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Research Article

GENOTYPIC CHARACTERIZATION OF THE *MYCOBACTERIUM TUBERCULOSIS* COMPLEX RESISTANT TO ANTI-TUBERCULOSIS DRUGS ISOLATED FROM BREEDERS IN SIX TOWNS IN SOUTHERN CHAD

[1]Togde Gildas le Djimbaye, [1,3]*Bessimbaye Nadlaou, [4]Bassirou Diarra, [1]DjimenanBoilengar, [2]Rimtebaye Kimassoum

[1]Department of Biological and Pharmaceutical Sciences, Faculty of Human Health Sciences (FSSH), Laboratory of Research, Diagnostics and Scientific Expertise (LaboReDES), Bacteriology Unit, University of N'Djamena BP 1117 N'Djamena/Chad.
[2]Department of Medicine, Faculty of Human Health Sciences (FSSH), University of N'Djamena BP 1117 N'Djamena/Chad.
[3]Laboratory of the National Reference University Hospital Center (CHU-RN) of N'Djamena, Mycobacteria Unit, BP 130 N'Djamena.
[4]University Clinical Research Center (CURC), University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako BP: 5445 - Point G, Bamako - Mali.

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ABSTRACT

Monitoring risk factors associated with tuberculosis transmission and identifying the genetic basis of *Mycobacterium tuberculosis* complex strains resistant to first-line anti-tuberculosis drugs could help provide important information to optimize care and reduce family contact. tuberculosis among breeders. The present study was an observational, cross-sectional and analytical study aimed at determining the prevalence of resistance genes of strains of the *Mycobacterium tuberculosis* complex circulating in the environments of breeders practicing nomadism and transhumance in Chad. Microscopy using the Ziehl Neelsen staining technique made it possible to identify 274 (21.8%) strains of *Mycobacterium tuberculosis* complex among 1256 breeders consulted for the search for tuberculosis including 67 cases in relapse and 207 new cases under treatment or not without clinical improvement. The average age of the patients was 47.5 years with the extremes ranging from 15 to 80 years. The M/F sex ratio was 1.6 in favorof the male sex. The GeneXpert MTB/RIF detected 268 *Mycobacterium tuberculosis*out of the 274 cases of tuberculosis that microscopy identified. Of 268 *Mycobacterium tuberculosis*, 24 (9%) were rifampicin resistant (RR-TB) and 244 (91%) rifampicin susceptible. Culture on MGIT (mycobacteria growth indicator tube) medium confirmed the isolation of 274 strains of mycobacteria. Molecular typing of the 274 strains of the *Mycobacterium* complex by Spoligotyping made it possible to detect 268 (98%) *Mycobacterium tuberculosis* and 6 (22%) *Mycobacterium bovis*. Spoligotyping also made it possible to detect three lines from *Mycobacterium tuberculosis* isolates (Lineage, Lineage 3 and Lineage 4 (L1, L3 and L4). Thirteen genotypic markers were detected by the Spoligotyping technique and *Mycobacterium tuberculosis* Cameroon (MtbCam) was predominant (27%) The GenoType MTBDR plus made it possible to detect the genes which code for resistance to rifampicin and isoniazid: rpoB (n=24), inhA (n=13) as well as the mutation genes associated with second-line anti-tuberculosis drugs(gyrA (n=5), gyrB (n=6), rrs (n=3), eis (n=3)). TB/HIV co-infection represented 5%. This study highlighted the emergence of lineages of strains of the *Mycobacterium tuberculosis* complex resistant to rifampicin and isoniazid as well as strains of *Mycobacterium bovis* transmitted from cattle to breeders. It raises the need to implement an effective surveillance system to detect the different lines of *Mycobacterium tuberculosis* resistant to anti-tuberculosis drugs in Chad, in Africa and even in the world.

Keywords: *Mycobacterium tuberculosis* complex, anti-tuberculosis drug resistance gene, breeder, conventional and molecular technique, Chad.

INTRODUCTION

Tuberculosis (TB), a pulmonary infection found in humans and cattle and one of the deadliest infections, remains a major threat to public health worldwide. The World Health Organization (WHO) estimates that in 2022, 10.6 million people will have developed tuberculosis worldwide, with 450,000 new cases of rifampicin-resistant tuberculosis [1]. In 2023, the WHO report based on data from 192 countries estimates the number of people diagnosed with TB at 7.5 million, the highest figure recorded since TB surveillance began in 1995 [2]. The COVID19 pandemic has led to a slowdown in updating data on tuberculosis in Africa [3]. The data increased from 7.1 million cases in 2019 to 5.8 million in 2020 [4]. The incidence of tuberculosis in Chad in 2022 was estimated at 140 cases per 100,000 inhabitants. This incidence would be 25,075, while the national tuberculosis surveillance system was only able to report 14,725 new cases per 100,000 inhabitants [4,5]. The incidence is higher in men (69%) than in women (31%). Children represented 7.2% of recorded tuberculosis cases [4]. Tuberculosis affects all categories of the population in Chad. This study focuses on breeders in ferriks who came to consult health facilities in the different study sites (Sarh, Doba, Moundou, Kelo, Bongor and Ndjamena). The herders are nomads and represent 40% of the population of Chad. Their activity (livestock breeding) is a sector which contributes significantly to the national economy [6]. The search for tuberculosis within this social stratum is difficult because of the transhumance and nomadism of the breeders. The species most incriminated in human and/or animal pathology are: *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium cannetii*, but the most dangerous in human pathology is *Mycobacterium tuberculosis* [7].

Resistance of the *Mycobacterium* complex to anti-tuberculosis drugs has been observed in several tuberculosis endemic regions. Drug-resistant tuberculosis can appear either by transmission of a resistant strain (primary resistance) or by mutation of the drug-sensitive strain during treatment (secondary resistance). Nine lineages (L1…L9) of the *Mycobacterium tuberculosis* complex with complete genomic sequences [8]. The lineages include genetic strains from diverse origins and lineage 3 strains are involved in multi-drug resistance to anti-tuberculosis drugs worldwide [8].

Corresponding Author: Bessimbaye Nadlaou,
1Department of Biological and Pharmaceutical Sciences, Faculty of Human Health Sciences (FSSH), Laboratory of Research, Diagnostics and Scientific Expertise (LaboReDES), Bacteriology Unit, University of N'Djamena BP 1117 N'Djamena/Chad. 3Laboratory of the National Reference University Hospital Center (CHU-RN) of N'Djamena, Mycobacteria Unit, BP 130 N'Djamena.

In Chad, few laboratories are involved in culture to allow better isolation and identification of different strains of the *Mycobacterium* complex [9]. However, previous work carried out in 2012 showed that lineages carrying MDR-TB are distributed in certain regions of southern Chad [10].

The objective of this study was to determine the frequency of resistance genes of species of the *Mycobacterium tuberculosis* complex to anti-tuberculosis drugs circulating among breeders in the six towns of Chad using conventional (Microscopic and culture) and molecular (GeneXpert, Spoligotyping) techniques. and the GenoType MTBDR plus) in order to better care for breeder tuberculosis patients in Chad.

MATERIAL AND METHODS

Setting, type, sites, study population and conduct of research work

This was an observational, cross-sectional and analytical study, carried out in six training courses in six provinces for the recruitment of breeders affected by tuberculosis in Chad.Sputum samples were taken from breeders with signs suggestive of tuberculosis for the search for *Mycobacterium* in health facilities in the six provinces of Chad (Sarh, Doba, Moundou Kélo, Bongor and N'Djamena) and tested:

- In the hospital laboratories of the six health districts using microscopic techniques (Ziehl Neelsen staining) and the detection of sensitivity and resistance to rifampicin using the GeneXpert MTB/RIF.
- At the Laboratory Mycobacteria Unit of the National Reference University Hospital Center (CHU-RN) of N'Djamena, the samples were decontaminated using the Kubica method and stored at -20°C, then transported under standard conditions of the World Health Organization (WHO).
- At the laboratory of the University Clinical Research Center (CURC) of Bamako in Mali, the samples were cultured on MGIT (Mycobacterium Growth Inhibitor Tube) medium and the suspected Mycobacterium colonies were subjected to molecular tests (Spoligotyping and Genotyping).

A total of 274 non-duplicate *Mycobacterium*isolates were collected from 1256 samples screened for resistance genes (rpoB, katG, gyrA, gyrB, rrs, eis and inhA) as well as associated mutations. Patient volunteers (each of whom signed an informed consent form) were recruited by convenience sampling at different stages of treatment with the aim of searching for resistant strains with mutant *Mycobacterium* genes. The study included 12 relapsed patients and 262 new cases under treatment or not without clinical improvement. The study population consisted of men and women, new cases of tuberculosis and previously treated cases of tuberculosis (relapse), all aged 15 years and over.

Approximately 1256 samples were tested by microscopy after Ziehl Neelsen heat staining for the detection of acid-fast bacilli (AFB). After microscopy, the GeneXpert confirmed the presence of 274 *Mycobacterium tuberculosis* (MTB), sensitivity and resistance to rifampicin. Isolation, identification and detection of mycobacteria by conventional and molecular techniques.

Ziehl Neelsen coloring technique

The sputum was spread on the slide (smear) and passed 2 to 3 times quickly through the blue of the Bunsen burner flame, smear upwards. This allows gentle smear fixation without altering the bacilli. The smear was completely covered by the fuchsin and heated for 5 minutes until steaming without boiling or drying the slide. Then, the slide was rinsed and drained then covered with 3% acid alcohol for 3 minutes and drained. This step was repeated three times until complete discoloration. A second rinse was performed before covering the slide with methylene blue for one minute. A third rinse was carried out before reading the slide. A slide should be examined for 15 minutes on average (at least 300 fields). The tuberculosis bacilli are colored red on a blue background, straight or slightly curved, often arranged in groups of 3 to 10. The results were expressed according to WHO standards.

Detection of mycobacteria by the GeneXpert MTB/RIF automated system

Principle: it is based on amplification of a fragment of the rpoB gene containing the central region at 81 base pairs and fragments of the target sequences of the IS1081 and IS6110 insertion elements with multiple copies by primers.

Using a Pasteur pipette, 2 mL of sputum and 4 mL of the reagent were taken and mixed in another sterile jar. The mixture was vortexed and then incubated at room temperature for 10 minutes. The pot was vortexed again and incubated at room temperature for 5 minutes. Then, 2 mL of the liquefied mixture was aspirated and transferred into the Xpert® MTB/RIF ULTRA cartridge and the test was run for 1 hour 30 minutes.

Isolation of mycobacteria by culture

The culture on the MGIT was done at UCRC and incubated in the BACTEC 960. Approximately 174 samples were inoculated on the MGIT media with an average incubation period of 7 to 14 days in the BACTEC 960. We added 800µl of PENTA and 500µl of the decontaminated sample in MGIT medium. The identification was made 7 days after sowing and the first colonies were directly recorded by the BACTEC 960. The BACTEC, using an infrared signal system, indicates the position of the positive samples in culture.

Detection of mycobacteria using the Spoligotyping technique [11-15]

All culture-positive samples were used for spoligotyping. Spoligotyping was performed on boiled bacterial lysates using a commercially available kit (Ocimum), or using a membrane prepared in-house. In short, this technique amplifies the polymorphic region called DR (Direct Repeat) using two external primers (DRa and DRb). We performed real-time PCR for amplification. Hybridization was then carried out on an Isogen membrane. Development was carried out using a streptavidin conjugate and the Enhanced Chemi-Luminescence (ECL) detection kit. A strip of film was used on the Isogen membrane in a darkroom for development and detection. Results were given in octal code on the film strip and the comparison of the strains was carried out with SPOTCLUST (based on SpolDB3), and SITVIT2 (available at http://www.pasteur-guadeloupe.fr:8081/SITVIT2). This database is currently a large database of genotypic markers with more than 111,635 genotypes isolated in 163 countries. Approximately 174 isolates were used for Spoligotyping, the results of which are in octal code.

Detection of mycobacteria using the GenoType MTBDR v2.0 technique

The GenoType MBDRs v2.0 is a qualitative invitro test for the identification of resistance to anti-tuberculosis drugs (rifampicin,

izoniazid and other second-line anti-tuberculosis drugs). DNA extraction was carried out on mycobacteria colonies on MGIT+). Approximately 110 µL of Mix A was prepared in a sterile tube and 385 µL of Mix B in a second sterile tube. In each PCR tube, 45 µL of solution are aliquoted: 10 µL of Mix A and 35 µL of Mix B plus 5 µL of DNA. The genetic material and mix solutions were amplified in thermocycler 96 for 3 hours. We then prepared the PCR plate (has 12 PCR wells) by adding 20 µL of DEN solution (denaturation) and 20 µL of the amplicon. The two solutions were mixed for 5 min, then we added the hybridization solution (HYB) and stirred gently to homogenize. GenoType strips were labeled and placed in each well with a positive control. Incubation at 45°C for 30 min was observed and then the HYB solution was aspirated. We added 1mL of the STR solution and incubate for 15 min at 45°C. After aspiration of STR, the RIN solution (1 mL in each well) was used to rinse the strips. Approximately 10 µL of CON-C + 990 µL of CON-D or 12 mL of CON-D and 120 µL CON-C for the 12 samples in a sterile tube. We then took the diluted conjugate mixture (CON-C+CON-D) and distributed approximately 950 µL into each well. After 30 min of incubation at room temperature, the CON solutions were aspirated. The revelation on the strip was done after rinsing with the RIN solution.

Ethical considerations

The data included in this study were collected under protocols approved by the Bioethics Research Institution of the Ministry of Public Health (N°1961/CMT/PC/PMT/MSPSN/SE/DGM/DGTPML/DL/2021). Participants in our study were recruited after signing written informed consent and they were free to withdraw at any time. We respected the confidentiality of their clinical care.

Written and signed informed consent

Mr/Mrs/Miss, we would like to have your agreement for the use of your sputum that you will emit into this jar in order to conduct research on tuberculosis in your community. If you agree that we use your sputum, it could help you know if you are sick or not, but also could help save many people in your family and in your community. The results that we will obtain using your sputum will be able to help the government in health decisions in our country. You will be informed about the progress of our study and the results at the end of this investigation.

Sir/Madam, your participation is essential for the completion of this study which will allow us to contribute to improving your care.

Patient's signature and telephone number

Data and statistical analysis

Microsoft office Word and Microsoft office Excel were used to enter and analyze the results. The chi-square test was used to study the correlations between the variables at the margin of error limited to 5%.

RESULTS

Mapping of Mycobacterium complex lineages of study sites Figure 1 is an illustration of different sample collection areas: Sarh, Doba, Moundou, Kelo, Bongor Ndjamena. Chad is a country in central Africa, with an area of 1,284,000 km2. It is located between the 7th and 24th degrees of North latitude and between the 13th and 24th of East longitude and shares its borders with Sudan to the East, the Central African Republic to the South, Libya to the North, Nigeria-Cameroon and Niger to the West. The geolocation of Chad would certainly have contributed to the transhumance and nomadism of breeders from neighboring countries and therefore to the transmission of tuberculosis and the dissemination of resistance genes to first-line anti-tuberculosis drugs by strains of the *Mycobacterium tuberculosis* complex (figure 1).

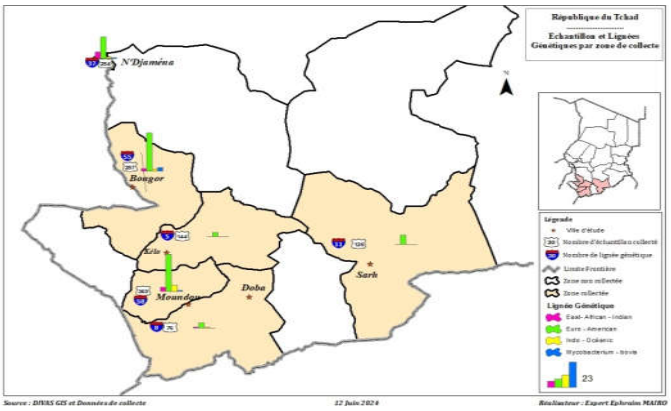


Figure 1: Mapping of the different lineages of the *Mycobacterium tuberculosis* complex of the study sites

A total of 1256 sputum was collected from ferrik breeders in the six study sites for tuberculosis research. The microscopy results revealed 274 (21.8%) positive cases and 982 (78.2%) negative cases with a significance threshold in favor of negative cases (p=0.01). Farmers with positive HIV status represented 98 (7.8%). Microscopy made it possible to detect 274 (21.8%) strains of *Mycobacterium tuberculosis* complex among breeders consulted for tuberculosis research, including 67 cases in relapse and 207 new cases under treatment or not without clinical improvement.

Distribution of positive cases of new cases and relapsed cases of tuberculosis by age, age group and HIV+ status

Of the 274 cases positive by microscopy, 168 (61.3%) were men and 106 (38.7%) were women (p = 0.02: significant difference in favor of the male sex). The average age of the patients was 47.5 years with the extremes ranging from 15 to 80 years. The M/F sex ratio was 1.6 in favor of the male sex. The age groups most affected were [15-25] with 27.4% followed by 20% for the age group of [26-35]. Fourteen (14) breeders among the 274 had an HIV+ status, i.e. 5.1%.

Table 1:Distribution of new cases and relapsed cases of tuberculosis by age group, sex and TB/HIV co-infection

	Age range						
Parameter	[15-25] N(%)	[26-35] N(%)	[36-45] N(%)	[46-55] N(%)	[56-65] N(%)	[66 et plus] N(%)	Total
Sex							
H	43 (15.7)	42 (15.3)	36 (13.1)	30 (10.9)	14 (5.1)	3(1.1)	168
F	32 (11.7)	13 (4.7)	22 (8.0)	28 (10.2)	4 (1.5)	7 (2.6)	106
HIV status (n=98)	1 (0.4)	2 (0.7)	1 (0.4)	1 (0.4)	2 (0.7)	0	7
H	2 (0.7)	1 (0.4)	1 (0.4)	3 (1.1)	0	0	7
F					0		
New	52 (19)	45 (16.4)	41 (15)	56 (20.4)	11 (4.0)	2 (0.7)	207
Relapse	23 (8.4)	10 (3.6)	17 (6.2)	2 (0.7)	7 (2/6)	8 (2.9)	67
Total							**274**

N=number, M=Male, F=Female, Pv-HIV=Person living with Human Immunodeficiency Virus

Result of the field survey on the risk factors associated with the transmission of tuberculosis among breeders

According to the field survey, nomadism, transhumance and promiscuity with animals were almost 100% among ferrik breeders. The unsanitary environment (huts: table 4: figure (a and b)) and the consumption of raw milk from cows infected with mastitis were common practices observed during the survey. Also the level of education among breeders was very low (5 to 10%).

Distribution of positive cases by study sites

Figure 2 illustrates the distribution of positive cases of *Mycobacterium tuberculosis* complex by study sites. The town of Moundou was the most represented with 33% followed by Bongor (26%).

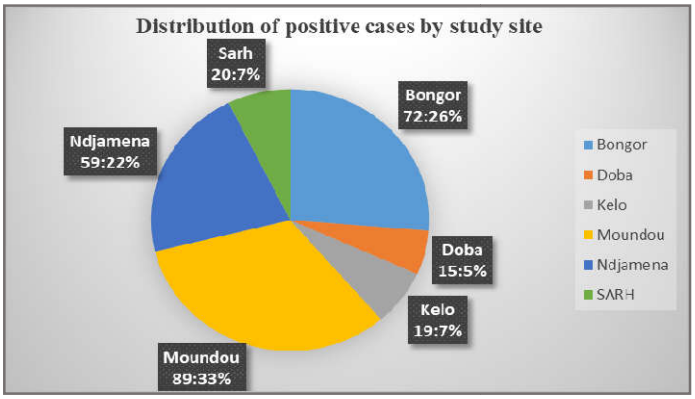


Figure 2: Distribution of positive cases by study site

Resistance profile and sensitivity to first-line anti-tuberculosis drugs

The Xpert MTB/RIF detected 268 *Mycobacterium tuberculosis* out of the 274 cases of tuberculosis that microscopy identified. Of 268 *Mycobacterium tuberculosis*, 24 (9%) was rifampicin resistant (RR-TB) and 244 (91%) rifampicin susceptible.

Culture result

Of the 274 tubercle bacilli identified by microscopy in Chad, and sent to the laboratory of mycobacteriology and hemorrhagic fevers (LMFH) in Bamako in Mali, the culture confirmed the isolation of the 274 strains of mycobacteria.

Molecular typing of *Mycobacterium tuberculosis* complex strains detected by spoligotyping and genotyping

Of the 274 mycobacteria strains confirmed positive by culture, spoligotyping detected 268 (97.8%) isolates of *Mycobacterium tuberculosis* and 6 (2.2%) of *Mycobacterium bovis*. Likewise, spoligotyping made it possible to detect three lineages of *Mycobacterium tuberculosis* isolates (lineage 1, lineage 3 and lineage 4 (L1, L3 and L4) out of 9 lineages detected in the world. Of the twelve (12) genotypic markers of MTBc identified, the frequency of *Mycobacterium tuberculosis* Cameroon (MtbCam) isolates was predominant (27%).

Spoligotyping of the isolates resulted in 5 groups, ranging from 04 to 09 isolates per group.Of the twelve octal codes, three lineages presented the same genotypic characteristics with the major genotypic lineages (Euro-American, Indo-Oceanic, East-African-Indian). The result of Spoligotyping of some strains from different sites in Chad could be consulted on the website (SITVI2) of the Pasteur Institute of Gouadeloupe in France.

The GenpType MTBDR plus made it possible to detect the genes which code for resistance to rifampicin and isoniazid: rpoB (n=24), inhA (n=13). Multi-resistance was approximately 7.5% (n=13).

Table 2: Distribution of *Mycobacterium tuberculosis* complex strain lineages detected by Spoligotyping and compared to isolates of major lineages

Results of Spoligotyping of 274 Isolates from Chad			Major genotypic lineages	Phylogenetic link	Percentage
Code ID	Genotypic isolates	Lineage			
676773777677600	BOV_1	*M.bovis*	*Mycobacterium bovis*	Ancient	3.4
700000000000051	Beijing	L3	East-African-Indian	Modern	1.1
400003743760771	T1-RUS2	L4	Euro-American	Modern	0.4
603777743760771	CAM	L4	Euro-American	Modern	27
703777700003171	CAS1-Dehli	L3	East-African-Indian	Modern	5.1
700777747413771	EAI6-BGD1	L1	Indo-Oceanic	Ancient	2.6
777777770003171	EAI7-BGD2	L1	Indo-Oceanic	Ancient	1.8
767777570020731	H1	L4	Euro-American	Modern	4
777777777720771	H3	L4	Euro-American	Modern	4
577355760160771	T	L4	Euro-American	Modern	1.5
677737777560721	T2	L4	Euro-American	Modern	3
777777777760771	T1	L4	Euro-American	Modern	3.6
777776777760601	X2	L4	Euro-American	Modern	7.7

L = lineage, ID code = identification code, M.*bovis* = *Mycobacterium bovis*

Distribution of genotypic lineages by sex of new cases and relapsed cases of tuberculosis

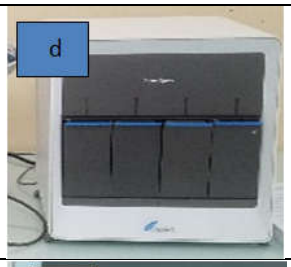
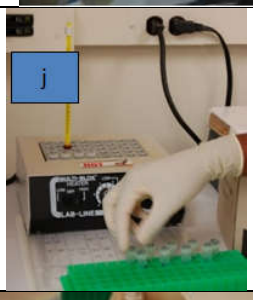
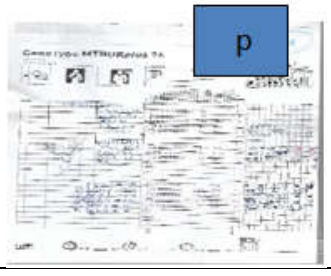
Table 3 shows the distribution of genotypic lineages by sex of new cases and relapsed cases of tuberculosis. Lineage 4 was the most represented among new cases (31%) men and (31%) men relapsed cases (11.3%) of tuberculosis. Resistance to rifampin was 5.8% in lineages.

Table 3: Distribution of genotypic lineages by sex of new cases and relapsed cases of tuberculosis

		Genetic lineage				
		L1 N (%)	L3 N (%)	L4 N (%)	*M. bovis* N (%)	Total
New cases	H	17 (6.2)	28(10.2)	85(31)	2(0.7)	132
	F	13(4.7)	20(7.3)	40(14.6)	2(0.7)	75
Relapse Case	H	2(0.7)	10(3.6)	31(11.3)	2(0.7)	44
	F	1(0.4)	7(2.6)	15(5.5)	0	23
RR-TB		0	8(2.9)	16(5.8)	0	
MDR-TB		0	4(1.5)	9(3.3)	0	
Total						**274**

L: Genetic lineage, N: Number, M: Male, F: Female RR-TB = Rifampin Resistant-Tuberculosis, MDR-TB = multi-drug resistance to tuberculosis.

Table 4: biotechnological steps in the detection of *Mycobacterium tuberculosis* strains and the rpoB, katG, gyraA, gyrB, rrs, eis and inhA genes by conventional and *molecular* techniques

1	**a:** awareness session for breeders to participate or not in the survey **b:** dwellings (huts) of breeders **c:** reading slides colored by the Ziehl Neelsen technique			
2	**d**: automaton: GeneXpert **e**: preparation of sputum under the safety hood **f** : prepared sputum transfer into GeneXpert cartridge			
3	**g**: introduction of the cartridge into the GeneXpert automaton **h** : reading the results on the GeneXpert automaton screen			
4	**i** : preparation of samples for spoligotyping **j** : heating of samples **k** :introduction, recording of samples on the automaton			
5	**l** : automaton for Genotyping **m** : depositing samples on a strip for migration **p** :results recorded			

Kelo, Bongor, Ndjamena. These study areas are located in southern Chad. The study showed that a third of the nine lineages of the *Mycobacteirum tuberculosis* complex known in the world circulate in the regions among breeders in the ferriks and the population of the six cities. While these genotypic lineages are recognized in maintaining tuberculosis pathogenicity and cases of multidrug resistance [16].

The study gave an overall rate of tuberculosis among breeders at around 21.8%. A recent study carried out by Djimenan et al in Chad found an overall prevalence of 25.1% [17]. This difference could be explained by the fact that Djimenan *et al.,* worked within the general population, while this study was only interested in breeders. The overall population of breeders carrying HIV in this study was 7.8%. While a study carried out in 2021 among sex workers, homosexuals and prisoners gave an overall HIV prevalence of around 23.8% according to the Global Fund report on the three diseases (Malaria, HIV and tuberculosis) [16]. HIV screening being systematic and important during a research study on tuberculosis, this population, which is predominantly uneducated, carries the virus without being informed. Resistance to rifampicin was 8.8%, this confirms recent studies carried out by Djimenan B *et al.,* (2024) who found 13% rpoB gene [17, 18]. This same study found 4% resistance to isoniazid while the present study showed 5.1% resistance to isoniazid. Although relatively low compared to other countries, MDR rates should be seriously monitored by Chad's national tuberculosis control program in order to plan further actions to avoid the uncontrolled spread of drug-resistant strains in the country. country. This rate of resistance to anti-tuberculosis drugs could be explained by the fact that the majority of breeders are uneducated, and therefore compliance with dosage and medication intake is not respected.

The study found 61.3% men and 38.7% women whose average age was 37.8 years. The age groups most affected were [15-25], [26-35] with respectively 27.4% and 20% of positive cases of tuberculosis. TB/HIV co-infection represented 5.1%. Gédéon O *et al.,* in their study noted 65.60% men and 34.40% women whose average age was 34.5 years [10]. This discrepancy in the results could be explained by the sample size which was relatively larger than this present study.

Nomadism and transhumance were considered 100% risk factors for the disease, because most of the herders in the study were nomads. The movement of this population category contributes to the transport of genotypic markers (isolates) of the *Mycobacterium tuberculosis* complex. Study areas such as Moundou and Bongor had a high concentration of study population even though these two areas share common borders with Cameroon and the Central African Republic. Of the 13 genotypic markers identified, *Mtb*.Cam which was discovered in 2003 by S Eyangoh *et al.,*2003 [19] was more represented at 27%. The study by Gédéon O et al in 2017 also noted 41.8% of this marker (Cameroon) in certain southern areas of southern Chad. Since the Republic of Cameroon has a wide border with Chad, it is difficult to control population movements across the border in response to economic and political pressures. As in the previous strain molecular typing study, we identify almost the same groups except other *M. tuberculosis* sublineages such as T5-RUS1, X1, S, LAM11-ZWE, H, T3 and T5 [10]. This similarity of the identified strains may suggest a relative stability and endemicity of disease transmission. One of the clinical uses of spoligotyping is also its speed in bacterial identification and its specificity, which is very useful in monitoring other strains of mycobacteria involved in the transmission of tuberculosis from animals to humans and vice versa. The results of Spoligotyping made it possible to detect 3 genotypic lines L1, L3 and L4. One of the particularities of this study, spoligotiping detected six (6) strains of *Mycobacterium bovis*. This is relatively diverse but not like the border region of West Africa which is only identified by the presence of two other lineages of *M. africanum*, L5 and L6 according to Togo et al. [20]. The data could be used to predict TB transmission and propose an effective public health response in the future.

CONCLUSION

Livestock breeders are a population group that seems to be most often overlooked in the monitoring of anti-tuberculosis treatment due to their nomadism and transhumance.

The results of this study not only show the resistance of strains of the *Mycobacterium tuberculosis* complex isolated from breeders, but this study also identified the *Mycobacterium bovis* strains transmitted from cattle to breeders as well as the different lineages carried by this category of population in Chad. The data from this study should encourage national programs to activate the surveillance system for resistant *Mycobacterium tuberculosis* complex in endemic neighboring countries.

Acknowledgments

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Conflict of interest declaration

The authors declare no conflict of interest.

Contribution of the authors

All authors contributed significantly to the writing and editing of this manuscript. It has been seen and approved by all the authors. This manuscript has not been sent for publication elsewhere.

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