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Molecular epidemiology and drug susceptibility profiles of *Mycobacterium tuberculosis* complex isolates from Northern Ghana

Samuel Kobina Ekuban Acquah[a,b,c,*], Prince Asare[a,**], Stephen Osei-Wusu[a], Portia Morgan[a], Theophilus Afum[a], Diana Asema Asandem[a], Emelia Konadu Danso[a], Isaac Darko Otchere[a], Linda Aurelia Ofori[c], Kwasi Obiri-Danso[c], Richard Kock[d], Adwoa Asante-Poku[a], Dorothy Yeboah-Manu[a,*]

[a] Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana
[b] Department of Clinical Microbiology, School of Medicine and Health Sciences, University for Development Studies, Tamale, Ghana
[c] Department of Theoretical and Applied Biology, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[d] Department of Pathobiology and Population Sciences, Royal Veterinary College, London

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ABSTRACT

Objective: We conducted a cross-sectional study in the five administrative regions of Northern Ghana to determine the diversity of *Mycobacterium tuberculosis* complex (MTBC) sub/lineages and their susceptibility to isoniazid (INH) and rifampicin (RIF).

Methods: Sputum specimens were collected and cultured from 566 pulmonary tuberculosis patients reporting to 17 health facilities from 2015 to 2019. Mycobacterial isolates obtained from solid cultures were confirmed as members of the MTBC by PCR amplification of IS*6110* and rpoß and assigned lineages and sub-lineages using spoligotyping.

Results: Of 294 mycobacterial isolates recovered, MTBC species identified were: *M. tuberculosis sensu stricto* (Mtbss) 241 (82.0%), *M. africanum* 41 (13.9%) and *M. bovis* four (1.4%) with eight (2.7%) unidentified. The human-adapted lineages (L) identified (N=279) were L1 (8/279, 2.9%), L2 (15/279, 5.4%), L3 (7/279, 2.5%), L4 (208/279, 74.5%), L5 (13/279, 4.7%) and L6 (28/279, 10.0%) with three unidentified lineages. Among the 208 L4, the dominant sub-lineages in the region were the Cameroon 120/208 (57.7%) and Ghana 50/208 (24.0%). We found 4.4% (13/294) and 0.7% (2/294) of the patients infected with MTBC isolates resistant to INH only and RIF only, respectively, with 2.4% (7/294) being infected with MDR strains. Whereas L6 was associated with the elderly, we identified that the Ghana sub-lineage of L4 was associated with both INH and MDR ($p<0.05$), making them important TB pathogens in Northern Ghana and a growing public health concern.

Introduction

Tuberculosis (TB) remains a leading cause of infectious diseases; the World Health Organisation estimates 10 million individuals comprising 5.6 million men, 3.2 million women, and 1.2 million children less than 15 years fell ill with TB in 2019 (WHO, 2020). About 1.2 million individuals died from TB in 2019, making it the leading cause of adult mortality from a single infectious agent globally (WHO, 2020). Several factors, including the emergence of drug-resistant (DR), strains of the causative pathogen, the HIV/AIDS syndemic, and urbanization, have been implicated in the persistence of TB in developing countries (Corbett et al., 2003, Ekaza et al., 2013, Kyu et al., 2018, Prasad et al., 2016). However, there is limited understanding of the contribution of pathogen genetic diversity to disease epidemiology and clinical phenotype (Gagneux, 2013,

* Corresponding author. Dorothy Yeboah-Manu. Bacteriology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, LG 581, Legon, Accra-Ghana.
** Co-corresponding author. Prince Asare. Bacteriology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, LG 581, Legon, Accra-Ghana.
E-mail addresses: pasare@noguchi.ug.edu.gh (P. Asare), DYeboah-Manu@noguchi.ug.edu.gh (D. Yeboah-Manu).

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Nicol and Wilkinson, 2008, Otchere et al., 2018, Yeboah-Manu et al., 2016).

Tuberculosis in mammals is caused by a group of homogenetic acid-fast bacteria referred to as *Mycobacterium tuberculosis* complex (MTBC). In humans, TB is mainly caused by *M. tuberculosis sensu stricto* (Mtbss), and *M. africanum* (Maf) herein referred to as human-adapted MTBC (hMTBC) (Gagneux et al., 2006, Kamerbeek et al., 1997). Nevertheless, animal species such as *M. bovis* and *M. orygis* occasionally cause TB in humans, resulting in zoonotic TB (Jenkins, 2011, Otchere et al., 2019, Rossi et al., 2015), which is seen chiefly in major livestock producing communities (Matteelli et al., 2017, Müller et al., 2009).

The hMTBC cluster in lineages (L) and sub-lineages are characterized by extensive sequence polymorphisms, single nucleotide polymorphisms, and insertion sequence polymorphisms. These lineages and sub-lineages are generally associated with specific geographical regions (Borrell et al., 2019, Brudey et al., 2006, Couvin et al., 2019, Malik and Godfrey-Faussett, 2005). Mtbss consists of six lineages (L1-L4 and L7-L8); L1 is mainly found in the Indo-Oceanic regions, L2 in Eastern Asia, L3 in Central Asia, whereas L4 is globally distributed. L7 has only been isolated from populations at the horn of Africa, and L8 has recently been identified in the Great Lake region of Africa (Brynildsrud et al., 2018, Hirsh et al., 2004, Merker et al., 2015, Tsolaki et al., 2005). On the other hand, Maf, which consists of lineages (L5 and L6), is generally restricted to West Africa (Asante-Poku et al., 2016, De Jong et al., 2010, Gagneux et al., 2006). However, due to human demographic dynamics and global migration for trade and other purposes, the geographical associations with some lineages may be disrupted. For example, the Beijing family of L2, which is endemic in Eastern Asia, has also been found in Europe, America, and Africa (Reed et al., 2009).

The impact of genetic variability within the MTBC and the geographical distribution of MTBC lineages is not entirely understood. Nevertheless, mounting evidence suggests that it may translate into clinically relevant phenotypic characteristics, including transmissibility, virulence, drug susceptibility, and treatment outcomes (Hershberg et al., 2008, Shanley et al., 2018). For example, Maf lineages showed a reduced rate of transmission (Asare et al., 2018), reduced rate of progression to active disease (de Jong et al., 2006), and reduced rate of clustering (Gehre et al., 2016) compared to their counterpart lineages of the Mtbss. Also, L1 is reported to be less virulent relative to other MTBC strains, and their transmissibility is further reduced when they acquire low- or high-cost drug resistance-conferring mutations, which decreases their overall fitness (Fenner et al., 2012).

Previous studies involving two of the five regions in Northern Ghana indicated that 79.1% of TB cases in the country were caused by Mtbss and 20% by Maf . One of the studies further observed that zoonotic tuberculosis was significantly higher in the Northern regions relative to regions in the South (Otchere et al., 2019, Yeboah-Manu et al., 2016).

To further define and understand the population structure of MTBC and their susceptibility to the two most important anti-TB drugs (i.e., isoniazid and rifampicin) in the northern regions, we conducted a hospital-based cross-sectional molecular epidemiology study among pulmonary TB patients reporting to health facilities in all the five (5) administrative regions.

Methods

Study area

The study was conducted from 2015 to 2019. Pulmonary tuberculosis patients attending health facilities in the five administrative regions of Northern Ghana, which comprise Upper East, Upper West, North East, Savanna, and Northern regions (Figure 1), were included in this study. The regions share boundaries with Cote D'Ivoire in the West, Togo in the East, and Burkina Faso in the North. Northern Ghana is predominantly an agrarian community, and the inhabitants are largely cereal and livestock farmers producing more than 80% of Ghana's cereal and 70% of its livestock (Asante et al., 2018, Karbo and Agyare, 2002).

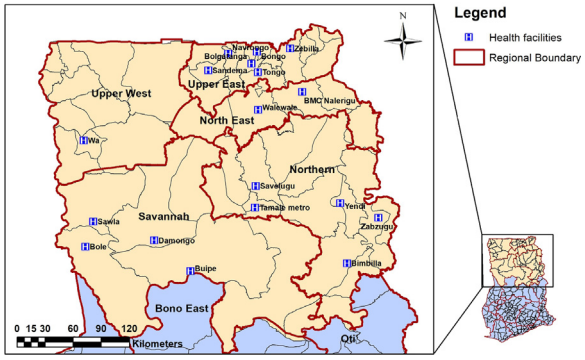

Figure 1. Map of study area and location of health facilities sampled.

Specimen and data collection

Clinical and demographic data, including mycobacterial load at diagnosis, age, sex, region, and district of origin, were retrieved from patients' records from their health facilities. Sputum samples were obtained following the National Tuberculosis Control Program (NTP) guidelines for routine diagnosis and treatment monitoring of TB in Ghana. Smear/GeneXpert positive sputum specimen was consecutively collected into labeled 100 mL wide-mouth containers. All collected samples were tightly capped, sealed with parafilm, packaged into a cold box, and transported to the bacteriology department of the Noguchi Memorial Institute for Medical Research (NMIMR) for further analyses.

Mycobacteria isolation

Approximately 5 ml of each sputum specimen was decontaminated using the 5% oxalic acid method (Yeboah-Manu et al., 2004) and inoculated on two pairs of labeled Lowenstein-Jensen (L-J) media slants supplemented with glycerol to enhance the growth of Mtbss and sodium pyruvate to enhance the growth of Maf and *M. bovis* (Asante-Poku et al., 2015, Keating et al., 2005). The inoculated media slants were incubated at 37 °C for a maximum period of 12 weeks for the appearance of confluent macroscopic growth of mycobacteria (Keating et al., 2005). Colonies suggestive of mycobacteria were stained for acid fastness by the Ziehl-Nielsen method (De Jong et al., 2010, Gehre et al., 2016).

Species identification and strain differentiation

A loopful of all acid-fast isolates was inactivated at 95°C for 1 hour in sterile distilled water, and DNA was extracted as previously described for molecular analysis (Asante-Poku et al., 2016). Confirmation of isolates as members of MTBC was done by PCR amplification of MTBC-specific Insertion Sequence 6110 (IS*6110*) and rpo*B* where necessary (Coros et al., 2008, Kabir et al., 2018, Yeboah-Manu et al., 2001). All confirmed MTBC isolates were further characterized by spoligotyping using appropriate primers; DRa (5'-CCG AGA GGG GAC GGA AAC-3') and biotinylated DRb (5'-GGT TTT GGG TCT GAC GAC-3') and protocols as previously described (Kamerbeek et al., 1997). Lineages, sub-lineages, and their shared

international type (SIT) numbers were assigned using the SITVIT-2 web database (http://www.pasteur-guadeloupe.fr:8081/SITVIT2) (Couvin et al., 2019) complemented with the MIRU-VNTR*plus* web database (http: //www. miru-vntrplus.org) (Brudey et al., 2006, Supply et al., 2006). All genotyping assays were controlled by including H37Rv and *M. bovis* BCG DNA as positive controls and nuclease-free water as a negative control.

Drug susceptibility testing

The drug susceptibility pattern of the isolates to the first-line anti-TB drugs INH and RIF was determined using the GenoType MTBDR*plus*. Similarly, resistance against the second-line anti-TB drugs, fluoroquinolones, and injectable aminoglycosides, was determined among the Multidrug-resistant (MDR) strains using GenoType MTBDR*sl* according to the manufacturer's protocol (Bruker-Hain Diagnostics Germany). Only reaction regions with band intensities equal to or stronger than that of the amplification control bands were considered positive. The absence of a wild-type band or the presence of a mutant band was indicative of a resistant strain (Bruker-Hain Diagnostics, Germany). We used the conventional definitions of MDR (resistance to INH and RIF), pre-XDR (MDR with additional resistance to either a fluoroquinolone or an aminoglycoside), and XDR (MDR with additional resistance to at least a fluoroquinolone and an aminoglycoside) strains for our analysis (Kaswa et al., 2014).

Data analysis

Each participant's clinical and demographic data were double entered into the Microsoft Access database and validated to correct for errors. Duplicate sputum samples from the same individual obtained from treatment follow-ups were not included in the analysis. Descriptive statistics were carried out for both the categorical and numerical variables. Cross-tabulations were further employed to explore the relationship between the different outcomes and selected variables using Chi-square and student t-test where applicable. We compared the estimated proportions from our current study in Northern Ghana to the reported proportions in Southern Ghana from a previous study (Yeboah-Manu et al., 2016) by performing a univariate logistic regression analysis. Where appropriate, the Fisher's exact or the chi-square tests were used to assess statistical significance. A *p-value* of less than 0.05 at a 95% confidence level was considered significant. The ArcMap tool employed in ArcGIS (Economic and Social Research Institute, version 10.1) (ESRI, 2010) was used for constructing maps.

Results

Clinical and demographic characteristics of study participants

A total of 600 sputum specimens, of which 34 were follow-up samples, were collected from 566 pulmonary TB patients reporting to 17 districts, regional and tertiary health facilities in the five administrative regions of Northern Ghana spanning 2015 to 2019. The majority of the samples (211; 37.8%) were obtained from the Tamale metropolitan area in the Northern region (Table 1). As expected, of the cases with data on gender, 71.7% (367/512) were males, and 28.3% (145/512) were females (Table 1). The ages of the patients ranged between 4 - 95 years (mean = 43.8 ± 17.2 years; median=40 years). However, the majority (190; 39.7%) were between 26 - 40 years. Female patients were significantly younger than the male patients (median age 35 vs. 42 years, $p<0.001$). Most patients (85.3%) reported a smear grade of at least 1+, an indication of late reporting.

Table 1
Clinical and demographic characteristics of study participants

Variable (Total number analyzed)	Number (%)
Year diagnosed (566)	
2015	16 (2.8)
2016	70 (12.4)
2017	86 (15.2)
2018	241 (42.6)
2019	153 (27.0)
Gender (512)	
Male	367 (71.7)
Female	145 (28.3)
Age Category (478)	
<26	61 (12.8)
26-40	190 (39.7)
41-60	140 (29.3)
>60	87 (18.2)
Region (515)	
North East	108 (21.0)
Northern	236 (45.8)
Savannah	8 (1.5)
Upper East	126 (24.5)
Upper West	37 (7.2)
Smear grade (462)	
3+	173 (37.5)
2+	98 (21.2)
1+	123 (26.6)
Scanty	68 (14.7)
District of diagnosis (558)	
Bimbilla	9 (1.6)
Bole	5 (0.9)
Bolgatanga	99 (17.7)
Bongo	8 (1.4)
Jirapa	4 (0.7)
Nalerigu	104 (18.6)
Navrongo	15 (2.7)
Sandema	9 (1.6)
Savelugu	9 (1.6)
Sawla	3 (0.5)
Tamale_Metro	211 (37.8)
Tongo	4 (0.7)
Wa	39 (7.0)
Walewale	7 (1.3)
Yendi	5 (0.9)
Zabzugu	22 (3.9)
Zebilla	5 (0.9)

There were 54, 88, 51, 104, 8 missing data, respectively for gender, age, region, smear grade, and district.

Population structure and spatial distribution of MTBC in Northern Ghana

We confirmed 294 isolates from the 566 sputum samples cultured as members of the MTBC. Three MTBC species were identified; the most dominant was Mtbss (241, 82.0%) followed by Maf (41, 13.9%) and *M. bovis* (4, 1.4%) with (8, 2.7%) unidentified isolates. Spacer oligonucleotide typing (spoligotyping) of the 294 MTBC isolates produced a total of 89 different patterns; 58 patterns with SIT's available in the SITVIT-2 database and 31 orphan/new strains that did not match any pattern in both SITVIT-2 and miru-vntr*plus* database (Table 2, FigureS1). The predominant shared type identified was the Cameroon sub-lineage SIT 61 (69, 23.5%) followed by the Ghana sub-lineage SIT 53 (35, 11.9%), the Cameroon sub-lineage SIT 772 (19, 6.5%), and the Beijing sub-lineage SIT 1 (15, 5.1%) (Table 2). The orphan patterns are available in the supplementary figure (Figure S1).

We observed varying distributions of MTBC across the five regions (Figure 2). For instance, the proportion of Mtbss ranged from 73.9% in the Northern region to as high as 100.0% in the Savannah region, whereas the proportion of Maf ranged from as low

Table 2
Distribution of identified spoligotypes

Species	Lineage	Spoligo family (sub-lineage)	Shared International Type (SIT)*	Octacode	Frequency, N (%)
Mbovis	Bovine lineage	Bovis	1037	676773777677600	1 (0.3%)
Mbovis	Bovine lineage	Bovis	2813	676773776277600	1 (0.3%)
Mbovis	Bovine lineage	Bovis	3760	466773777677600	1 (0.3%)
Mbovis	Bovine lineage	Bovis	Orphan or New_8	774077777777000	1 (0.3%)
Mtbss	L1	EAI	340	474377777413771	1 (0.3%)
Mtbss	L1	EAI	342	677777777413771	1 (0.3%)
Mtbss	L1	EAI	Orphan or New_28	777757777023771	1 (0.3%)
Mtbss	L1	EAI	Orphan or New_3	677777377413771	3 (1.0%)
Mtbss	L1	Manu2	54	777777777763771	1 (0.3%)
Mtbss	L1	Manu2	2698	777777743763771	1 (0.3%)
Mtbss	L2	Beijing	1	000000000003771	15 (5.1%)
Mtbss	L3	Delhi/CAS	2398	703777744037771	1 (0.3%)
Mtbss	L3	Delhi/CAS	203	703777740001771	2 (0.7%)
Mtbss	L3	Delhi/CAS	1199	703777740001171	2 (0.7%)
Mtbss	L3	Delhi/CAS	Orphan or New_6	703637740001771	2 (0.7%)
Mtbss	L4	Cameroon	403	777777743760731	1 (0.3%)
Mtbss	L4	Cameroon	852	400003743760771	1 (0.3%)
Mtbss	L4	Cameroon	1141	777777741760771	1 (0.3%)
Mtbss	L4	Cameroon	2429	777777743560771	1 (0.3%)
Mtbss	L4	Cameroon	2550	777737743760771	1 (0.3%)
Mtbss	L4	Cameroon	2834	377777743760771	1 (0.3%)
Mtbss	L4	Cameroon	2855	777777743760571	1 (0.3%)
Mtbss	L4	Cameroon	3193	777777703760771	1 (0.3%)
Mtbss	L4	Cameroon	3400	777770343760771	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_11	777777743760760	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_20	777773343740771	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_21	777777743660771	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_22	777777743360771	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_23	777771343760771	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_24	777773343760771	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_25	777357743760771	1 (0.3%)
Mtbss	L4	Cameroon	Orphan or New_26	777377747760771	1 (0.3%)
Mtbss	L4	Cameroon	772	777777743460771	19 (6.5%)
Mtbss	L4	Cameroon	Orphan or New_4	777777743740771	2 (0.7%)
Mtbss	L4	Cameroon	Orphan or New_5	777707743760771	2 (0.7%)
Mtbss	L4	Cameroon	Orphan or New_1	777777740360771	4 (1.4%)
Mtbss	L4	Cameroon	61	777777743760771	69 (23.5%)
Mtbss	L4	Cameroon	57	777777143760771	7 (2.4%)
Mtbss	L4	Ghana	205	737777777760771	1 (0.3%)
Mtbss	L4	Ghana	373	777777767760771	1 (0.3%)
Mtbss	L4	Ghana	Orphan or New_27	777717757760771	1 (0.3%)
Mtbss	L4	Ghana	504	777737737760771	10 (3.4%)
Mtbss	L4	Ghana	1475	777357777760771	2 (0.7%)
Mtbss	L4	Ghana	53	777777777760771	35 (11.9%)
Mtbss	L4	Haarlem	740	777777747720771	1 (0.3%)
Mtbss	L4	Haarlem	Orphan or New_15	077777770020731	1 (0.3%)
Mtbss	L4	Haarlem	3189	777777765720771	2 (0.7%)
Mtbss	L4	Haarlem	655	477777777720771	5 (1.7%)
Mtbss	L4	Haarlem	1498	777777776000371	5 (1.7%)
Mtbss	L4	Haarlem	50	777777777720771	6 (2.0%)
Mtbss	L4	LAM	765	777760077760771	1 (0.3%)
Mtbss	L4	LAM	2428	674017607760771	1 (0.3%)
Mtbss	L4	LAM	42	777777607760771	2 (0.7%)
Mtbss	L4	Ugandal	52	777777777760731	1 (0.3%)
Mtbss	L4	Ugandal	78	777777777760711	1 (0.3%)
Mtbss	L4	Ugandal	836	777777777700131	1 (0.3%)
Mtbss	L4	Ugandal	848	737777777760731	7 (2.4%)
Mtbss	L4	X	119	777776777760771	2 (0.7%)
Mtbss	L4	X3	70	700076777760671	1 (0.3%)
Mtbss	L4	X3	200	700076777760700	1 (0.3%)
Maf	L5	West African 1	319	574077607777071	1 (0.3%)
Maf	L5	West African 1	320	770003606377071	1 (0.3%)
Maf	L5	West African 1	2174	774037607777071	1 (0.3%)
Maf	L5	West African 1	Orphan or New_12	774077607774001	1 (0.3%)
Maf	L5	West African 1	Orphan or New_17	770033607777071	1 (0.3%)
Maf	L5	West African 1	330	774077607777031	2 (0.7%)
Maf	L5	West African 1	331	774077607777071	2 (0.7%)
Maf	L5	West African 1	438	774077777777071	2 (0.7%)
Maf	L5	West African 1	Orphan or New_7	374077607777031	2 (0.7%)
Maf	L6	West African 2	318	570777777777671	1 (0.3%)
Maf	L6	West African 2	1867	770777707777651	1 (0.3%)
Maf	L6	West African 2	3476	770777707777631	1 (0.3%)

(*continued on next page*)

Table 2 (*continued*)

Species	Lineage	Spoligo family (sub-lineage)	Shared International Type (SIT)*	Octacode	Frequency, N (%)
Maf	L6	West African 2	Orphan or New_18	770377777477671	1 (0.3%)
Maf	L6	West African 2	Orphan or New_19	170777777777671	1 (0.3%)
Maf	L6	West African 2	Orphan or New_30	773777747777771	1 (0.3%)
Maf	L6	West African 2	Orphan or New_31	777377777777771	1 (0.3%)
Maf	L6	West African 2	326	770777707777671	13 (4.4%)
Maf	L6	West African 2	2833	777777747777771	2 (0.7%)
Maf	L6	West African 2	181	770777777777671	3 (1.0%)
Maf	L6	West African 2	Orphan or New_2	770377707777671	3 (1.0%)
Mtbss	Unidentified	Manu ancestor	523	777777777777771	3 (1.0%)
Unidentified	Unidentified	Unidentified	1178	777771000000731	1 (0.3%)
Unidentified	Unidentified	Unidentified	3808	777737730000000	1 (0.3%)
Unidentified	Unidentified	Unidentified	Orphan or New_10	700036777760700	1 (0.3%)
Unidentified	Unidentified	Unidentified	Orphan or New_13	777737740000031	1 (0.3%)
Unidentified	Unidentified	Unidentified	Orphan or New_14	177750000000731	1 (0.3%)
Unidentified	Unidentified	Unidentified	Orphan or New_16	474001777037071	1 (0.3%)
Unidentified	Unidentified	Unidentified	Orphan or New_29	676773777677771	1 (0.3%)
Unidentified	Unidentified	Unidentified	Orphan or New_9	700076077760700	1 (0.3%)

M bovis: *Mycobacterium bovis*, Mtbss: *Mycobacterium tuberculosis* sensu stricto, Maf: *Mycobacterium africanum.*
L1: Lineage1, L2: Lineage 2, L3: Lineage 3, L4: Lineage 4, L5: Lineage 5, L6: Lineage 6
* Strains with no identified SIT are defined as orphan or New strains.

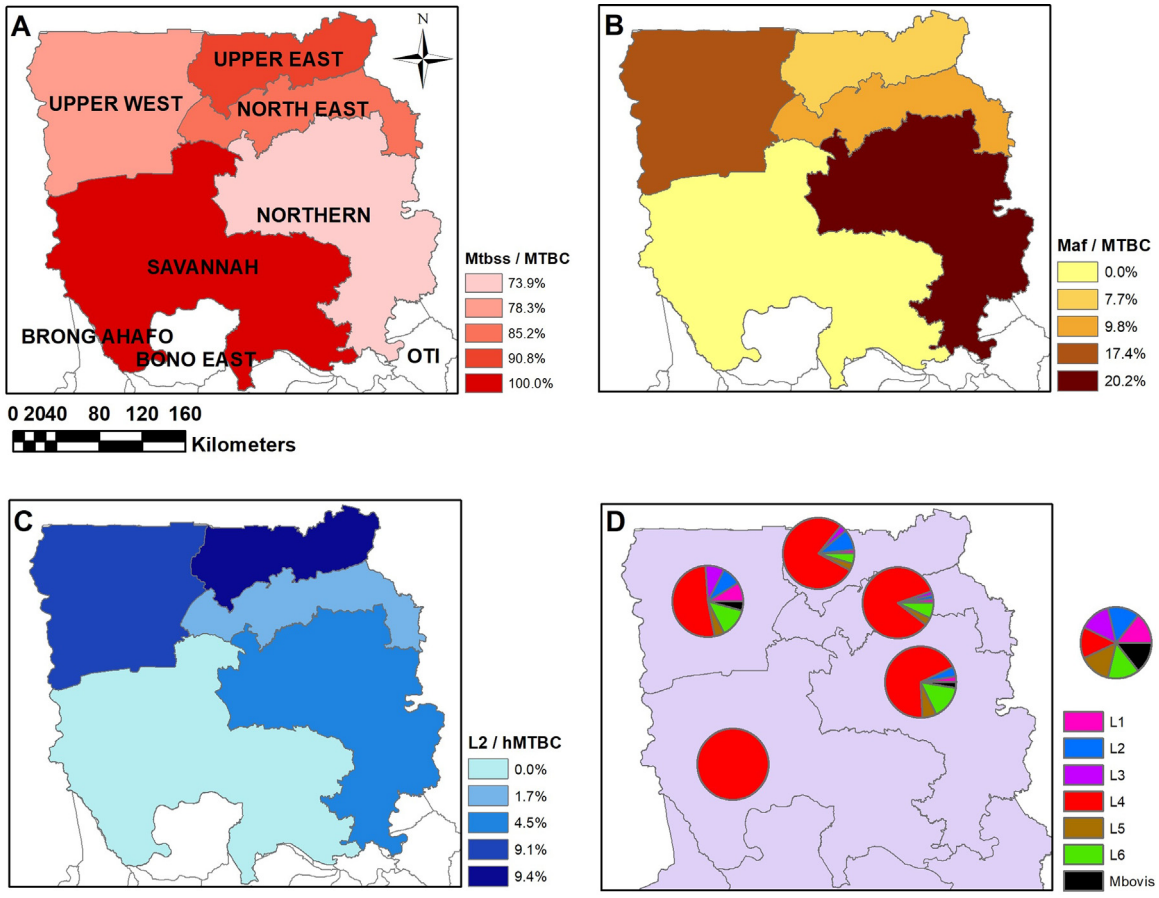

Figure 2. Spatial distribution and regional prevalence of MTBC and human-adapted lineages in Northern Ghana. *A-Mtbss strains, B-Maf strains, C-L2 strains of the hMTBC and D-hMTBC lineages, as well as M. bovis. The Mtbss strains were identified in all five regions and were proportionally dominant in the Savannah region. Maf strains were identified in four of the five regions but most prominent in the Northern region. L 2 was most dominant in the Upper East region but absent in the Savannah region. L4 was isolated in all five regions. L5 and L6 were present in all regions except the Savannah region, whereas M. bovis was identified in two regions (Upper West and Northern).*

as 0.0% in the Savannah region to 20.2% in the Northern region (Figure 2A and 2B). Among the identified human-adapted lineages, L2 was predominant in the Upper East region (6, 9.4%), followed by the Upper West (2, 9.1%) (Figure 2C). The *M. bovis* species were detected only in the Northern (3/4) and Upper West (1/4) regions (Figure 2D).

Significant variation of MTBC prevalence in Northern Ghana compared to Southern Ghana

We observed a significant difference in the proportions of Mtbss, Maf, and *M. bovis* between the two sectors. Whereas the proportion of Maf was significantly higher (p=0.0257) in South-

Table 3
Prevalence of MTBC in Northern Ghana in comparison with Southern Ghana

Variable	Northern Ghana, N (%)	Southern Ghana, N (%)	p-value
MTBC species	N=294	N=2118	
M. tuberculosis sensu stricto	241 (82.0)	1698 (80.2)	0.4657
M. africanum	41 (13.9)	410 (19.4)	0.0257
M. bovis	4 (1.4)	10 (0.5)	0.0602
Unidentified	8 (2.7)	-	-
Human adapted MTBC lineage	N=279	N=2108	
Lineage 1	8 (2.9)	34 (1.6)	0.1342
Lineage 2	15 (5.4)	51 (2.4)	0.0046
Lineage 3	7 (2.5)	25 (1.2)	0.0710
Lineage 4	208 (74.5)	1588 (75.3)	0.7766
Lineage 5	13 (4.7)	274 (13.0)	0.0001
Lineage 6	28 (10.0)	136 (6.5)	0.0261
Lineage_4 sub-lineage	N=208	N=1567	
Cameroon	120 (57.7)	969 (61.8)	0.2486
Ghana	50 (24.0)	326 (20.8)	0.2834
Haarlem	20 (9.6)	144 (9.2)	0.2300
LAM	4 (1.9)	43 (2.7)	0.4883
Uganda	10 (4.8)	39 (2.5)	0.0551
X	4 (1.9)	46 (2.9)	0.4070

ern Ghana (19.4% vs. 13.9%), the proportion of the animal adapted strain, *M. bovis*, was relatively higher (p=0.0602) in Northern Ghana (1.4% vs. 0.5%) (Table 3).

We identified six of the eight hMTBC lineages in the study area, with L4 being the most dominant (208, 74.5%). However, there was no significant difference (p=0.7766) in the proportion of L4 between the Northern (74.5%) and Southern (75.3%) regions. The proportion of L2 was significantly higher in the North (15, 5.4%) compared to the South (51, 2.4%) (p=0.0046). Among the Maf species, L6 was significantly more common in the Northern regions (10.0% vs. 6.5%, p=0.0261) (Table 3). However, the proportion of L5 was significantly higher in the South than in the North (p=0.0001) (Table 3).

The Cameroon and Ghana sub-lineages were the predominant L4 sub-lineages constituting 82% of all L4 in both population groups (Northern vs. Southern Ghana). Except for the Uganda sub-lineage of L4, where we observed close to borderline significance (p=0.0551), the relative proportions of all other L4 sub-lineages were not significantly different in both groups (Table 3).

The Ghana sub-lineage of L4 is associated with drug resistance

Twenty-two (22/294; 7.5%) of our isolates were resistant to either INH, RIF, or both INH and RIF. Thirteen (13/294, 4.4%) were resistant to INH only, two (2/294, 0.7%) were resistant to RIF only, and seven (7/294, 2.4%) were multidrug-resistant (MDR). We further observed that 7.9% (19/241) and 2.4% (1/41) of Mtbss and Maf were respectively resistant to INH (Table 4). Also, RIF resistance occurred in 3.3% (8/241) and 2.4% (1/41) of Mtbss and Maf isolates, respectively. Lineage 4 alone accounted for 81.8% (18/22) of the total DR-TB cases. Of the seven MDR isolates, five were identified as belonging to the Ghana sub-lineage (5/50, 10.0%), significantly higher compared to the single Cameroon MDR strain (1/120, 0.8%) (p=0.009). The Ghana sub-lineage was also significantly associated with INH drug resistance (p<0.001) (Table 4). None of the MDRs were resistant to any second-line anti-TB drugs using the MTBDR*sl* assay.

Logistic regression analysis identifies age as a risk factor for lineage 6 infection

We found no significant difference in the proportion of the three main lineages (L4, L5, and L6) when stratified by year of diagnosis, gender, or smear grade (Table 5). However, compared to Mtbss, it appears that L6 was more likely (OR = 2.5) to infect more males than females, though it was not statistically significant (p=0.107). One noteworthy observation was that, compared to younger (age range 26 - 40 years) individuals infected with Mtbss, older individuals within the ages of 41 to 60 years were four times more likely to be infected with an L6 strain (OR = 4.3, p=0.005) (Table 5, Figure 3). We also observed that compared to Mtbss, L6 was more likely to be isolated from the Northern region (OR = 3.7, p=0.045) than the Upper East region. On average, L4 infected patients were the youngest among the study participants (Table 5). The eight isolates with unidentified lineages were found to infect older individuals compared to the other infecting lineages (Figure 3).

Discussion

The progress and outcome of MTBC infections have primarily been associated with host and environmental factors.

Table 4
Correlation between MTBC and drug resistance

Variable (Total number analyzed)	INH^r, N (%)	p-value	RIF^r, N (%)	p-value	MDR, N (%)	p-value	ANY, N (%)	p-value
MTBC species (N=294)								
M. tuberculosis sensu stricto (241)	19 (7.9)	0.466	8 (3.3)	0.922	6 (2.5)	0.959	21 (8.7)	0.391
M. africanum (41)	1 (2.4)		1 (2.4)		1 (2.4)		1 (2.4)	
M. Bovis (4)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Unidentified (8)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Human adapted MTBC lineage (N=279)								
Lineage 1 (8)	2 (25.0)	0.306	0 (0.0)	0.889	0 (0.0)	0.929	2 (25.0)	0.321
Lineage 2 (15)	1 (6.7)		0 (0.0)		0 (0.0)		1 (6.7)	
Lineage 3 (7)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Lineage 4 (208)	16 (7.7)		8 (3.8)		6 (2.9)		18 (8.6)	
Lineage 5 (13)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Lineage 6 (28)	1 (3.6)		1 (3.6)		1 (3.6)		1 (3.6)	
Lineage_4 sub-lineage (N=208)								
Cameroon (120)	3 (2.5)	0.001	3 (2.5)	0.204	1 (0.8)	0.035	5 (4.2)	0.004
*Ghana (50)	11 (22.0)		5 (10.0)		5 (10.0)		11 (22.0)	
Haarlem (20)	1 (5.0)		0 (0.0)		0 (0.0)		1 (5.0)	
LAM (4)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Uganda (10)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
X (4)	1 (25.0)		0 (0.0)		0 (0.0)		1 (25.0)	

MTBC: *Mycobacterium tuberculosis* complex, INH: Isoniazid, RIF: Rifampicin, MDR: Multidrug resistance
* Compared to the Cameroon sub-lineage, the Ghana sub-lineage was associated with INH DR (p<0.001) and MDR (p=0.009).

Table 5
Univariate logistic regression analysis of MTBC lineages

Variable	Mtbss (L1 – L4) N (%)	Maf L5 N (%)	Maf L6 N (%)	OR L5 vs. Mtbss	p-value	OR L6 vs. Mtbss	p-value
Year diagnosed							
2015	3 (1.3)	0 (0.0)	1 (3.6)	-	-	2.9	0.364
2016	27 (11.3)	2 (15.4)	2 (7.1)	1.7	0.462	0.6	0.530
2017	31 (13.0)	2 (15.4)	8 (28.6)	1.0	0.967	2.1	0.135
2018	112 (47.1)	7 (53.8)	13 (46.4)	Reference		Reference	
2019	65 (27.3)	2 (15.4)	4 (14.3)	0.5	0.404	0.5	0.305
Gender							
Male	151 (70.2)	10 (83.3)	24 (85.7)	2.0	0.360	2.5	0.107
Female	64 (29.8)	2 (16.7)	4 (14.3)	Reference		Reference	
Age Category							
<25	34 (17.0)	0 (0.0)	2 (7.7)	-	-	0.9	0.847
26-40	94 (47.0)	4 (33.3)	6 (23.1)	Reference		Reference	
41-60	48 (24.0)	6 (50.0)	13 (50.0)	3.0	0.104	4.3	0.005
>60	24 (12.0)	2 (16.7)	5 (19.2)	2.0	0.432	3.4	0.061
Region							
North East	51 (23.4)	2 (16.7)	4 (14.8)	1.1	0.931	1.5	0.632
Northern	86 (39.4)	7 (58.3)	17 (63.0)	2.3	0.317	3.7	0.045
Savannah	4 (1.8)	0 (0.0)	0 (0.0)	-	-	-	-
Upper East	59 (27.1)	2 (16.7)	3 (11.1)	Reference		Reference	
Upper West	18 (8.3)	1 (8.3)	3 (11.1)	1.6	0.694	3.3	0.167
Smear positivity							
Scanty	18 (9.0)	1 (8.3)	1 (4.2)	3.2	0.213	0.38	0.360
1+	45 (22.4)	4 (33.3)	8 (33.3)	2.5	0.244	1.1	0.770
2+	54 (26.9)	4 (33.3)	2 (8.3)	2.1	0.333	0.2	0.072
3+	84 (41.8)	3 (25.0)	13 (54.2)	Reference		Reference	

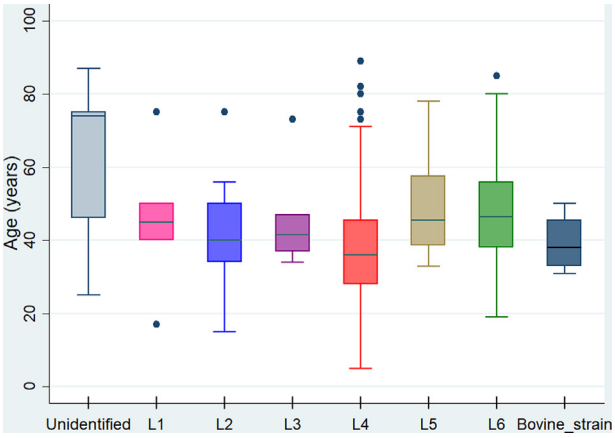

Figure 3. Age distribution stratified by MTBC lineage.

However, pathogenic factors cannot be over-emphasized. Molecular epidemiology of MTBC allows for a better understanding of specific strain distribution in the communities and the development of new interventions, including vaccines, drugs, and novel diagnostic tools (Gagneux et al., 2006, Hirsh et al., 2004, Malik and Godfrey-Faussett, 2005, Mendis et al., 2019). Through this cross-sectional study, we observed that pulmonary TB in the Northern regions of Ghana was predominantly caused by Mtbss (241, 82.0%). We further observed that, although six of the eight human-adapted lineages were in circulation in the region, the prevalence and distribution of Mtbss lineages and sub-lineages were markedly diverse and varied with each region.

The second most important MTBC species responsible for TB infection in the region was Maf accounting for 41/294 (13.9%) of the total MTBC species identified. Maf is generally endemic in West Africa, where it can cause up to 50% of the total TB cases (De Jong et al., 2010, Diarra et al., 2018, Lawson et al., 2012, Zumla et al., 2017). However, the prevalence of Maf varies from country to country as well as regions within countries. Rates ranging from approximately 5% in Ivory Coast to 66% in Benin, depending on the molecular typing methods used, have been documented in West Africa (Bentley et al., 2012, De Jong et al., 2010, Gehre et al., 2016, Gomgnimbou et al., 2012, Groenheit et al., 2011, Yeboah-Manu et al., 2016). In Ghana, previous studies from the southern regions reported rates of approximately 20% (Yeboah-Manu et al., 2011, Yeboah-Manu et al., 2016), which is significantly higher than the 13.9% we observed in the Northern regions. Furthermore, we noted that Maf L6 infected patients were more likely to be older (>40 years) which is consistent with reports from The Gambia (De Jong et al., 2005). This is expected as L6 is known to be relatively attenuated and associated with opportunistic infection, thus more common in immunocompromised patients relative to Mtbss (Bentley et al., 2012, De Jong et al., 2005). The concomitant predominance of the animal strain and L6 in Northern Ghana may be explained further by the fact that they both share genetic and phenotypic characteristics, including genome-wide diversity (Gehre et al., 2016, Gonzalo-Asensio et al., 2014, Otchere et al., 2018). *M. bovis* is a principal causative agent of TB in cattle. However, it occasionally shows cross-species infection in wildlife, other domesticated mammals, and humans (Matteelli et al., 2017, Ring et al., 2019, Thoen et al., 2009). Our report is consistent with previous studies conducted in the region that showed that zoonotic tuberculosis was significantly more common in Northern Ghana compared to the South (Otchere et al., 2019). We also found that three of the four clinical *M. bovis* strains identified shared the same SIT with other *M. bovis* strains obtained from animal carcasses [unpublished data]. This may suggest a possible zoonotic transmission of *M. bovis* strains in Northern Ghana, which is predominantly an agrarian community where the indigenes are generally cereal and livestock farmers. The proximity of farmers to livestock, consumption of uncertified meat and unpasteurized milk, and their products are not uncommon in the region (Yeboah-Manu et al., 2016).

Generally, the sub-lineages of Mtbss L4 are widely diverse and geographically widespread (Brynildsrud et al., 2018, Stucki et al., 2016). In the current study, more than 74% of human-adapted MTBC lineage was attributable to L4. Furthermore, the Ghana (24.0%) and the Cameroon (57.7%) sub-lineages of L4 were the most dominant sub-lineages, accounting for approximately 81.7% of the total infections. Our observations are similar to findings from Southern Ghana, where up to 82.6% of TB cases were caused

by the combined infection of the Ghana sub-lineage (20.8%) and the Cameroon sub-lineage (61.8%). Our results are also consistent with reports from neighboring countries in the sub-region, including Burkina Faso (Godreuil et al., 2007), Cameroon (Niobe-Eyangoh et al., 2003), Mali (Diarra et al., 2018), and Nigeria (Lawson et al., 2012).

A remarkable observation from the current study was the significant presence of the L2 in Northern Ghana. The proportion of L2 (5.4%) in the North was significantly higher (p=0.0046) relative to the South (Table 3). It is worth noting that L2 was the third predominant lineage in Northern Ghana compared to it being the fourth in Southern Ghana. While L2 infections are primarily common to patients of Asian (East or Southeast) origin, it is currently one of the predominant MTBC lineages gaining worldwide attention (Tamaru et al., 2012), probably due to its high transmissibility and association with drug resistance. Even though only one (1/15, 6.7%) out of the 15 L2 isolates was drug-resistant (INH mono resistant) in our study, the proportion (6.7%) is indeed high compared to the likelihood of a Ghanaian MTBC isolate being resistant to at least one anti-TB drug (Almeida Da Silva and Palomino, 2011, Yeboah-Manu et al., 2016). Our findings affirm the widespread reports of L2 and its association with outbreaks of multidrug-resistant tuberculosis (MDR-TB) in various parts of the world (Mendis et al., 2019, Merker et al., 2015, San et al., 2018). Furthermore, the proportion of Maf L5 in the North (4.7%) was significantly lower than in the South but compares to L5 in Burkina Faso (De Jong et al., 2010).

We observed that eight of our isolates, though confirmed as MTBC by having the IS*6110*, could not be differentiated to the species level using the SITVIT database. Interestingly, all eight isolates were obtained from cases that were associated with older age; further characterization by whole-genome sequencing will provide more insights. We acknowledge that spoligotyping, though a PCR-based typing method that is very fast, cost-effective, and can be performed directly on clinical samples, has low discriminatory power (Ali et al., 2019, Cousins et al., 1998, Driscoll, 2009, Ravansalar et al., 2016). Therefore, employing robust molecular tools with high discriminatory power such as SNP typing and whole genome sequencing and analysis will be helpful for accurate characterization. (Comas et al., 2009, Kato-Maeda et al., 2011).

The emergence of drug-resistant strains of MTBC is threatening the effective management and control of TB, especially in limited-resource communities (Parsons et al., 2011, Sambandan, 2012, Yeboah-Manu et al., 2004). The proportions of TB cases with INH mono resistance and MDR/RR-TB have remained relatively stable in recent times (Almeida Da Silva and Palomino, 2011, Asante-Poku et al., 2015, WHO, 2020). Notwithstanding, the rate of drug resistance at the regional level may vary. We report that INH mono-resistance (4.4%) and MDR/RR-TB (3.1%, 9/294) in Northern Ghana was relatively lower than the South (7.5% INH mono-resistance and 3.3% MDR/RR-TB) (Otchere et al., 2016). We again observed that drug resistance was relatively more common with Mtbss compared to Maf, which was consistent with earlier reports (Asante-Poku et al., 2015). Furthermore, consistent with earlier reports(Otchere et al., 2016) (Otchere et al., 2016), the Ghana sub-lineage of Mtbss L4 was associated with drug resistance compared to the other sub-lineages in Northern Ghana.

Conclusion

Our study underscores the importance of the Ghana and Cameroon sub-lineages of Mtbss L4 as the dominant causative agents of pulmonary tuberculosis in Northern Ghana. The Ghana sub-lineage was observed to be associated with both INH and MDR ($p<0.05$), making it a critical TB pathogen in Northern Ghana with a growing public health concern. There is, therefore, the need for close monitoring and evaluation of patients' treatment response to assess the magnitude of drug-resistant TB in the region.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical consideration

The Scientific and Technical Committee and the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research, the University of Ghana, with a federal-wide assurance number FWA00001824, reviewed and approved the protocols and procedures for this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.07.020.

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