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Prevalence of drug resistance and genetic characterization of *Mycobacterium tuberculosis* complex strains from pulmonary tuberculosis patients co-infected with malaria at Jamot Hospital in Yaoundé

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Tuberculosis (TB) is caused by the *Mycobacterium tuberculosis* complex (MTBC) and remains a major global public health concern. This study aimed to determine the prevalence of drug resistance and the genetic variation among MTBC population in pulmonary tuberculosis patients co-infected with Malaria at Jamot Hospital in Yaoundé-Cameroon. This was a 12 months cross-sectional study that enrolled 336 participants aged 15 years and above. Following sputum culture on solid media, drug resistance was detected using the proportion method and later confirmed by the Line Probe Assay. Isolates were further subjected to molecular characterization using spoligotyping. Amongst the 336 TB patients

registered in this study, there were 17 (5.05%) TB-Malaria co-infected patients. Overall, in 25 (12.88%) patients the bacteria were resistant to at least one anti-TB drug, of which, 3 (1.54%) were co-infected with malaria. Multidrug-resistance (MDR) was observed in 2 cases (1.02%), 1 (0.51%) of which was in a TB-Malaria co-infected patient. *M. tuberculosis* was the only species identified. The T1 (60%) and the LAM10_CAM (27.5%) families were the most prevalent genetic families both in TB-malaria co-infected and in mono-infected TB patients. The description of drug resistance prevalence and of the *M. tuberculosis* genetic diversity is expected to contribute to improving the TB case management in Cameroon.

Key words: Pulmonary tuberculosis, multidrug resistance, line probe assay, spoligotyping, genetic diversity, HJY, co-infection.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis (M. tuberculosis)*, remains a major public health problem worldwide. According to reports from the World Health Organization (WHOb) in 2019, 10 million new cases of tuberculosis were identified in the world, resulting in 1.2 million deaths. A number of 2.5 million new cases were and 377,000 deaths were reported in Africa in 2020 (WHO Report, 2020).

Tuberculosis also represents one of Cameroon's major threats to public health and a significant cause of preventable mortality in the adult population (NTCP, 2019). In 2018, 47,000 cases of tuberculosis were registered, with 7,700 cases of death (WHO Report, 2020).

On the other hand, malaria was responsible for 229 million cases in 2018 in the world, with 93% of cases occuring in Africa (WHO, 2019a). In 2017, malaria accounted for 24% of consultations and for 12.8% of deaths in Cameroon (National Malaria Control Strategic Plan in Cameroon, 2019). In co-infected patients, symptoms such as anemia, respiratory stress, cough, and fever are similar and delay diagnosis and initiation of treatment of either (Bahbahani and Al-Rashed, 2014). Malaria co-infection increases the mortality of TB patients. The disease increases the bacillary load, the hemozoin load, and disrupts the granuloma with altered responses (Colombatti et al., 2011).

Efforts to control TB were hindered in recent years due to the emergence of multidrug-resistant strains (MDR) to the first-line drugs. In 2018, the WHO identified 490,000 cases of MDR tuberculosis worldwide (WHO, 2019b). In 2016, the WHO estimated the number of rifampicin (RIF)-resistant MDR-TB (RR/MDR-TB) incident cases to be 1200 (1000-2200) corresponding to a prevalence rate of 6.8 (4.3-9.4) per 100,000 population in Cameroon (WHO, 2017). Further contribution to the increased death rate due to TB in the country was associated with the emergence and spread of drug resistant strains to nearly all first line therapies (Kuaban et al., 2000a). RIF, isoniazid (INH), streptomycin (STM), and ethambutol (EMB) are components of first-line multidrug therapy in Cameroon (Soini and Musser, 2001). The rising prevalence of MDR strains has resulted in outbreaks and cases that are not only marginally treatable, but also often fatal. Following the awareness generated by the previous studies and the reorganization of the National TB Control Programme with focus on proper treatment methods, it has been necessary to reassess the levels of resistance to the main anti-TB drugs in the country. A systematic review analysis of drug resistance in Cameroon from 1998 to 2014 revealed the prevalence of drug resistance across some regions of the country. This analysis showed that in 2014, drug resistance reached 10.1% in the Centre region (Titanji and Assam, 2016). In addition, for 6 years, no further studies on resistance medication were conducted to get an idea of how the situation has evolved over the last few years. Futhermore, the long duration of culture (8 weeks), delays the detection of resistance and the proper management of TB patients. Molecular assays such as the line probe assay (LPA) have been recommended by WHO for the rapid detection of drug resistance to the most important first-line drugs (RIF and INH) (WHO, 2016).

Information on the *M. tuberculosis* complex (MTBC) genotypes is useful for understanding the spread and phylogeographic specificity of predominant clones, as MTBC lineages have differences in virulence, transmissibility, and capacity of acquiring drug-resistance conferring mutations (Coscolla et al., 2010). In this context, spoligotyping proves to be a very practical and reproducible tool. This method is based on PCR, which tests for the presence or absence of a set of target sequences in the Direct Repeat (DR) locus (Kamberbeek et al., 1997). The resulting genotype is a simple binary format, which has recently led to the construction of large

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databases, intended to facilitate the recognition and origin of a particular clinical isolate (Filliol et al., 2002). As in most countries with limited resources, the epidemiology of tuberculosis in Cameroon has so far largely consisted of reporting the number of cases detected and their demographic data. Thus, very little information is available on the strains of MTBC circulating in the country and more particularly in patients co-infected with TB and Malaria patients in Centre region. Indeed, for more than 5 years, no study has sought to determine the spoligotypes responsible for transmission in this region. The aim of this study was to describe the molecular drug resistance and assess the genetic diversity of isolates of the MTBC in TB/Malaria co-infected patients in the Centre region of Cameroon using spoligotyping.

MATERIALS AND METHODS

This was a cross-sectional study with descriptive and analytical aims and lasted from April 2018 to March 2019. All consented smear-positive pulmonary tuberculosis patients, aged 15 years and above and consulting at the Jamot Hospital in Yaoundé were included in the study. Well-designed study questionnaires were used to capture clinical and epidemiological data. Sputum samples collected from patients were processed and confirmed for TB using microscopy in the hospital laboratory. Serology and malaria analyses were also performed under the same conditions.

Culture, drug susceptibility testing, molecular analysis and quality control (internal and external) were carried out at the Laboratory for Tuberculosis Research and Pharmacology (LTRP), located at the Biotechnology Center of the University of Yaoundé I.

Sample processing

Samples were collected from each participant with productive cough on two consecutive days following standard procedures. Ziehl-Neelsen and/or auramine smears (Degommier, 1957) were performed at the recruitment site. Only the samples with the highest smear grade were transported in a cooler within one day to the LTRP for microscopic confirmation and culture. Each specimen was subjected to a decontamination step with cetylpyridinium chloride/NaCl. Anti-HIV antibodies were determined in whole blood by the immunochromatographic method the ALERE Determine kit, and all positive cases were confirmed using Oraquick. Each consenting participant received appropriate counseling prior to blood collection. Whole blood collected in the dry tube was used to perform the rapid diagnostic test for malaria using the Histidin Rich Protein2 (HRP2) kit specific to *Plasmodium falciparum* (Care Start^{TM}Malaria *pf* Ag RDT). The results were confirmed by microscopy following staining of the slides with 5% Giemsa (Quakyi et al., 2000).

Sputum culture, drug susceptibility test and identification

Following centrifugation of the sputum specimens, three to four drops of the suspended decontaminated sediment inoculate in 2 tubes, namely, the Löwenstein Jensen tube (LJ) without pyruvate and another one supplemented with 0.4% pyruvate. The inoculums were made on sloping media with inclination. The cultures were incubated at 37°C, followed by a weekly observation for growth and counting of colonies. The absence of a colony after 10 weeks of incubation was considered negative. For all positive cultures, drug susceptibility testing was performed using the indirect proportion method on Löwenstein Jensen media at the following drug concentrations: INH (H1: 1 mg/ml and H2: 2 mg/ml), STM (S: 4 mg/ml), RIF (R: 40 mg/ml), EMB (E: 2 mg/ml). Drug resistance was defined as growth on a drug containing medium greater than or equal to 1% for INH and RIF, and 10% for STM and EMB (Canetti et al., 1963).

DNA extraction

A loop full of mycobacterial colonies was scraped from Löwenstein-Jensen's media using a sampling loop, and introduced into eppendorf tubes containing Tris-EDTA (10 mM, 1 mM, pH 8) and heated for 30 min at 90°C. After centrifugation at 13,000 ×g, the supernatant was collected in a new tube and stored at -20°C until further use.

Molecular detection of drug resistance by LPA

Molecular detection of drug resistance was performed with *MTB/DRplus* (Hain Diagnostics), an LPA that targets wild-type regions and mutations in *rpoB* codons 516, 526 and 531 associated with RIF resistance; and in *katG* codon 315 and *inhA* positions 16, associated with INH resistance (Molina-Moya et al., 2015). TB Resistance assay was performed following the manufacturer's instructions. Hybridization and detection were performed with LPA (*MTB/DRplus* version 1.0 kit). The presence of all wild-type hybridization bands and absence of mutation bands indicated a susceptibility to the drug considered. The absence of at least one wild-type hybridization band and/or the presence of any mutation band indicated resistance to the drug considered. The presence of all wild-type hybridization bands in combination with a mutation band in a target gene indicates heteroresistance, a combination of both susceptible and resistant *M. tuberculosis.*

Genotyping of MTBC by spoligotyping

All the isolates were genotyped with a commercial spoligotyping kit (Isogen Bioscience, BV Maarsen, The Netherlands) as described previously by Kamerbeek et al. (1997). Briefly, the DR region of the genome of the tuberculosis isolates was amplified using the primers DRa, 5'-GGTTTTGGGTCTGACGAC-3 '(biotinylated end 5) and DRb, 5'-CCGA-GAGGGGACGGAAAC-3 ". PCR products were hybridized with a set of 43 spacer oligonucleotides covalently linked to the spoligotyping membrane (Isogen Life Sciences, The Netherlands) according to the manufacturer's instructions. The hybridized PCR products were then incubated with streptavidin-peroxidase conjugate and the membrane exposed to chemiluminescence (Amersham ECL Direct ™ Nucleic Acid Detection and Labeling System, GE Healthcare Limited, UK). The X-ray film was developed using standard photochemical procedures after 20 min exposure in the darkroom. DNA extracts from *M. tuberculosis* H37Rv and *M. bovis* BCG were used as controls.

Data analysis

The sample size was calculated by the formula $n = z^2 \times p(1-p)/m^2$. With $p = 0.27$, $z = 1.96$, m = margin of error (5%). By calculation we have 303 TB patients, and we have maximized the sample size to 336 patients.

Socio-demographic and clinical data obtained through questionnaires and the results of laboratory tests were entered, cleaned and analyzed using the statistical software for social sciences (SPSS) version 22.1, data record files. Spoligotype

patterns in binary format were entered into a Microsoft 2016 Excel sheet, and compared to the SpolDB4 database using *MIRU-VNTRplus* software. The Hunter Gaston Discriminant Index (HGDI) was used to calculate the discriminating power of the spoligotyping method. Fisher's Chi-square or Exact Test was used to assess the correlation between epidemiological data and isolate genotypes. P-values less than 0.05 were considered statistically significant.

Ethical consideration

This study complied with the standards of the National Research Ethics Committee for Human Health (CNERSH) N°2018/01/970/CE//SP. Administrative authorization was obtained from Jamot Hospital (N°: 00001478/L/MINSANTE/SG/DHJY). All study procedures were carefully explained before and during the study. Written, informed and signed consent (provided in French and English languages) was obtained from each enrolled patient who incurred no cost for sample processing. All drug susceptibility test results were reported to the respective health facilities for further management of the patients. Furthermore, the confirmed MDR-TB case identified in this study was referred to the MDR-TB treatment center for further management.

RESULTS

Socio-demographic characteristics of the subjects of study

Out of a total of 336 tuberculosis patients included in this study, 215 (63.98%) were males, while 121 (36.01%) were females, with a male to female ratio of 1.77:1. The average age of the patients was (35.16 ± 14.04 years), with minimum age of 15 years. Regarding the marital status and level of education, 222 (66.07%) were singles, 94 (27.97%) were married, 14 (4.16%) were widowed and 6 (1.78%) were divorced, while 17 (5.05%) were out of school. On the level of education, 77 patients (22.91%) had a primary level, 194 (57.73%) had a secondary level and 48 (14.28%) had university level studies. Regarding the HIV status of the study participants, 99 (29.46%) tested positive for HIV.

A total of, 17/336 (5.05%) cases of TB-Malaria co-infection were found with a prevalence of co-infection comparable in males 9/17 (52.94%) and females 8/17 (47.05%). The most affected were found the patients aged between 30 and 50 years 8/17 (47.05%). Most of the co-infected individuals were single 13/17 (76.47%), had higher educational training 12/17 (70.58%), worked in the informal sector 14/17 (82.35%) and resided in the urban area 13/17 (76.47%). Table 1 presents the socio-demographic characteristics of the patients in this study.

Results of tuberculous mycobacteria cultures and resistance pattern to first line anti-TB drugs

The study of the distribution of cultures of *M. tuberculosis* showed that out of the 308 sputum samples cultured, 194 (62.98%) were positive cultures. Of these, there were 12 positives cultures among co-infected patients. Drug sensitivity test to the first line anti-TB drugs INH, RIF, EMB and STM was carried out on a total of 308 *M. tuberculosis* isolates. The highest proportion of mono resistance was observed against STM 12/25 (7.2%) followed by EMB 7/25 (3.6%) and INH 2/25 (1.03%). There was no observed mono-resistance to RIF (0 %). Poly-drug resistance among new TB patients was observed with INH + EMB only 1/25 (0.51%), INH + STM only 1/25 (0.51%) and INH+RIF+EMB only 1/25 (0.51%). Two isolates 2/25 (1.02%) were resistant to RIF and INH (MDR). Initial and acquired resistance was 11/25 (7.17%) and 14/25 (5.67%), respectively. Three co-infected TB/Malaria participants 3/25 (1.54%) were resistant to EMB. Only one case of MDR INH+RIF+EMB 1/25 (0.51%) was found among co-infected patients (Table 2).

Distribution of the different genetic families

A total of 9 genetic profiles were identified within 4 large genetic families among the 80 isolates. Thus, 22/80 (27.5%) of the isolates were from the LAM10_CAM family, 2/80 (2.5%) from the Haarlem family, 1/80 (1.25%) from the Uganda family, 48/80 (60%) from the T1 Ghana family and 7/80 (8.75%) cases were not identified by the database as indicated in Table 3.

Predominant spoligotypes

Sixteen (16) spoligotypes were identified amongst which, nine (9) represented Shared Types (ST) already known and already listed in the SpolDB4 database. The remaining seven (7) spoligotypes were identified for the first time. Among these Shared Types, the ST 53 (Ghana family) member of the T1 family, and the ST 61 member of the LAM10_CAM family were, respectively represented by 47.5 and 26.25% shown in Table 4.

Distribution of spoligotypes (Share type) according to patient types (mono-infected TB and co-infected TB-malaria)

These results showed that the co-infected participants were infected with strains belonging to the LAM10_CAM families and the T1 family. In co-infected participants, the LAM10_CAM family was represented exclusively by the ST61 spoligotype. This spoligotype was also dominant in the mono-infected participants. However, the presence of a strain with the spoligotype represented by ST838 was also noted. Within the T1 family, the presence of 2 spoligotypes was observed in the co-infected patients and represented by: ST53 and ST123. Moreover, in the mono-infected participants, apart from these 2 spoligotypes, the spoligotypes represented by ST205, ST774 and ST498 were also recalled. In view of these

Table 1. Sociodemographic characteristics of the study population.

Characteristics	Total number	Percentage	TB/Malaria Co-infected	Percentage
Sex				
Female	121	36.01	8	47.05
Male	215	63.98	9	52.94
Total	336	100	17	100
Age (years)				
<20	28	8.33	3	17.64
21-30	112	33.33	4	23.52
31-40	96	28.57	3	17.64
41-50	49	14.58	5	29.41
51-60	28	8.33	1	5.88
61-70	18	5.35	1	5.88
71 and above	5	1.48	0	0
Total	336	100	17	100
Marital status				
Singles	222	66.07	13	76.64
Married	94	27.97	0	0
Divorced	6	1.78	3	17.64
Widowers	14	4.16	1	5.88
Total	336	100	17	100
School level				
Out of school	17	5.05	1	5.88
Primary	77	22.91	3	17.64
Secondary	194	57.73	12	70.58
University	48	14.28	1	5.88
Total	336	100	17	100
Type of infection				
TB	237	65.49	-	-
TB-HIV	99	29.46	-	-
TB/Malaria	17	5.05	-	-
Total	336	100	-	-

Source: Microsoft word office 2010

results, co-infected participants showed low genetic diversity compared to mono-infected participants (Table 5).

Predominant families and transmission rate in TB/Malaria co-infected patients and mono-infected TB

The genetic profile was generated by the SpolDB4 database and SITVIT WEB, and allowed to calculate the transmission rate (88.75%) and the Hunter Gaston Discriminating Power Index (HGDI) of 64.6%. Overall, this transmission rate implies that 88.75% of the strains are involved in a recent transmission chain. Thus, the disease reactivation rate was 11.25%. In co-infected patients the rate of transmission of the disease was lower (83.3%) and the rate of reactivation higher (16.07%). Only 2 genotype families were found in cases of coinfection, namely the LAM10_CAM and T1 family (Table 6).

Correlation between drug resistance and genetic diversity of *M. tuberculosis* strains

A statistically significant difference was found between the T1 family and streptomycin monoresistance (p=0.046).

Table 2. Resistance pattern to first line anti-TB drugs among new smear positive pulmonary TB and co-infected patients at Jamot Hospital in Yaoundé Cameroon.

Variable	Number/Frequency of appearance (%)	TB/Malaria co-infected patients/Frequency of appearance (%)
Resistance to only one drug		
INH only	2 (1.03)	-
RIF only	-	-
STM only	12 (7.2)	-
EMB only	7 (4.2)	2 (1.02)
Poly-resistance	-	-
INH+EMB	1 (0.51)	-
INH+STM	1 (0.51)	-
INH+RIF+EMB (MDR)	1 (0.51)	1 (0.51)
INH+RIF(MDR)	1 (0.51)	-
MDR Global	2 (1.02)	-
Total	25 (12.88)	3/25 (1.54)
Type of resistance		
Initial resistance	11 (5.67)	1 (0.51)
Acquired resistance	14 (7.17)	2 (1.03)
Total	25	3

Source: Microsoft word office 2010

Table 3. Distribution of the different genetic families identified from 80 isolates of the *Mycobacterium tuberculosis* complex.

Genetic family	No. of Isolates	Frequency of appearance (%)
LAM10_CAM	22	27.5
Haarlem 3	2	2.5
Uganda 1	1	1.25
T1	48	60
Not identified	7	8.75

Source: Microsoft word office 2010

These 12 cases of streptomycin monoresistance were identified mainly within ST53 suggesting that particular attention should be paid to this spoligotype. The T1 family presented a case of MDR among the 2 cases of MDR that we observed in this study. The only case of MDR among the co-infected patients was also resistant to ethambutol and belonged to the LAM10_CAM family (Table 7).

DISCUSSION

The overall objective of this study was to determine the prevalence of drug resistance to anti-tuberculosis drugs and genotyping the isolates of MTBC among TB-Malaria co-infected patients at Jamot Hospital in Yaoundé (Cameroon).

A total of 336 patients with microscopically positive pulmonary tuberculosis were enrolled. The majority of the patients were males, 215 (63.98%), which corroborates the work of Pokam, Kuaban, and Assam Assam (Pokam et al., 2020; Kuaban et al., 2020; Assam Assam et al., 2011). In this study, the prevalence of malaria among TB patients was low 17/336 (5.05%). Similar studies have reported a low prevalence of TB/Malaria co-infection. In Cameroon, Anyangwe (Anyangwe, 2016) found a co-infection prevalence of 1.5%. A similar study by Range (Range et al., 2007) in Tanzania found a coinfection prevalence of 4.3%. Similarly, Baluku's study in 2019 in Uganda also found a low coinfection prevalence of 2.2% (Baluku et al., 2019). This low prevalence might be due to the fact that tuberculosis results in the production of interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and humoral factors that are protective of malaria (Page et al., 2005; Murphy, 1980).

One of the major problems responsible for treatment failure in tuberculosis patients is the emergence of strains resistant to anti-tuberculosis drugs. In the present study,

Table 4. Distribution of Shared Type identified from the collection of 80 isolates of the *Mycobacterium tuberculosis* complex.

Genotype family	Share type	Spoligotypes	No. of isolate(s)	Frequency (%)
LAM10_CAM	61		21	26.25
	838		1	1.25
T1 family	53		38	47.5
	123		6	7.5
	205		1	1.25
	774		1	1.25
	498		2	2.5
Uganda	237		1	1.25
Haarlem3	50		2	2.5
Not identified	-		7	8.75
	-			
	-			
	-			

Source: Microsoft word office 2010

Table 5. Distribution of predominant spoligotypes (Share type) according to the types of patients: co-infected TB-malaria and mono-infected TB.

Genotypes family	Co-infected TB/Malaria		Mono-infected	
	ST	proportion	ST	Proportion
LAM10_CAM	ST61	4	ST61	17
	ST838	-	ST838	1
T1 family	ST53	4	ST53	34
	ST123	4	ST123	2
	ST205	-	ST205	1
	ST774	-	ST774	1
	ST498	-	ST498	2
		N=12		N=58

Source: Microsoft word office 2010

the overall rate of resistance (to one or several drugs) was 12.88% corresponding to the global resistance. This resistance rate is close to that reported earlier (13.93%) by Tchatchouang et al. (2015) in the Centre region of Cameroon. This rate is slightly higher than those found by Assam et al. (2011) and Sidze et al. (2014), which were 8.1 and 10.1%, respectively. However, high rates have been found by several authors in Africa namely: 23% in Ethiopia, 46.7% in Nigeria (Seyoum et al., 2014; Otokunefor et al., 2018). The primary resistance rate in this work was 5.67% while the secondary resistance rate was 7.17%. A study conducted in the Centre Region in 1998 showed initial and acquired resistance rates to be 31.8 and 55.8%, respectively, another study conducted in 2011 had also shown initial and acquired resistance of 7.35 and 16.66% (Bercion and Kuaban, 1998; Assam et al., 2011). This drop could be the fall-out of the reorganization of the National TB Control Programme, which emphasises the implementation of the Directly Observed Treatment Strategy (DOTs). The highest resistance rates to a drug were observed with streptomycin (7.2%). A similar study in the Centre region of Cameroon in 2015 showed the frequency of resistance to streptomycin was 8.86% (Tchatchouang et al., 2015). Moreover, another study in Mozambique in 2017 showed an overall resistance to streptomycin of 4% (Valencia et al., 2017). The high prevalence to streptomycin may be due to its use in the treatment for other infections in Cameroon. The lowest resistance rate was found with isoniazid (1.02%). Some work carried out in the Centre

Table 6. Summary of predominant families involved in TB-Malaria co-infection and transmission percentage.

Genotype	LAM10_CAM		Family T1		Percentage of transmission	
	Frequency	Proportion (%)	Frequency	Proportion (%)	Rate of recent transmission	Rate of reactivation
Mono-TB	18	81.81	40	83.33	96.25	3.50
TB/Malaria Co-infected	4	18.18	8	16.66	83.3	16.07
P-values	0.402		0.634			

Source: Microsoft word office 2010

Table 7. Relationship between *M.tuberculosis* families and resistance to anti-TB drugs.

Resistance to:	Type of infections	Anti-TB drug	Families	Spoligotyping (Share type)	No. of isolate(s)	P-value
One anti-TB drug	Mono-infected TB	INH	H3	ST50	1	0.644
			LAM10_CAM	ST61	1	0.066
		RIF	-	-	-	-
			Orphan	-	2	-
		STM	T1	ST205 (1) ST53 (1) ST53 (10)	12	0.046
			LAM10_CAM	ST61	1	0.893
			Orphan	-	1	-
		EMB	T1	ST498 (1) ST123 (1) ST53 (2)	6	0.587
	Co-infected TB-Malaria	EMB	T1	ST53 (2)		
Two anti-TB drugs	Mono-infected TB	STM-EMB	LAM10_CAM	ST61	1	0.217
		INH-RIF	T1	ST53	1	0.241
Three anti-TB drug	TB-Malaria Co-infected	INH-RIF-EMB	LAM10_CAM	ST61	1	0.257

Source: Microsoft word office 2010

region (Jamot hospital and Mbalmayo district hospital) has shown high rates of isoniazid resistance of 5.06 and 4.7%, respectively (Tchatchouang et al., 2015). Despite the low rate of resistance to isoniazid in this study, it remains permanent, the constant presence of resistance to isoniazid can be justified by its long period of use in the treatment of tuberculosis (Assam et al., 2011). A more serious aspect of the TB drug

problem is when the infecting organism is resistant to both INH and RIF, referred to as MDR-TB (Canetti et al., 1963). Under this condition, the duration of treatment is prolonged from 6 to 18-24 months, and the cure rate could decrease from nearly 100% to less than 60%. This makes the treatment of MDR cases particularly challenging (Warren et al., 2006). MDR-TB was demonstrated in 2 patients (1.02%). Kuaban in 2000 noted an MDR rate of 4.1% in the West region (Kuaban et al., 2000b), Assam Assam noted an MDR rate of 6.6 % in the same region, but the Centre region in noted an MDR of 1.1% (Assam et al., 2011; Sidze et al., 2014). The lower resistance rates for the Centre region in the present study could be accounted for, at least in part, by the fact that the region is the seat of the capital city of Cameroon, Yaoundé where better health care facilities may be found.

Molecular characterization of *MTBC* strains was performed using the spoligotyping technique. In this study, *M. tuberculosis* was the only species found. This result is similar to that obtained by Assam (2020) in western Cameroon which showed that 100 % of the strains belonged to the species *M. tuberculosis* (Assam et al., 2020). However, this result was slightly different from the 97.65, 98.8 and 97.3% obtained the rate of *M. tuberculosis*, respectively by Kamgue (2013), Assam Assam et al., (2013) and Onana et al., (2018).

However, mass BCG vaccination applied for decades could shape the structure of the *M. tuberculosis* population (Hermans et al., 1995). In addition, the same studies mentioned earlier reported low prevalence of *M. africanum* in the following proportions 2.03, 1.2 and 2.74% (Kamgue et al., 2013; Assam et al., 2013; Onana et al., 2018). This strong presence of *M. tuberculosis* and the virtual absence of *M. africanum* does not corroborate with the studies carried out in 1970 which showed that the majority of cases of TB were caused by *M. africanum* (56%) (Huet et al., 1971). Three decades after, the study conducted by Niobe-Eyangoh et al. (2003) suggests that the downward trend observed over the past decades probably reflects a real regression of *M. africanum* (56 to 9%) as an etiologic agent of tuberculosis in Cameroon (Niobe-Eyangoh et al., 2003). This drop in *M. africanum* for the benefit of *M. tuberculosis* has been confirmed by the studies of Simon et al. (1989), Ledru et al. (1996) and Bourahima et al. (2022) which have shown a regression of *M. africanum* in Burkina-Faso and in Mali. This study reveals the highly diverse *M. tuberculosis* population structure, it confirms a predominance of the Cameroon lineage in the Centre region of Cameroon and the disappearance of *M. africanum* in Cameroon. The causes of this regression are not known. However, the treatment protocol applied to the patients could play an essential role in this selection (Vekemans et al., 1999). Likewise, the *M. bovis* species was not found in this study. This result corroborates with work carried out in Cameroon and Burkina-Faso which shows the low prevalence or the absence of *M. Bovis* in humans (Niobe-Eyangoh et al., 2003; Ledru et al., 1996). This low prevalence can be explained by the fact that the majority of TB cases due to the *M. bovis* species are associated with extrapulmonary TB cases. In this study, we considered only pulmonary tuberculosis. In addition, cases of TB due to *M. bovis* are more frequent in rural areas (Vekemans et al., 1999), but the study was conducted in an urban area. Similarly, the culinary practices that are popular in Africa, consisting in cooking meat well before consumption can also explain the low prevalence of *M. bovis*. The factors that may explain the adaptability of *M. tuberculosis* to a particular area are poorly understood.

The search for the genetic profile of our 80 isolates was done using the database (SpolDB4) and SITVIT WEB and this enabled us to identify 4 major spoligotypes families namely LAM10_CAM (27.5%), Haarlem (2.5%), T1 (60%), Uganda (1.25%) and unidentified cases (8.75%). The major family in this study is the T1 family (60%), contrary to those obtained (27%) by Assam et al. (2013) in the Centre region. The same is true of the 31.66% obtained by the latter (2020) in the western region and 31.84% obtained by Bourahima (Assam Assam et al., 2020; Bourahima et al., 2022). The high prevalence of the T1 family in particular in this study is intriguing and should be followed in studies based on a large population. Of these 60%, the most represented clade was Shared Type 53 or Ghana family with a proportion of (47.5%) compared to other isolates. However, we noticed a strong propensity of ST 53 (47.5%) compared to 11.3% obtained by Pokam in Littoral region of Cameroon in 2020 and 14.7% obtained by Bourahima in Centre of Bamako (Pokam et al., 2020; Bourahima et al., 2022). This therefore implies a strong infiltration of the Ghana family during the last 18 years in Cameroon, probably due to the phenomenon of cross-border migration of strains. The second family mainly represented was the LAM10_CAM family (28.20%) which corroborates with the 34% obtained by Assam et al. (2013), but was different from the 43.33% obtained by Assam et al. (2020) and 46.73% obtained by Niobe-Eyangoh et al. (2003) as well as 51.01% by Kamgue et al. (2013). Beyond our borders, 63% obtained by Molina-Moya et al. (2018) in Nigeria and 45.1% obtained by Bourahima et al. (2022) in Mali. The strong regression of the LAM10_CAM family in this study in favor of the T1 family is intriguing and should be followed in studies based on a larger population. Within the LAM10_CAM family, a diversity was found and the Share Type 61 (26.25%) was predominant within this family. This result corroborates with those of Koro Koro et al. (2013) who showed a predominance of ST 61 within the LAM10_CAM family.

Conclusion

The description of drug resistance prevalence and of the *Mycobacterium tuberculosis* genetic diversity is expected

to contribute to improving the TB case management in Cameroon.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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