favor of the L4 LAM_10 Cameroon genotype as the etiologic agent of pulmonary TB in Cameroon. Drug-resistance profiling revealed new mutations: Ser441Ala, His445Asp and Pro454Ser in the *rpoB* gene, the 386 delG, 115 delG and 315 delG in the *gid* gene, the *panD*Val138Ala in the *panD* gene and the 1402 C>A mutation in *rrs* gene. Although RIF mono-resistance as a proxy marker for MDR indicated a low rate of resistance in newly acquired pulmonary TB, there is a need for continuous surveillance of circulating resistant variants in the population. Further investigation would provide additional insight into the genetics, epidemiology, and transmission dynamics of the L4 LAM_10 Cameroon genotype, which may furnish clues to the evolutionary success of modern MTBC lineages.

Consent for Publication

Not applicable.

Availability of Data and Materials

All datasets used to generate the present report are available upon reasonable request from the corresponding author and will be deposited into public databases.

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Authors' Contributions

Below are the respective contributions of each author in no order: D. N. A., N. T. M., C. M., D. G. K. T.: Co-investigator, led data collection in the field, the laboratory and analysis. D. N. A.: Drafting of the manuscript for publication. F. F. F., A. J. N., M. R., M. J. N., A. A., A. H., E. I. G., S. W.: Co-investigators, participated in the revision of the manuscript. F. N. N.: Generation of the map of study sites. T. M. N., J. F. C.: Co-investigator, supervision of field and laboratory research activities and revision of manuscript for publication. A. G. W., T. D. K., D. H., Y. K.

H., B. A.: Co-investigator, supervision of laboratory research activities, and coordination of bioinformatics data analysis. K. B., S. W.: Principal Investigator, in charge of conceptualization and the design of the study, leading and directing research activities and revision of the manuscript for publication. This manuscript was read and approved by all authors involved in this study prior to publication.

Ethics Approval and Consent to Participate

Ethical clearance for this study, was obtained from the Cameroon National Ethics Committee, Ref No: 2019/03/1154/CE/CNERSH/SP. The administrative authorization was obtained from the Ministry of Public Health, Ref No: D30-368/AAR/MINSANTE/SG/DROS/NJ. The research authorization was obtained from the Regional Delegation of Public Health for the Littoral, Ref No: 018/AR/MINSANTE/DRSPL/BCASS/CNT. The protocol was reviewed and approved by the Institutional Review Board of the Faculty of Health Sciences, University of Buea. The written and verbal authorizations were obtained from the various TB diagnostic and treatment centres. Informed consent was obtained from participants ≥ 21 years. Assent was obtained for participants between 18 and 20 years and informed consent was obtained from their parent or legal guardian. Confidentiality of each participant's information was ensured by using individual codes.

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Conflicts of Interest

The authors declare no competing interests.

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