Research Article

Genetic Structure and Drug Susceptibility Patterns of *Mycobacterium tuberculosis* Complex Strains Responsible of Human Pulmonary Tuberculosis in the Major Rearing Region in Cameroon

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Background. Cameroon this last decade continues to present a low contribution of *M. africanum* and *M. bovis* in human tuberculosis (TB), while *M. bovis* was prevalent in cattle but all these pieces of information only concerned West and Center regions. *Methods*. We carried out the first study in Adamaoua, one of the most rearing regions of Cameroon, on the genetic structure and drug susceptibility of the MTBC strains isolated from newly diagnosed sputum smear-positive patients aged 15 years and above. For that purpose, spoligotyping, a modified 15 standard MIRU/VNTR loci typing, and the proportion method were used. *Results*. Four hundred and thirty-seven MTBC isolates were analyzed by spoligotyping. Of these, 423 were identified as *M. tuberculosis*, within the Cameroon family being dominant with 278 (65.7%) isolates; twelve (2.75%) isolates were classified as *M. africanum* and two as *M. bovis*. MIRU/VNTR typing of the most prevalent sublineage (SIT 61) suggested that this lineage is not a unique clone as thought earlier but could constitute a group of strains implicated to different pocket of TB transmission. Only *M. tuberculosis* sublineages were associated with antituberculosis drug resistance. *Conclusion*. These results showed the weak contribution of *M. africanum* and *M. bovis* to human active pulmonary tuberculosis in Cameroon even in the rearing region.

1. Introduction

Cameroon is an intermediary country with regard to the incidence of tuberculosis (TB) in sub-Saharan Africa, which is estimated at 122 TB cases per 100,000 per year [1]. Cameroon is also situated in the Central West African region, where there is a particular dominance of a closely related group of *Mycobacterium tuberculosis* strains, called Cameroon family and the decline of *M. africanum* in human TB [2, 3]. The reasons of this successful adaptation of *M. tuberculosis*, the genuine regression of *M. africanum*, and the constant absence of Beijing strains, need to be explored for a better understanding of the major driving forces of the dynamics in transmission within specific populations. This may significantly impact TB control and vaccine strategies development.

It is known that molecular typing of *M. tuberculosis* complex strains can greatly enhance our understanding of population structure and strains circulation of the MTBC and may

help improve TB control [4], especially in Cameroun where there seems to be a rapid dynamic evolution or change in the *M. tuberculosis* complex strain population structure since 1970.

In fact, in Cameroon, the application of spoligotyping used in sparse studies showed a striking regression of *M. africanum* as etiologic agent of pulmonary tuberculosis in the West and Center regions [2, 3, 5]. These studies also showed the predominance of a group of *M. tuberculosis* strains named "Cameroon family" [2, 3]. This can be intriguing considering the population structure or the prevalence of *M. africanum* strain in Nigeria, a neighbouring country to Cameroon, and in other West African countries.

Adamaoua is a breeding region of Cameroon with an approximate population of 703,432 and with an estimated incidence of 121 TB cases per 100,000 per year [1]. It is also neighboring Nigeria and Central African Republic, two high TB burden countries in Africa [6].

Nowadays, there is very limited data available pertaining strains circulating and the population structure of *M. tuberculosis* in other regions of Cameroon apart from the West and Center regions.

The aims of this study were to evaluate for the first time the genetic population structure of *M. tuberculosis* complex strains in Adamaoua and to estimate the implication of *M. bovis* in human pulmonary tuberculosis, in order to gain better understanding of the population structure of MTBC and strains circulation in Cameroon.

2. Methods

2.1. Ethical Considerations. Institutional permission, reference number 052/CNE/DNM/07, to conduct the study was obtained from the National Ethics Committee of the Public Health Ministry. The patients were included in the study after understanding the aims of the research and having signed an informed consent.

2.2. Study Design. This was a cross-sectional study which included all consecutive newly presenting Ziehl-Neelsen (ZN) smear-positive patients between June 2008 and December 2009, aged 15 years and above. The patient recruitment and sample collection process was done as described in our previous report [2, 3]. Briefly, all patients underwent a standardized interview and three consecutive sputum samples were collected from each. Only the samples with positive ZN smear were further analyzed. Samples were stored in cetyl bromide pyrinidium 0.6% (V/V) at the recruitment DCT clinics and transported according to International Union Against Tuberculosis and Lung Diseases recommendations [7] by train to the NTRL for processing, confirmatory Ziehl-Neelsen microscopy, and culture and molecular analysis.

2.3. Sputum Sample Processing. Specimens (2–5 mL) were washed three times with sterile distilled water in proportion 1 : 3 (V/V) and concentrated each time at 4000 × rpm for 20 minutes. The sediment, irrespective of the original sample volume, was reconstituted to three-milliliter sterile distilled water to make the inoculums for the smears and cultures.

2.4. Culture and Identification. Three Lowenstein-Jensen (L-J) slants, two containing 0.75% glycerol without pyruvate and one containing 0.4% pyruvate, were inoculated with the sediment and incubated at 37°C. Cultures were considered negative when no colonies were seen after eight weeks of incubation. Isolates were harvested and DNA extraction was performed as described in our previous report [2, 3].

The drug susceptibility pattern of all identified mycobacterial isolates to isoniazid (0.2 mg/mL and 1 mg/mL), rifampicin (40 mg/mL), streptomycin (4 mg/mL), and ethambutol (2 mg/mL) was determined phenotypically by the indirect proportion method on L-J slants, as described previously [8]. Drug resistance was expressed as the proportion of colonies that grow on drug containing medium to drug-free medium and the critical proportion for resistance was 1% for all drugs.

2.5. Spoligotyping. Standard spoligotyping was done generally as described by Kamerbeek and colleagues [9] using a commercially available kit (Isogen Bioscience BV, Maarssen, The Netherlands).

2.6. MIRU/VNTR Typing. A modified standard 15-MIRU/VNTR locus system MIRU04, ETRC, QUB-26, QUB-11b, MIRU24, MIRU20, MIRU40, ETRA, MIRU27, MIRU26, MIRU31, MIRU39, Mtub30, Mtub34, and Mtub21 were individually amplified and analyzed as previously described [10]. This system was chosen because it has a more reliable discrimination power between MTBC species, lineage, and clad when we analyze MTBC strains isolated from human and animal in our laboratory (personal data) compared to the conventional 12 and 15 loci that had been used before and gave the same haplotype for different species, lineages, or clad. Moreover this system permits the recovery of another polymorphic locus compared to that given by 12 and 15 standard system when we analyze Cameroonian strains. Results from each of the 15 loci were combined to form a 15-digit allele profile.

2.7. Data Analysis. The rate of diversity was calculated by dividing the number of spoligotypes by the number of isolates. The Hunter-Gaston discriminatory index (HGDI) was used to estimate the discriminatory power of spoligotyping and MIRU VNTR [11]. Fisher's exact and chi square test were used to estimate the association between resistance profile and genotype or sex or age or to evaluate the repartition of genotypes between Adamaoua and West region, using statistical software R version 2.15.3 (https://www.r-project.org/). Two-sided p values of 0.05 or lower were considered statistically significant.

2.8. Family Assignment. The spoligotypes patterns were introduced to the SITVIT_WEB database (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/) and assigned a SIT number. Major spoligotyping-based phylogeographic clades

TABLE 1: Antituberculosis drug resistance profiles in Adamaoua region.

Resistance to	Antituberculosis drugs	Total number of resistant strains	% according to all new cases ($n = 437$)	% according to resistant cases ($n = 38$)
	H	13	2,97	34,21
One drug	R	2	0,46	5,26
	S	12	2,74	31,6
	Total	*27*	*6,99*	*71,05*
	HR	2	0,46	5,3
Two drugs	HS	3	0,68	7,9
	Total	*5*	*1.10*	*13,16*
	HRS	1	0,23	2,63
Three drugs	RSE	2	0,46	2,63
	Total	*3*	*0,68*	*5,3*
Four drugs	HRSE	3	0,68	7,9
Total resistance		*38*	*8,70*	*100*
Total sensitive strains		*399*	*91,30*	
	Total	*437*	*100*	

were assigned according to signatures provided by Brudey and collaborators [12].

3. Results

3.1. Studied Patients Data. This was a cross-sectional study of 452 newly diagnosed TB patients aged between 15 and 85 years with mean age of 32.4 years. The female to male sex ratio was approximately 2 : 3. The TB-HIV coinfection rate was 26.55% (94/354) of which 54.3% (51/94) were women and 45.8% (43/94) were men.

3.2. Drug Susceptibility Result. The primary resistance rate of isolates was 7.20% while primary resistance to one, two, three, or four antituberculosis drugs was observed in 6.99%, 1.1%, 0.67%, and 0.67%, respectively (Table 1). The primary monoresistance to isoniazid and streptomycin was the most prevalent (2.58% both) followed by monoresistance to rifampicin (0.52%). No monoresistance was recorded for ethambutol. Multidrug-resistance rate to at least isoniazid and rifampicin was 0.77%. There was no statistical association between resistance, age, and sex (Table 1).

3.3. Spoligotype Results. Molecular typing of the 437 recovered strains by spoligotyping revealed that 423 strains belonged to *M. tuberculosis* species, 12 to *M. africanum* species, and two to *M. bovis* species.

The twelve *M. africanum* strains were regrouped into nine distinct spoligotypes patterns (Table 2) among which eight patterns were not clustered and one pattern was clustered into four strains, while the two *M. bovis* strains revealed two distinct spoligotypes patterns (Table 2).

Spoligotyping of the 423 *M. tuberculosis* isolates revealed 54 distinct spoligotypes, among which 29 were not clustered and the remaining 25 patterns were clustered into 2 and 217 strains.

The comparison between the spoligotypes found in our study and those of the SITVIT_WEB database revealed that only 41 had a SIT number. The remaining 24 were orphan within which 20 were not clustered and four clustered with 2-3 strains.

Among the *M. africanum* spoligotype patterns, only four were already described in SITVIT2. The other five were not clustered and had no SIT number. AFRI-2 lineage accounted for eight strains among which four were clustered (SIT 101) while AFRI-3 accounted for four strains representing four spoligotypes not clustered (Table 2).

The two *M. bovis* spoligotypes belonged to BOVIS1 (SIT 1037) and BOV (SIT 308) lineage.

Of the 423 *M. tuberculosis* strains, a total of 278 (65.7%) belonged to the Cameroon family, of which 223 (80.2%) strains were from the most prevalent clade called SIT61. The other described *M. tuberculosis* families were the ubiquitous T (69 strains), represented by lineage T1 (42 strains) and T2 (27 strains), the Haarlem (40 strains), represented by lineage H1 (20 strains) and H3 (20 strains), the U family represented by lineage U (16 strains) and U (Likely H) (13 strains), the CAS family represented by CAS1_Delhi (one strain), and the X family represented only by lineage X1 (one strain) which has never been described before in Cameroon. None of the strains had the spoligotype of the worldwide-expanded epidemic Beijing family (Table 2).

3.4. Drug Susceptibility Results among Mycobacterium tuberculosis Complex Species and Lineages. Regarding cluster analysis in drug resistant isolates, none of the *M. africanum* and *M. bovis* strains was responsible of drug resistance, while all drug resistance observed was exclusively due to *M. tuberculosis* strains. The U, T, and Cameroon family and the H families were principally responsible for 10.71%, 10.14%, 8.63%, and 2.50% of all forms of antituberculosis drug resistance in Cameroon (Table 3). When looking for the MDR cases, the

TABLE 2: Families and lineage among the 437 *M. tuberculosis* complex strains isolated from TPM+ patients from Adamaoua, Cameroon.

Genetic families	Lineages	Total number of spoligotype	Total number of strains	% according to total number of strains	% according to total number of strains in the family
LAM	LAM1	1	4	0.91	100
Cameroon	LAM10_CAM	9	278	63.61	100
	T1	7	42	9.61	60.9
T	T2	12	27	6.18	39.1
	ND	1	1	0.22	—
Haarlem	H1	8	20	4.57	50.0
	H3	5	20	4.57	50.0
U	U	8	16	3.66	55.2
	U (likeli H)	1	13	2.97	44.8
Africanum	AFRI 2	5	8	1.83	66.7
	AFRI 3	4	4	0.91	33.3
Bovis	BOV	1	1	0.22	50
	BOVIS 1	1	1	0.22	50
CAS	CAS1_Deli	1	1	0.22	100
X	X1	1	1	0.22	100
Total		*65*	*437*	*100*	

ND = not determined.

TABLE 3: *Mycobacterium tuberculosis* complex species and lineage drug susceptibility.

Family or lineage	Total strains	Total primary resistance	Percentage of Primary monoresistance			MDR tuberculosis
			INH	RIF	STR	
M. tuberculosis	**423**	**8.27 (35/423)**	**3.07 (13/423)**	**0.47 (2/423)**	**2.84 (423)**	**1.42 (6/423)**
Cameroon family	278	**8.63** (24/278)	**3.95** (11/278)	**0.36** (1/278)	**3.24** (9/278)	**0.36** (1/278)
T1	42	**14.29** (6/42)	**4.76** (2/42)	0	**4.76** (2/42)	**7.14** (3/42)
T2	27	**3.70** (1/27)	0	0	**3.70** (1/27)	0
U	16	**6.30** (1/16)	0	0	0	**6.30** (1/16)
U (likely H)	13	**15.38** (2/13)	0	**7.7** (1/13)	0	**7.70** (1/13)
H3	20	**5.00** (1/20)	0	0	0	0
H1	20	0	0	0	0	0
X1	1	0	0	0	0	0
CAS1_Delhi	1	0	0	0	0	0
LAM1	4	0	0	0	0	0
M. africanum	**12**	**0**	**0**	**0**	**0**	**0**
M. bovis	**2**	**0**	**0**	**0**	**0**	**0**
Total	*436*					

Cameroon family, the U lineage, the U (likely H) lineage, and the T1 lineage were responsible for 0.36%, 6.30%, 7.10%, and 7.70% of the MDR case, respectively (Table 3). There was no statistical association between resistance and MTC genotypic families ($p > 0.05$).

3.5. Comparison of Genetic Population Structures of MTC Strains between Adamaoua and West Region in Cameroon. In order to understand strains circulation in Cameroon, we compared these data to those of our previous studies in the West region of Cameroon. We found that twenty-nine (44.6%) spoligotypes among the sixty-five found in Adamaoua were also found in West region. This represents 395 (90.4%) of all isolates. From this, clades SIT61 and SIT53 were more distributed (Table 4) in the two regions. This result shows a common sharing of strains between these two regions of Cameroon.

On the other hand, strains of some lineages seemed to be more adapted in one of the two regions. For example, 73 strains of SIT 50 belonging to H3 lineage were isolated in West

TABLE 4: MTBC population structure comparison between Adamaoua and West region in Cameroon.

Spoligotype profiles	SIT	Lineage	Strains number	
			Adamaoua	West region
	523	U	2	0
	Orphan	AFRI_2	1	0
	Orphan	ND	1	0
	53	T1	34	46
	7	T1	2	0
	498	T1	1	0
	2577	T1	1	0
	118	T2	0	9
	119	X1	1	0
	Orphan	ND	0	1
	44	T5	0	4
	1913	T5	0	2
	Orphan	ND	1	0
	Orphan	ND	0	1
	42	LAM_9	0	2
	20	LAM1	4	5
	93	LAM_5	0	1
	17	LAM_2	0	1
	150	LAM_9	0	4
	Orphan	ND	0	1
	774	T1	2	1
	61	Cameroon	223	184
	Orphan	Cameroon	0	1
	Orphan	Cameroon	0	1
	Orphan	Cameroon	2	0
	Orphan	Cameroon	0	1
	115	Cameroon	2	18
	Orphan	Cameroon	0	1
	Orphan	Cameroon	0	1
	Orphan	Cameroon	0	1
	Orphan	Cameroon	0	1
	Orphan	Cameroon	2	12
	852	Cameroon	1	1
	Orphan	Cameroon	1	4
	Orphan	Cameroon	0	2

TABLE 4: Continued.

Spoligotype profiles	SIT	Lineage	Strains number	
			Adamaoua	West region
	Orphan	Cameroon	0	2
	144	T1	0	1
	516	T1	1	0
	Orphan	ND	0	1
	Orphan	ND	0	1
	50	H3	3	78
	655	H3	0	1
	849	H3	0	1
	75	H3	0	7
	47	H1	11	1
	Orphan	H1	1	0
	742	H3	1	0
	Orphan	U	1	0
	25	CASE1_DELHI	1	1
	839	U	0	4
	1338	U	1	0
	Orphan	U	1	0
	Orphan	Cameroon	0	1
	Orphan	Cameroon	0	3
	Orphan	AFRI_3	0	1
	Orphan	ND	0	1
	370	T1	1	0
	438	AFRI_3	1	1
	Orphan	ND	0	1
	331	AFRI_2	0	2
	319	AFRI_2	1	1
	860	AFRI_2	0	1
	Orphan	AFRI	0	2
	Orphan	AFRI_2	1	0
	101	AFRI_2	4	3
	Orphan	AFRI_2	1	0
	Orphan	AFRI_2	1	1
	Orphan	AFRI_3	1	3
	Orphan	AFRI_2	0	1
	856	AFRI_3	1	0
	52	T2	7	16
	853	T2	2	17
	848	T2	1	1
	Orphan	T2	3	4
	Orphan	ND	0	1
	2088	T2	1	0
	Orphan	T2	1	0

Table 4: Continued.

Spoligotype profiles	SIT	Lineage	Strains number	
			Adamaoua	West region
	117	T1	0	7
	Orphan	ND	0	2
	Orphan	ND	0	2
	Orphan	T2	1	1
	125	T2	1	0
	403	Cameroon	6	2
	844	Cameroon	0	1
	Orphan	Cameroon	0	1
	Orphan	Cameroon	0	2
	2867	T2	3	0
	Orphan	ND	0	1
	317	T2	4	4
	233	T2	2	1
	Orphan	T2	1	0
	49	H3	1	8
	307	H3	1	0
	448	H3	0	1
	62	H1	2	0
	316	H3	14	4
	Orphan	H1	1	0
	314	H3	0	1
	Orphan	H1	1	0
	Orphan	ND	0	1
	1204	U	3	1
	Orphan	ND	0	1
	Orphan	ND	0	1
	Orphan	ND	0	1
	Orphan	H1	2	0
	330	AFRI	0	1
	Orphan	AFRI_2	0	1
	Orphan	AFRI_2	0	1
	Orphan	AFRI_3	1	0
	838	Cameroon	40	18
	Orphan	Cameroon	1	0
	Orphan	Cameroon	1	0
	Orphan	ND	0	1
	Orphan	Cameroon	0	1
	Orphan	Cameroon	0	1
	Orphan	H1	1	0
	1037	BOVIS1	1	0
	Orphan	ND	0	1
	308	BOV	1	0

Table 4: Continued.

Spoligotype profiles	SIT	Lineage	Strains number	
			Adamaoua	West region
	237	U likely H3	0	12
	Orphan	ND	0	1
	46	U (likely H)	13	4
	Orphan	U	0	0
	1656	U	0	2
	450	U	6	2
	Orphan	U	0	1
	842	U	0	1
	786	U	0	8
	569	U	0	1
	Orphan	U	0	1

ND = not determined.

region while only three were isolated in Adamaoua (Table 4). Moreover 36 (55.4%) spoligotypes among the sixty five found in Adamaoua were not found in West region. But these represent only 9.8% (43/437) of all isolated strains. Among these, 22 have never been described either in Cameroon or in the SITVIT_WEB databases.

3.6. Clonal Structure Analysis of the Most Prevalent Clade SIT61. The 34 (15%) strains clonal structure of clade SIT61, randomly chosen, were analyzed with MIRU/VNTR using a modified standard 15-locus set. 19 haplotypes differing by more than two loci were identified (Table 5). This corresponds to allelic diversity of 0.93. The discriminatory power between each locus varied between 0.00 and 0.67. Seven loci were polymorphic: QUB-11b (H = 0.67), ETRA (H = 0.59), MIRU40 (H = 0.47), ETRC (H = 0.34), MIRU 27 (H = 0.34), QUB-26 (H = 0.33), and MIRU 26 (H = 0.16).

4. Discussion

It is now known that characterization of prevailing *M. tuberculosis* lineages and clones focusing on different geographical levels such as continents, countries, regions, or cities is important for locating the origin, evolution, and spreading dynamics of a particular *M. tuberculosis* clone [13]. Cameroon is a country of Golf of guinea with a high incidence of tuberculosis. It is particularly characterized by an expansion of a particular family of *M. tuberculosis* called "Cameroon family" and the decline of *M. africanum* which was previously widespread in the 1970s [14]. However all studies given this assertion had been only done in two of the ten regions that counts Cameroon. Systematic typing of MTBC population strains in another region of Cameroon was therefore needed in order to verify this assertion and for a better understanding of this damage disease's epidemiology in Cameroon.

We used for that purpose spoligotyping which is a rapid, simple, and reproducible molecular technic and which can be performed with nonpurified DNA [15–17]. It permits also the simultaneous distinction between MTBC strains at subspecies and lineage level. We also used MIRU/VNTR typing in order to analyze the clonally nature of the most prevalent clade SIT 61.

Ninety-seven percent of all TB cases reported in this study were caused by *M. tuberculosis* with the dominance of Cameroon family, largely represented by the lineage ST61. This result shows once again the prevalence of Cameroon family in Cameroon even in a rearing region as described in other studies [2, 3, 10]. The same result was obtained in neighboring Nigeria [6, 18], Chad [19], and other West African Countries, among which are Ghana [20] and Burkina Faso [21, 22].

The other *M. tuberculosis* families (T, Haarlem, and U) have also been identified in the Adamaoua region of Cameroon but with lesser proportion [3]. However some lineages that have never been described before in Cameroon were identified. This was X1 lineage which is ubiquitous in Anglo-Saxon countries and the U lineage ST523 clade which is scarce and only described in few countries like Nigeria and USA. The occurrence of these families is probably due to the migration trade between Nigeria and Cameroon or to indirect zoonosis since this clade has been identified in strains causing tuberculosis in cattle in the same region [23]. None of the strains presenting the spoligotype of the worldwide-expanded epidemic Beijing family was identified. This result remains intriguing given the expansion capacity of this genotype and it identification in young girls in Kumba subdivision in Cameroon. We also identified only two spoligotypes as *M. bovis*; this was very intriguing because *M. bovis* is prevalent in cattle intended for human consumption in Cameroon [24], in Nigeria [6], and in Chad [25], two neighboring countries to Cameroon. Moreover Adamaoua region is the most rearing region of Cameroon. This low contribution of *M. bovis* and the dissemination of Cameroon family need to be further explored but we think as was the case of Beijing strain that the stain belonging to these species has different selective habits including virulence and pathogenesis or that systematical introduction of vaccination in new born baby in Cameroon may have selected some species or genotypes as Cameroon family. This situation could also be explained by differences in host reaction of African citizens. We also think that culinary habits of the Cameroon populations which eat well boiled and cooked meat could also explain this low contribution of *M. bovis* to human tuberculosis in Cameroon.

This result shows very low contribution of *M. africanum* to human pulmonary tuberculosis. But this contribution was lower than that obtained in other regions of Cameroon [2, 3]. This remains controversial since *M. africanum* was described as endemic in Cameroon in 1970s [14]. This result has been also observed in Guinea-Bissau a West African countries [26]. Conversely, other West African countries continue to report high proportions of *M. africanum*. Among them are Ghana with 20% [20] and 23% [27] of *M. africanum* and Nigeria with 13% [6] and 12.35% [18] of *M. africanum*.

The fact that 89% of *M. africanum* strains were not clustered argues in favor of reactivation of the old case than the ongoing transmission in Adamaoua. However, one clade of AFRI_2 lineage was clustered to four strains, suggesting that the strain of this clade might be actively implicated in TB transmission. But considering the few number of strains obtained, this assertion needs to be verified with other data and the use of other DNA markers different from the DR region. The same results were notified in Nigeria [18].

We also found in this study that 67% of *M. africanum* strains belonged to AFRI_2 lineage. This results were the same as those observed in West Cameroon where this lineage was also dominant and in neighboring Nigeria [18], but this was not the case in Guinea-Bissau where 99% of *M. africanum* strains responsible for active tuberculosis belonged to the AFRI_1 lineage.

The molecular investigation of Adamaoua's *M. africanum* strains did not show different results compared to those observed in West region of Cameroon after using the same techniques [2, 3]. However, when looking at the global genetic structure, Cameroon family was significantly ($p = 0.015$) more represented in West region while H1 was significantly represented in Adamaoua ($p = 0.033$).

TABLE 5: Population structure of Cameroon family prevalent lineage SIT 61 strains analyzed with a modified standard 15-locus MIRU/VNTR system.

Haplotypes	MIRU 04	ETRC	QUB-26	QUB-11b	MIRU 24	MIRU 20	MIRU 40	ETRA	MIRU 27	MIRU 26	MIRU 31	MIRU 39	Mtub 30	Mtub 34	Mtub 21	Total
C1	2	4	4	6	1	1	2	4	1	5	4	3	2	3	3	**3**
C2	2	4	5	5	1	1	2	4	1	5	4	3	2	3	3	**5**
C3	2	6	5	4	1	1	3	3	1	5	4	3	2	3	3	**4**
C4	2	4	4	6	1	1	3	1	2	5	4	3	2	3	3	**2**
C5	2	4	5	4	1	1	3	4	1	4	4	3	2	3	3	**1**
C6	2	4	5	6	1	1	3	3	1	5	4	3	2	3	3	**1**
C7	2	4	5	4	1	1	3	3	0	5	4	3	2	3	3	**1**
C8	2	4	4	5	1	1	3	4	1	5	4	3	2	3	3	**1**
C9	2	3	4	6	1	1	3	1	2	5	4	3	2	3	3	**1**
C10	2	4	5	6	1	1	3	4	1	5	4	3	2	3	3	**2**
C11	2	4	4	6	1	1	3	4	1	4	4	3	2	3	3	**2**
C12	2	6	5	6	1	1	3	3	1	5	4	3	2	3	3	**2**
C13	2	4	4	5	1	1	4	4	2	5	4	3	2	3	3	**1**
C14	2	4	5	5	1	1	3	4	1	5	4	3	2	3	3	**2**
C15	2	4	5	4	1	1	3	3	1	5	4	3	2	3	3	**2**
C16	2	4	5	3	1	1	3	1	2	5	4	3	2	3	3	**1**
C17	2	4	5	6	1	1	4	3	1	5	4	3	2	3	3	**1**
C18	2	4	5	5	1	1	3	1	2	5	4	3	2	3	3	**1**
C19	2	4	5	6	1	1	2	4	1	5	4	3	2	3	3	**1**
Total																**34**

Clade ST61 in our study, based on spoligotyping results, seems to be associated with recent transmission since more than 80% (223/278) of strains of Cameroon family belong to this clade. The same result was found in neighboring Nigeria [18] and Burkina Faso [21]. But when analyzing molecular structure of the population of the strains belonging to this clade using independent markers (MIRU/VNTR), we obtained a high genetic diversity and a very high allelic diversity. This result suggested that this clade is not the true clone but that some of the strains were not implicated in the direct transmission of tuberculosis in Cameroon but it seems to be implicated in the reactivation of cases or in imported cases. However, some haplotypes such as C1, C2, and C3 were clustered with three, five, and four strains, respectively. This result could traduce the implication of strains of these haplotypes to different pockets of ongoing TB transmission in Adamaoua region.

The high diversity obtained in these lineages was very different to that obtained in the last study done in West region of Cameroon [10]. This difference might be due to the system of loci used in this first study in which the 12 standard MIRU/VNTR loci were used. In fact, four new polymorphic loci have been identified irrespective of what was obtained in the latest study.

Three other major families and lineages observed argue in favor of three different pockets of ongoing TB transmission; most of it is due to the ubiquitous T (T2 and T1) family, Haarlem family (H1 and H3 lineages), and U (U and U Likely H) family phylogeographically specific for European and North American regions. We think that the presence of these families in Cameroon might be imported cases of the disease or strains that initially arrived through settlers but adapted to the local population. The same results have also been noticed in Nigeria [18].

The high prevalence of *M. tuberculosis* species and the very little prevalence of *M. africanum* and *M. bovis* in the Adamaoua lead us to evaluate the association between MTBC species or lineages and drug susceptibility. We found that only *M. tuberculosis* strains were responsible for all form of TB drug resistance observed in this study similarly to Ghana where *M. tuberculosis* species was also more likely resistant to any of the tested drugs compared to *M. africanum* [20]. The comparison of the drug resistance phenotypic proportions to different lineages did not permit us to find any statistical significant difference. Nevertheless only Cameroon family strains appeared to be associated with all phenotypic drug resistance observed, but in fewer proportions compared to other lineages like T1 when considering the phenotypic profile individually. This result also shows that Cameroon family presents the less proportion of MDR profiles compared to other *M. tuberculosis* lineages. This result can be intriguing considering the endemicity of this family in Cameroon but further studies analyzing the implication of this family to secondary drug resistant need to be done in order to better understand its endemicity in Cameroon.

5. Conclusion

Our study provides the first genetic structure of *M. tuberculosis* complex strain populations in the rearing region of Cameroun. Results show an expansion of the *M. tuberculosis* family endemic called Cameroon family in other regions and confirm a very low contribution of *M. bovis* and *M. africanum* to active pulmonary tuberculosis infection, even in the most rearing region of Cameroon.

Competing Interests

With the submission of this manuscript, the authors would like to undertake the responsibility that, for this submitted manuscript, they did not receive reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future. They did not hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future. Moreover they did not hold or were currently applying for any patents relating to the content of the manuscript or even received reimbursements, fees, funding, or salary from an organization that holds or had applied for patents relating to the content of the manuscript. To the best of their knowledge, they did not have any other financial and nonfinancial competing interests.

Authors' Contributions

Eyangoh Sara Irène, Um Boock Alphonse, Kuaban Christopher, Etoa François-Xavier, and Koro Koro Francioli conceived and designed the experiments. Koro Koro Francioli, Kaiyven Afi Leslie, and Noeske Juergen performed the experiments. Eyangoh Sara Irène and Gutierrez Cristina, Koro Koro Francioli, and Etoa François-Xavier analyzed the data. Koro Koro Francioli prepared the first draft of paper, and Gutierrez Cristina and Koro Koro Francioli designed the figures. All authors provided critical input.

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