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Review Article

Exploring the usefulness of molecular epidemiology of tuberculosis in Africa: a systematic review

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Abstract: Background: Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* complex (MTBC) and remains a serious global public health threat, especially in resource-limited settings such as the African region. Recent developments in molecular epidemiology tools have significantly improved our understanding of TB transmission patterns and revealed the high genetic diversity of TB isolates across geographical entities in Africa. This study reports the results of a systematic review of current knowledge about MTBC strain diversity and geographical distribution in African regions. Methods: Search tools (PubMed, Embase, Popline, OVID and Africa Wide Information) were employed to identify the relevant literature about prevalence, strain diversity, and geographic distribution of MTBC infection in Africa. Results: A total of 59 articles from 739 citations met our inclusion criteria. Most articles reported about patients with presumptive pulmonary TB (73%), fewer reports were on retreatment and treatment failure cases (12%), and presumptive drug resistance cases (3%). Spoligotyping was the most used, alone in 21 studies and in parallel with either the Mycobacterial Interspersed Repetitive Units Variable Number of Tandem Repeats or the Restriction Fragment Length Polymorphism. Various TB lineages were observed across the African continent, with the originally European lineage 4 spotted in all countries studied. Conclusion: TB molecular epidemiology tools have substantially improved our understanding of the MTBC circulating isolates, their evolution, and diversity in this highly endemic region of Africa. We found that only TB lineage 4 is present throughout all the continent and the clusters identified provides an extended insight into the disease transmission dynamics.

Keywords: *Mycobacterium tuberculosis* complex, molecular epidemiology, Africa

Introduction

Tuberculosis (TB), an infectious disease caused by the *Mycobacterium tuberculosis* complex (MTBC), is the leading infection causing death in the world, particularly, in resource-limited settings [1]. According to the World Health Organization (WHO), in 2018, there were an estimated 10 million new cases of TB infection and 1.5 million reported deaths, worldwide, including 0.3 million deaths due to co-infection with HIV [1]. In 2018, the incidence of TB in Africa was 275 (238-314) per 100,000 population compared to 10.7 per 100,000, for example, in the European Union. In addition, among the 30 most affected countries by TB in the world, three are in West Africa, three in Central Africa, six in East Africa and four in Southern African countries [1]. In 2018, an estimated 484,000 people developed multidrug-resistant tuberculosis (MDR-TB), which is a simultaneous resistance to at least rifampicin and isoniazid, demonstrating that drug resistance is also becoming an increasingly serious threat to eradication of the disease in the short- and medium terms. Although the TB epidemic has decreased modestly in the past decade, the incidence remains very high in low- and middle-income countries (LMICs). Furthermore, the burden of disease is expected to persist or

even increase in Africa for many years because of the lack of an effective vaccine and short treatment regimens.

The key TB species that have adapted to the human species are *Mycobacterium sensu stricto* and *Mycobacterium africanum.* A total of seven phylogenetics lineage exist including Lineage 1 (L1 = Indo-Oceanic); Lineage 2 (L2 = East-Asian, include Beijing); Lineage 3 (L3 = East Africa-India); Lineage 4 (L4 = Euro-American); Lineage 5 (L5 = *M. africanum* West African 1); Lineage 6 (L6 = *M. africanum* West African 2); Lineage 7 (L7 = Ethiopia) [2-4]. However, given the limited number of laboratories capable of rapidly and accurately diagnosing the disease and the lack of specialized care centers and physicians, gaining a better understanding of the epidemiology of TB, particularly in Africa, is essential to allow for more targeted interventions to ultimately overcome the chains of transmissions and to successfully mitigate or eliminate current trends of the disease [5].

Recent development of modern molecular epidemiological techniques, however, offer a better understanding of the dynamics of TB transmission, through identification and evaluation of circulating mycobacterium strains, their molecular characteristics, resistance profiles, and disease associated factors [6]. Molecular typing of MTBC isolates has proven to be a valuable technique to better understand the epidemiology of TB [6-10] with the potential to significantly impact both clinical and public health management of the disease [11].

The Restriction Fragment Length Polymorphism (RFLP) typing method is considered to be the gold standard for molecular epidemiologic investigation of TB [12, 13], but is known to have limited DISCRIMINATORY POWER FOR ISOLATES harboring few copies of IS6110 [14]. Spacer oligonucleotide typing or Spoligotyping is a genotyping method based on the amplification of the direct repeat (DR) region of the MTBC genome [6, 15]. It is a relatively simple, fast, cost-effective, and a high-throughput polymerase chain reaction (PCR)-based method for MTBC strain typing. Although Spoligotyping has a lower DISCRIMINATORY power than RFLP typing [16], this tool, during the past decade, has been used exclusively to describe the molecular epidemiology of TB in Africa, and is limited by inaccuracy and non-reproducibility [17]. The Mycobacterial Interspersed Repetitive Units Variable Number of Tandem Repeats (MIRU-VNTR) [18-20] typing method is a PCR-based genotyping method used to differentiate MTBC strains. MIRU is a MTBC-specific name of a multiple locus VNTR (Variable Number Tandem of Repeats). MIRU-VNTR is a 100% reproducible, sensitive, and specific TB typing method [16]. Both Spoligotyping and MIRU-VNTR typing methods are widely used and the results of these assays are maintained in international databases [21-23]. Future study of epidemiological links (transmission chains) and clusters need to include these new techniques [24]. The combination of Spoligotyping with one or more of the above-mentioned methods has demonstrated better precision and resolution in molecular characterization of mycobacterial strains [25].

This systematic review focuses on studies that report on the genetic diversity of MTBC strains in patients with pulmonary TB, from different regions of Africa, using Spoligotyping alone or in combination with other molecular typing tools.

Material and methods

Identification strategy, data abstraction and inclusion criteria

We searched the PubMed, EMBASE, Web of science, Scopus, Science Direct, and Google Scholar databases for articles published between January 1, 1940 and March 12, 2018, using the following search terms and strategy: Molecular [All Fields] AND ("mycobacterium tuberculosis" [MeSH Terms] OR ("mycobacterium" [All Fields] AND "tuberculosis" [All Fields]) OR "mycobacterium tuberculosis" [All Fields]) AND ("Africa" [MeSH Terms] OR "Africa" [All Fields]). There was no limitation on language. The systematic review was performed, based on the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) Statement [26].

Seven hundred thirty-six (739) citations were identified by the database search (**Figure 1**). Articles were screened by the lead author (BK), based on their relevance by review of the title, abstract, and manuscript. The review included studies that reported about pulmonary TB in

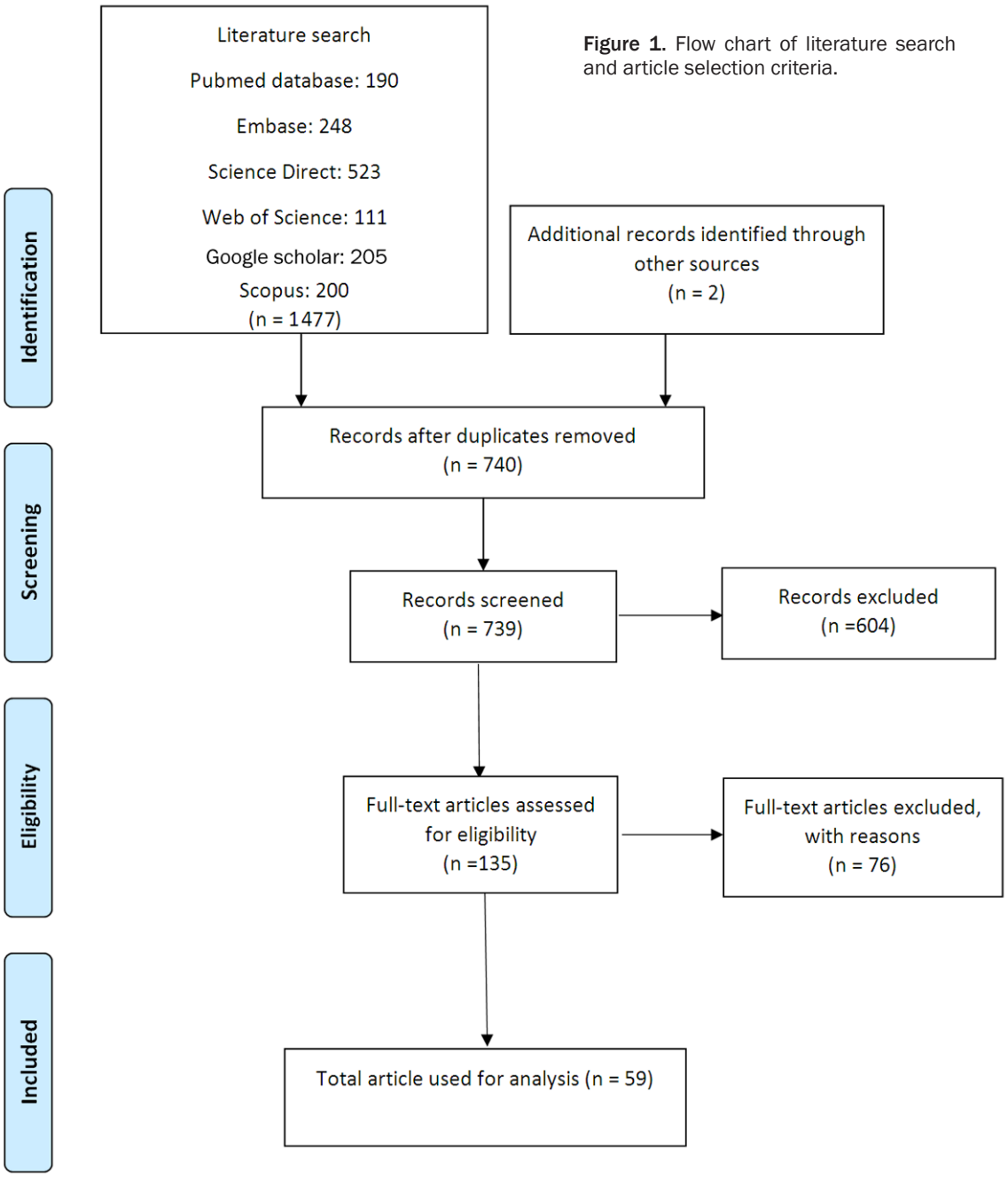

Figure 1. Flow chart of literature search and article selection criteria.

humans in Africa, which involved treated and non-treated TB cases. The review also focused on the molecular methods used to detect MTBC, particularly, Spoligotyping (the traditional method) alone or in combination with other molecular techniques, such as the MIRU-VNTR typing method. Studies that detailed the genetic diversity, clusters and their rates in Africa were included. For all relevant articles, the following data were extracted using a data extraction sheet that included: (1) Region of Africa; (2) Study period; (3) Type of patient: pulmonary TB, treatment naïve, or previously treated, and patient HIV status; (4) Characteristics of genetic diversity: numbers of isolates, MTBC species isolated; (5) Tool used for MTBC identification; Spoligotyping, other tool; and (6) Infection characteristics: presence of clusters, predominant strain identified, and lineage/clade.

In order to minimize the risk of bias, data were extracted from the studies, using Coviden-

Table 1. Distribution of isolates by country in Africa from January 2007 to Mar 2018

Country	Studies	Number of isolates
Benin [74, 88]	2	301
Burkina Faso [89]	1	120
Cameroon [79, 90]	2	734
Côte d'Ivoire [81]	1	194
Djibouti [67]	1	62
Egypt [65]	1	230
Ethiopia [64, 70, 71, 75, 77, 91-95]	10	1597
Gabon [59]	1	159
Gambia [66, 96]	2	1260
Ghana [42, 44, 46]	3	2227
Guinea [97]	1	359
Guinea-Bissau [43]	1	414
Madagascar [68, 98]	2	768
Malawi [34]	1	1194
Morocco [99]	1	592
Mozambique [31, 84]	2	512
Nigeria [83, 100-102]	4	863
Rwanda [33]	1	151
Sierra Leone [78]	1	97
South Africa [27-30, 69, 103-106]	9	4416
Tanzania [76, 107-109]	4	772
Uganda [32, 45, 60, 110-112]	6	1705
Zambia [80]	1	273
Zimbabwe [113]	1	224
Total	59	19,224

ce software (https://community.cochrane.org/help/tools-and-software/covidence) and inserted into a data sheet by the lead author (BK) and cross-checked by another author (AMS), with discrepancies verified by a third author (MM). Data were exported into Epi Info 7.2 for analysis of frequencies of phenotypic and genotypic diversity of MTBC strains.

Results

Description of studies

As described in **Figure 1**, 1,479 potential research articles were identified. After removing duplicates, a total of 739 articles were reviewed, and 604 were not retained, based on the inclusion criteria and fifty-nine (59) articles that represented 59 distinct studies were retained from which data were abstracted (**Table 1** and Supplementary Table 1). Studies, conducted in Southern Africa (Mozambique, Zambia, Zimbabwe, Malawi, Madagascar, South Africa), Northern Africa (Morocco, Egypt), Eastern Africa (Ethiopia, Rwanda, Tanzania, Uganda, Djibouti), Western Africa (Gambia, Guinea, Guinea Bissau, Sierra Lomé, Côte d'Ivoire, Burkina Faso, Ghana, Nigeria, Benin), and Central Africa (Cameroon, Gabon), were included, of which 16 studies were from Southern Africa (7,387 isolates), 2 studies from Northern Africa (822 isolates), 22 studies from Eastern Africa (4,287 isolates), 16 studies from Western Africa (5,835 isolates) and 3 studies from Central Africa (893 isolates).

Characteristic of patients

In the studies, 85% of patients were newly diagnosed with pulmonary TB, whereas 15% were previously treated, including 3% with MDR/XDR-TB. There were 19,224 MTBC isolates from sputum specimens from 59 studies. Patient HIV status was reported in 26 studies of 44% seropositivity. The co-infection with HIV rate was particularly high in certain countries, above 40% in South Africa (n = 4 studies) [27-30], Mozambique (n = 1 study) [31], Uganda (n = 1 study) [32], Rwanda (n = 1 study) [33] and Malawi (n = 1 study) [34].

Molecular typing techniques used

Among the 59 studies, from 24 countries, included in the review, all used the Spoligotyping technique alone or in association with other molecular techniques for characterization of the *MTBC* strains, despite its low discriminatory power [35]. MIRU was used as a second typing method in 18 studies (30%) from 14 countries to identify the epidemiological relationship between strains, including similarities between isolates and potential chains of transmissions. Only 8 studies (13%) from five countries used RFLP typing, 3 (5%) used Large sequence polymorphisms (LPS) and Regions of Difference (RD)-9 Analysis (2%) (**Table 2**). Comparison of molecular tying methods was described in **Table 3**.

Diversity of MTBC strains

Using the TB lineage classification [36] in West Africa there was a predominance of lineage 5 in Gambia, lineage 6 in Guinea Bissau, lineage

Table 2. Different techniques used in molecular epidemiology of *Mycobacterium tuberculosis complex* in Africa

Molecular diagnostic assay	Reference	Isolates n (%)	Number of studies n (%)
Spoligotyping, ONLY	[28, 30, 60, 64, 66, 68, 74, 76, 79, 84, 91, 94, 95, 97, 98, 100, 103, 104, 106, 108, 112]	8426 (43.8)	21 (35.6)
Spoligotyping, MIRU-VNTR	[31, 67, 69, 70, 78, 80, 81, 83, 88-90, 95, 99, 101, 102, 105, 107, 109, 113]	3712 (19.3)	19 (32.2)
Spoligotyping, RFLP	[27, 29, 34, 42-46]	5147 (26.8)	8 (13.6)
Spoligotyping, RD Analysis	[32, 33, 71, 75, 77, 92, 111]	486 (2.5)	7 (11.8)
Spoligotyping, LSP	[96, 110]	1064 (5.5)	2 (3.4)
Spoligotyping, MIRU-VNTR typing, LSP, MLST	[65]	230 (1.2)	1 (1.7)
Spolygotyping, MIRU-VNTR, GeneXpert, Hain GenoType	[59]	159 (0.8)	1 (1.7)
Total		19,224	59

Table 3. Comparison of molecular tying methods

Characteristic	IS6110-RFLP	Spoligotyping	MIRU-VNTR	WGS
Turnaround time	Time consuming	rapid	Lower than RFLP	Medium
Discriminatory ability	No [114]	Yes [114, 115]	No [114, 115]	No [114, 115]
Technique Complexity	Complex [114]	Simple [114]	Less complex	Less complex
Technique Cost	Expensive [116]	Less expensive [116]	fairly expensive	Very high [116]
Competence of personnel needed	Yes and high [116]	Yes [116]	Yes [116]	Yes [116]
Quantity of DNA	Need high amount of DNA [114]			Need high amount of DNA [114]
Easily reproductive	No	Yes	No	No

4 in Guinea, Sierra Leone, Burkina Faso, Cote d'Ivoire, Ghana, Benin, and Nigeria. In Central Africa, lineage 4 was predominant in Cameroon and Gabon. In South African countries, lineage 2 was the most represented in South Africa, lineage 4 in Zambia and Madagascar, and lineage 1 in Mozambique. In East Africa, lineage 3 was predominant in Tanzania and Ethiopia, and lineage 4 in Rwanda, Uganda and Djibouti. In North Africa, lineage 4 was present in Morocco (**Figures 2** and **3**). Overall, in West Africa, there was a predominance of lineages 4, 5 and 6. In Central Africa, lineage 4 was the predominant strain. In Southern African countries, lineages 1, 2 and 4 were the most represented. In East-Africa, the lineages 3 and 4 were predominant, while in North Africa, lineage 4 was identified.

Drug resistance profile

Drug sensitivity testing (DST) was reported in 31 studies on of 12,347 isolates from 19,224 total isolates (64.22%). Mono-resistance to isoniazid was reported in 20 studies (64.5%), to rifampicin in 23 (74.2%), to streptomycin in 21 (67.7%), and to ethambutol in 18 (58.1%) studies. MDR-TB was reported in 28 (90.3%) and XDR TB in 3 studies (9.7%). All XDR cases were reported in studies from South Africa (**Figure 4**).

Discussion

This review describes the molecular epidemiology tools and methods used for MTBC identification in African countries, a major epicenter of TB disease. In most endemic countries, including in Africa, molecular typing is not being routinely used, despite some research studies showing its usefulness not only to identify strains, but also to better understand the dynamics of TB transmission, which involves the identification and evaluation of circulating mycobacterial strains and their molecular characteristics, including similarities between strains and resistance profiles.

Molecular fingerprinting techniques for MTBC have evolved from IS6110-based restriction fragment length polymorphism to Spoligotyping and MIRU-VNTR typing. More recently, whole genome sequencing (WGS) [14, 37-39] has been introduced.

This review assessed studies that report on the molecular epidemiology of TB in Africa and the diversity of strains. Molecular epidemiology has been shown to be powerful to study

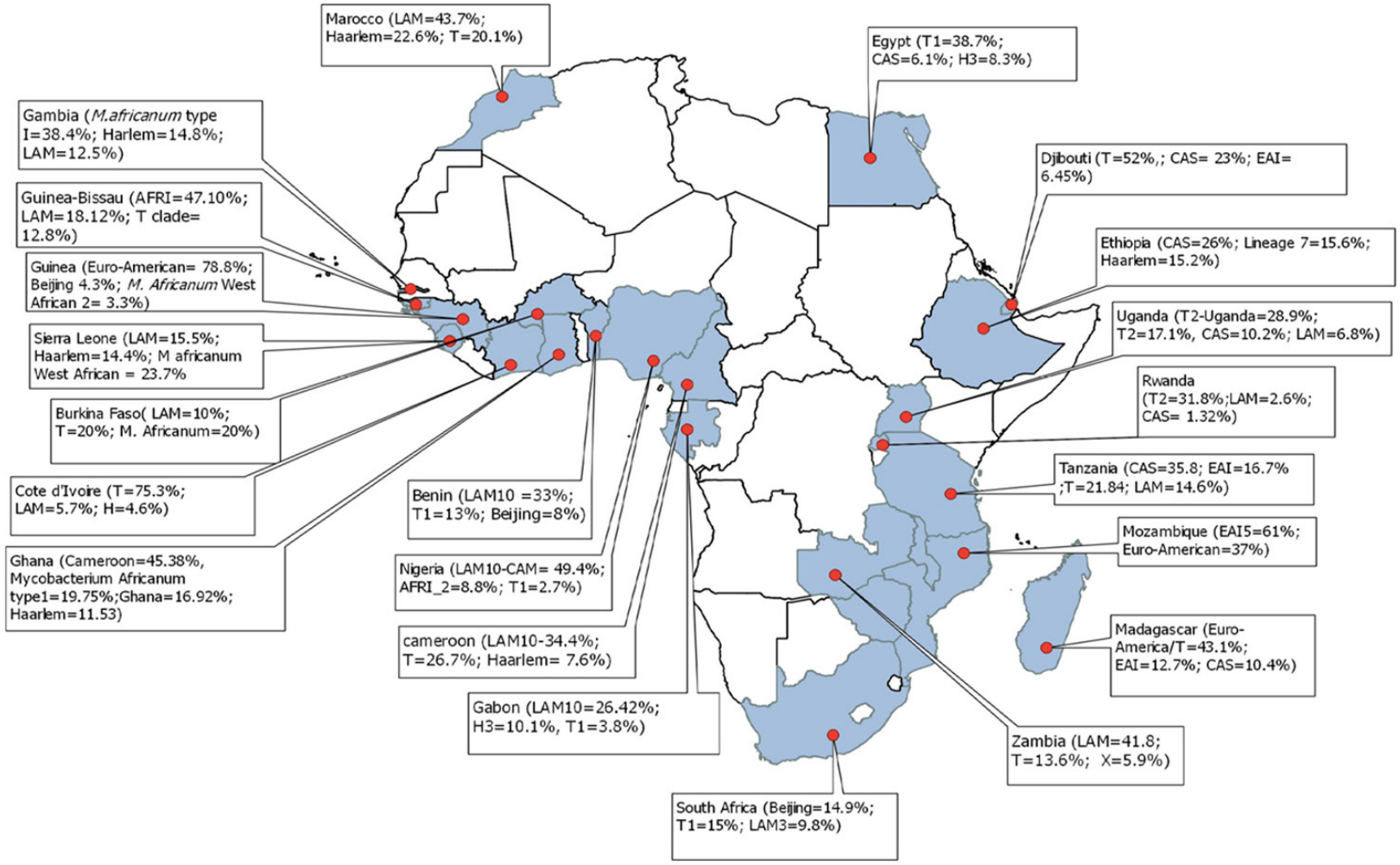

Figure 2. Distribution of predominant strain of *Mycobacterium tuberculosis* complex in Africa countries included in this study.

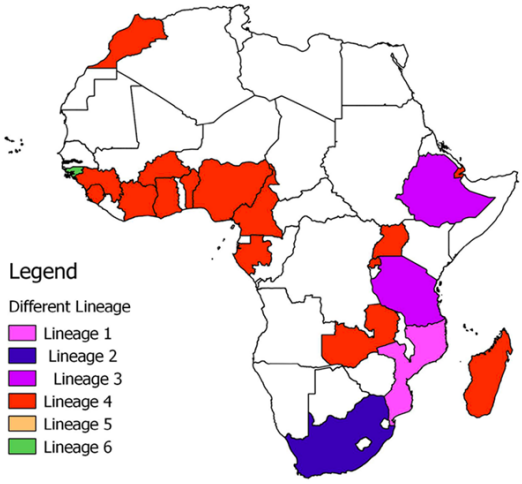

Figure 3. Distribution of Lineages of *Mycobacterium tuberculosis* complex in Africa.

aspects of infectious diseases, including the identification of circulating isolates and transmission chains.

In this study, lineage 4, representing 19,224 TB isolates, and originally a European TB lineage, was the most expanded strain in Africa, as it is worldwide [40]. The global expansion to Africa and to the world of this European lineage can potentially be explained by European migration and colonization of Africa, America, and Asia. The high virulence of this lineage relative to the other strains and the associated high mutation rates may explain the increasing likelihood of drug resistance [40]. This also explains the high and increasing prevalence of TB drug resistance in Africa. The review includes 31 studies reporting on multidrug resistance and 3 studies from South Africa about extensive drug resistance. This is worrisome, as treatment regimens are limited for drug resistant cases, expensive, take longer, and require the use of drugs with many side effects.

The most common genotyping methods used in African countries are Spoligotyping, followed by the MIRU-VNTR typing, and IS6110-RFLP. Spoligotyping and MIRU are currently the most common and accurate combination of TB strain typing. This approach has proven to be helpful for in-depth understanding of the epidemiological profile of MTBC, with an accuracy close to whole sequencing, especially in places where sequencing is not routinely available [41].

Restriction fragment length polymorphism (RFLP)

RFLP is an old technique that has been used in biology and is considered as the gold standard for molecular epidemiology of MTBC [27, 29, 34, 42-46]. Botstein *et al.*, published in 1980 the genetic map of the human genome using genetic markers derived from RFLP [47].

Since the 1990s, IS6110-RFLP genotyping of *M. tuberculosis* has become well-established and is considered as the standard method of TB strain typing [48-52]. The method consists of amplification of a fragment containing the variation of the number of copies of TB insertion sequences. It is the gold standard of molecular epidemiology of MTBC, the most reliable method compared to conventional phenotypic molecular methods and the most stable [52]. Its disadvantage is that it requires specific endonucleases, is complex to perform, and time consuming [53-55]. However, a recent study reported that the new semi-automated RFLP method could be a promising, robust, first-line method for the routine typing of *M. tuberculosis* [56].

We should mention that, despite the use of this method since the 90's, few studies reported its association with Spoligotyping (15.3% (9/59), likely due to the high specificity of RFLP alone but also in part because of the complexity of RFLP which limits its availability.

Spoligotyping

Spoligotyping is the most popular, rapid, cost-effective TB molecular typing used in Africa. It allows a distinction of different strains of MTBC. Forty three types of mycobacterial spacers are well known and present in the direct repeat (DR) locus of the MTBC genome [6]. The number and order of spacers are different from one strain to another, which is the basis of differentiations. The sensitivity and specificity are, 63% and 49%, respectively, compared to the standard RFLP methods [57]. This technique doesn't require a big amount of DNA. The results are easily converted into octal codes [58]. and the method is useful for ongoing and recent outbreaks and clusters. However, Spoligotyping has a low DISCRIMINATORY power than RFLP typing for the same strain over years because the spacers in the RD locus change overtime [16]. Our data revealed that

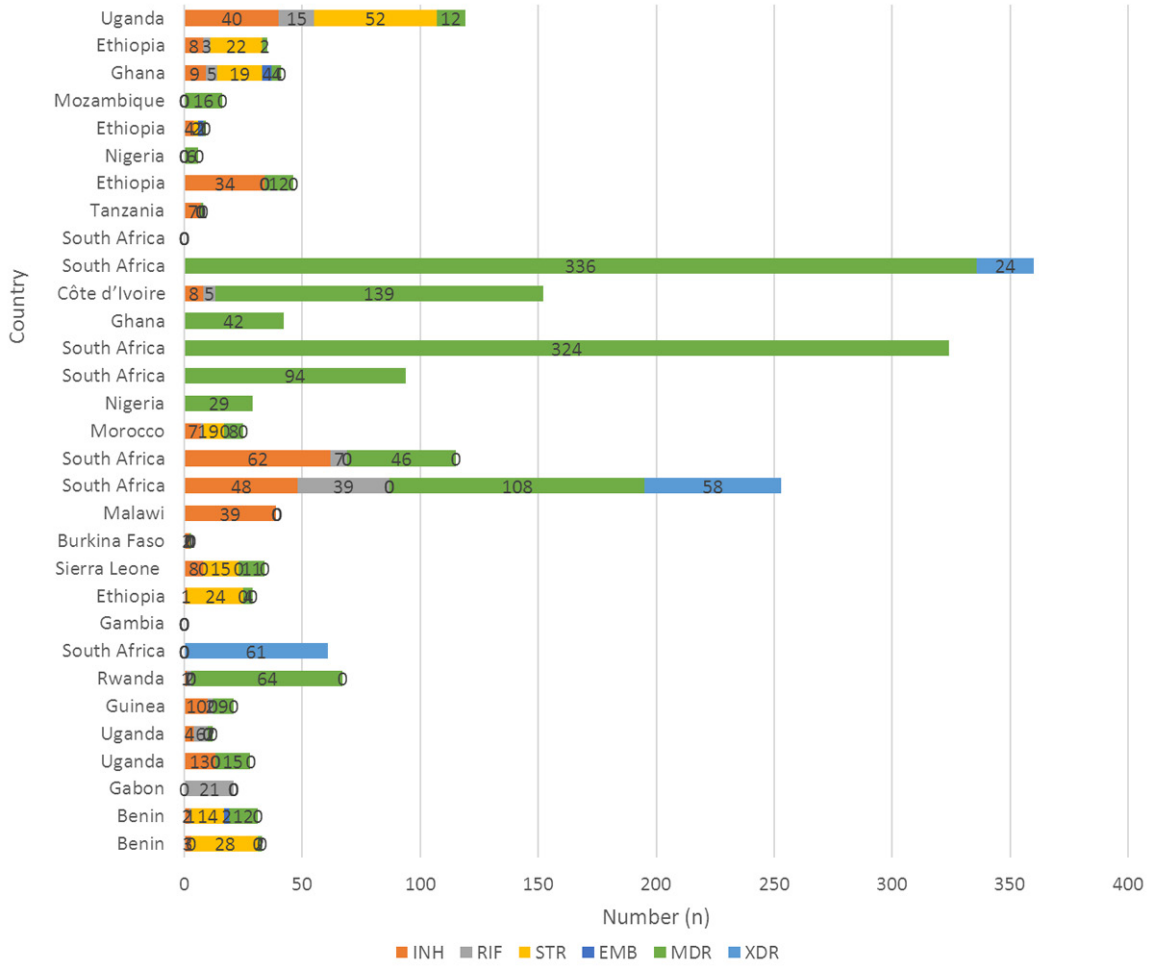

Figure 4. Drug resistance of MTBC in Africa, January 2007 to Mar 2018. INH: isoniazid, RIF: rifampicin, STR: streptomycin, EMB: ethambutol, MDR: Multidrug Resistant, and XDR: Extensively Drug Resistant.

Spoligotyping was combined with other methods in many studies, which is well advised because of the limitations mentioned above. Nevertheless, Spoligotyping was used alone in 36% (21/59) of the studies [4, 13, 14, 21, 34, 41, 55, 59-71], making it the most frequently used typing method in Africa.

MIRU-VNTR typing

MIRU-VNTR typing is widely used in TB molecular typing. It consists of evaluating the variable sizes of repeat regions in the TB genome as a way to differentiate strains. It is useful for the identification of polyclonal infections, its discrimination power is similar to RFLP, and is more specific than Spoligotyping [18, 20, 72, 73]. The sensitivity is 35% and the specificity 65% compared to RFLP [17, 55]. MIRU combined with Spoligotyping was used in 32% (19/59) of the studies reviewed [10, 17, 33, 47, 51, 63, 65, 74-84].

Whole genome sequencing (WGS)

Genome sequencing is widely used for strain classification, lineage determination, studying dynamic transmissions, understanding strain characteristics [85]. In contrary to many tests, it allows a deeper and comprehensive analysis of isolates' genome structure. The WGS characterizes various aspects of TB strains, from virulence to pathogenesis, and from evolution to genome structure [61, 86, 87]. The WGS also helps to determine "hot" areas where targeted interventions may be needed. However, the WGS is expensive and requires sophisticated equipment and experienced biologists and bioinformatics to perform assays, analysis and interpret the data.

Strengths and limitations

This study has some limitations, including, identification of papers from a limited number of sources (**Figure 1**) and papers in English only. Articles in French journals, in from French-speaking countries of Africa, were not identified from sources used. However, many francophone researchers and scientists write their articles in English and many are represented, as evidenced by the studies from Burkina Faso, Benin, Cote d'Ivoire and Guinea. In addition, the absence of clusters in some manuscripts (an exclusion criterion), slightly limited the number of studies. However, the inclusion criteria selected make sense first, because, these molecular typing tools and methods are the most useful for comparing TB isolates from potential same source of transmission chain and clusters and second, because pulmonary TB is most common presentation of the disease.

Conclusion

This review of TB molecular epidemiology in Africa and of the tools being used improves our understanding, not only of the typing methods being used in highly endemic African regions but also of the features and transmission dynamics of the disease. This study found that Spoligotyping is the most commonly used TB typing method in Africa. Overall, these molecular typing methods are being increasingly being used in endemic countries in Africa, which should increase the availability of essential information and help to reduce the transmission chains and disease incidence. Lineage 4 was found in all regions of Africa, but further studies are still needed to better understand the host-pathogen interactions and other factors leading to the widespread of this strain in Africa and beyond.

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Disclosure of conflict of interest

None.

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References

[1] World Health Organization. Global tuberculosis report 2019. WHO 2019.

[2] Coscolla M and Gagneux S. Consequences of genomic diversity in Mycobacterium tuberculosis. Semin Immunol 2014; 26: 431-444.

[3] Gehre F, Kumar S, Kendall L, Ejo M, Secka O, Ofori-Anyinam B, Abatih E, Antonio M, Berkvens D and de Jong BC. A mycobacterial perspective on tuberculosis in West Africa: significant geographical variation of M. africanum and other M. tuberculosis complex lineages. PLoS Negl Trop Dis 2016; 10: e0004408.

[4] Yimer SA, Birhanu AG, Kalayou S, Riaz T, Zegeye ED, Beyene GT, Holm-Hansen C, Norheim G, Abebe M, Aseffa A and Tonjum T. Comparative proteomic analysis of mycobacterium tuberculosis lineage 7 and lineage 4 strains reveals differentially abundant proteins linked to slow growth and virulence. Front Microbiol 2017; 8: 795.

[5] Small PM. Tuberculosis: a new vision for the 21st century. Kekkaku 2009; 84: 721-726.

[6] Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M and van Embden J. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol 1997; 35: 907-914.

[7] Almeida SM, Malaspina AC, Leite CQF and Saad MHF. Usefulness of 3'-5' IS6110-RFLP genotyping and spoligotyping of Mycobacterium tuberculosis isolated in a tertiary hospital: a retrospective study detecting unsuspected epidemiological events. Rev Inst Med Trop Sao Paulo 2019; 61: e51.

[8] Andersen AB, Lillebaek T, Soborg C, Johansen IS and Thomsen VO. Tuberculosis and molecular biology. Ugeskr Laeger 2003; 165: 920-922.

[9] Cannas A, Mazzarelli A, Di Caro A, Delogu G and Girardi E. Molecular typing of mycobacterium tuberculosis strains: a fundamental tool for tuberculosis control and elimination. Infect Dis Rep 2016; 8: 6567.

[10] Comas I, Homolka S, Niemann S and Gagneux S. Genotyping of genetically monomorphic bacteria: DNA sequencing in Mycobacterium

tuberculosis highlights the limitations of current methodologies. PLoS One 2009; 4: e7815.
[11] Allix C, Walravens K, Saegerman C, Godfroid J, Supply P and Fauville-Dufaux M. Evaluation of the epidemiological relevance of variable-number tandem-repeat genotyping of Mycobacterium bovis and comparison of the method with IS6110 restriction fragment length polymorphism analysis and spoligotyping. J Clin Microbiol 2006; 44: 1951-1962.
[12] Jagielski T, van Ingen J, Rastogi N, Dziadek J, Mazur PK and Bielecki J. Current methods in the molecular typing of Mycobacterium tuberculosis and other mycobacteria. Biomed Res Int 2014; 2014: 645802.
[13] Kremer K, van Soolingen D, Frothingham R, Haas WH, Hermans PW, Martin C, Palittapongarnpim P, Plikaytis BB, Riley LW, Yakrus MA, Musser JM and van Embden JD. Comparison of methods based on different molecular epidemiological markers for typing of Mycobacterium tuberculosis complex strains: interlaboratory study of discriminatory power and reproducibility. J Clin Microbiol 1999; 37: 2607-2618.
[14] van Deutekom H, Supply P, de Haas PE, Willery E, Hoijng SP, Locht C, Coutinho RA and van Soolingen D. Molecular typing of Mycobacterium tuberculosis by mycobacterial interspersed repetitive unit-variable-number tandem repeat analysis, a more accurate method for identifying epidemiological links between patients with tuberculosis. J Clin Microbiol 2005; 43: 4473-4479.
[15] Streicher EM, Victor TC, van der Spuy G, Sola C, Rastogi N, van Helden PD and Warren RM. Spoligotype signatures in the Mycobacterium tuberculosis complex. J Clin Microbiol 2007; 45: 237-240.
[16] Ei PW, Aung WW, Lee JS, Choi GE and Chang CL. Molecular strain typing of mycobacterium tuberculosis: a review of frequently used methods. J Korean Med Sci 2016; 31: 1673-1683.
[17] Kremer K, Arnold C, Cataldi A, Gutierrez MC, Haas WH, Panaiotov S, Skuce RA, Supply P, van der Zanden AG and van Soolingen D. Discriminatory power and reproducibility of novel DNA typing methods for Mycobacterium tuberculosis complex strains. J Clin Microbiol 2005; 43: 5628-5638.
[18] Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rusch-Gerdes S, Willery E, Savine E, de Haas P, van Deutekom H, Roring S, Bifani P, Kurepina N, Kreiswirth B, Sola C, Rastogi N, Vatin V, Gutierrez MC, Fauville M, Niemann S, Skuce R, Kremer K, Locht C and van Soolingen D. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol 2006; 44: 4498-4510.
[19] Supply P, Lesjean S, Savine E, Kremer K, van Soolingen D and Locht C. Automated high-throughput genotyping for study of global epidemiology of Mycobacterium tuberculosis based on mycobacterial interspersed repetitive units. J Clin Microbiol 2001; 39: 3563-3571.
[20] Weerasekera D, Pathirane H, Madegedara D, Dissanayake N, Thevanesam V and Magana-Arachchi DN. Evaluation of the 15 and 24-loci MIRU-VNTR genotyping tools with spoligotyping in the identification of Mycobacterium tuberculosis strains and their genetic diversity in molecular epidemiology studies. Infect Dis (Lond) 2019; 51: 206-215.
[21] Allix-Beguec C, Harmsen D, Weniger T, Supply P and Niemann S. Evaluation and strategy for use of MIRU-VNTRplus, a multifunctional database for online analysis of genotyping data and phylogenetic identification of Mycobacterium tuberculosis complex isolates. J Clin Microbiol 2008; 46: 2692-2699.
[22] Demay C, Liens B, Burguiere T, Hill V, Couvin D, Millet J, Mokrousov I, Sola C, Zozio T and Rastogi N. SITVITWEB--a publicly available international multimarker database for studying Mycobacterium tuberculosis genetic diversity and molecular epidemiology. Infect Genet Evol 2012; 12: 755-766.
[23] Mansoori N, Yaseri M, Vaziri F and Douraghi M. Genetic diversity of Mycobacterium tuberculosis complex isolates circulating in an area with high tuberculosis incidence: using 24-locus MIRU-VNTR method. Tuberculosis (Edinb) 2018; 112: 89-97.
[24] Doitsidou M, Poole RJ, Sarin S, Bigelow H and Hobert O. C. elegans mutant identification with a one-step whole-genome-sequencing and SNP mapping strategy. PLoS One 2010; 5: e15435.
[25] Liu K. Genetic diversity and drug resistance of 133 Mycobacterium tuberculosis isolates from Jiangxi Province, China. Molecular Biology 2015; 05.
[26] Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P and Stewart LA; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 2015; 4: 1.
[27] Gandhi NR, Weissman D, Moodley P, Ramathal M, Elson I, Kreiswirth BN, Mathema B, Shashkina E, Rothenberg R, Moll AP, Friedland G, Sturm AW and Shah NS. Nosocomial transmission of extensively drug-resistant tuberculosis in a rural hospital in South Africa. J Infect Dis 2013; 207: 9-17.

[28] Marais E, Mlambo CK, Lewis JJ, Rastogi N, Zozio T, Grobusch MP, Duse A, Victor T and Warren RW. Treatment outcomes of multidrug-resistant tuberculosis patients in Gauteng, South Africa. Infection 2014; 42: 405-413.

[29] Mathema B, Lewis JJ, Connors J, Chihota VN, Shashkina E, van der Meulen M, Graviss EA, Ha NP, Kreiswirth BN, Grant AD, Fielding KL, Dorman SE and Churchyard GJ. Molecular epidemiology of Mycobacterium tuberculosis among South African gold miners. Ann Am Thorac Soc 2015; 12: 12-20.

[30] Stavrum R, Mphahlele M, Ovreas K, Muthivhi T, Fourie PB, Weyer K and Grewal HM. High diversity of Mycobacterium tuberculosis genotypes in South Africa and preponderance of mixed infections among ST53 isolates. J Clin Microbiol 2009; 47: 1848-1856.

[31] Saifodine A, Fyfe J, Sievers A, Coelho E, Azam K and Black J. Genetic diversity of Mycobacterium tuberculosis isolates obtained from patients with pulmonary tuberculosis in Beira city, Mozambique. Int J Mycobacteriol 2014; 3: 94-100.

[32] Bazira J, Matte M, Asiimwe BB and Joloba LM. Genetic diversity of Mycobacterium tuberculosis in Mbarara, South Western Uganda. Afr Health Sci 2010; 10: 306-311.

[33] Gafirita J, Umubyeyi AN and Asiimwe BB. A first insight into the genotypic diversity of Mycobacterium tuberculosis from Rwanda. BMC Clin Pathol 2012; 12: 20.

[34] Glynn JR, Crampin AC, Traore H, Chaguluka S, Mwafulirwa DT, Alghamdi S, Ngwira BM, Yates MD, Drobniewski FD and Fine PE. Determinants of cluster size in large, population-based molecular epidemiology study of tuberculosis, northern Malawi. Emerg Infect Dis 2008; 14: 1060-1066.

[35] van der Zanden AG, Kremer K, Schouls LM, Caimi K, Cataldi A, Hulleman A, Nagelkerke NJ and van Soolingen D. Improvement of differentiation and interpretability of spoligotyping for Mycobacterium tuberculosis complex isolates by introduction of new spacer oligonucleotides. J Clin Microbiol 2002; 40: 4628-4639.

[36] Florian LM, Guislaine R, Christophe S, Jérôme A, Kristin K and Soolingen Dv. Welcome on TB-miner Université Paris-Sud, France (IGM and LRI), LIRMM (Montpellier, France) and RIVM, Netherlands involving [cited 2020 2020-02-08 14:20:17]. Available from: http://info-demo.lirmm.fr/tbminer/index.php.

[37] Gauthier M, Bidault F, Mosnier A, Bablishvili N, Tukvadze N, Somphavong S, Paboriboune P, Ocheretina O, Pape JW, Paranhos-Baccala G and Berland JL. High-throughput mycobacterial interspersed repetitive-unit-variable-number tandem-repeat genotyping for Mycobacterium tuberculosis epidemiological studies. J Clin Microbiol 2015; 53: 498-503.

[38] Meehan CJ, Moris P, Kohl TA, Pecerska J, Akter S, Merker M, Utpatel C, Beckert P, Gehre F, Lempens P, Stadler T, Kaswa MK, Kuhnert D, Niemann S and de Jong BC. The relationship between transmission time and clustering methods in Mycobacterium tuberculosis epidemiology. EBioMedicine 2018; 37: 410-416.

[39] Merker M, Kohl TA, Niemann S and Supply P. The evolution of strain typing in the Mycobacterium tuberculosis complex. Adv Exp Med Biol 2017; 1019: 43-78.

[40] Brynildsrud OB, Pepperell CS, Suffys P, Grandjean L, Monteserin J, Debech N, Bohlin J, Alfsnes K, Pettersson JO, Kirkeleite I, Fandinho F, da Silva MA, Perdigao J, Portugal I, Viveiros M, Clark T, Caws M, Dunstan S, Thai PVK, Lopez B, Ritacco V, Kitchen A, Brown TS, van Soolingen D, O'Neill MB, Holt KE, Feil EJ, Mathema B, Balloux F and Eldholm V. Global expansion of Mycobacterium tuberculosis lineage 4 shaped by colonial migration and local adaptation. Sci Adv 2018; 4: eaat5869.

[41] Pole I, Trofimova J, Norvaisa I, Supply P, Skenders G, Nodieva A, Ozere I, Riekstina V, Igumnova V, Storozenko J, Jansone I, Viksna L and Ranka R. Analysis of Mycobacterium tuberculosis genetic lineages circulating in Riga and Riga region, Latvia, isolated between 2008 and 2012. Infect Genet Evol 2019; 78: 104126.

[42] Goyal M, Lawn S, Afful B, Acheampong JW, Griffin G and Shaw R. Spoligotyping in molecular epidemiology of tuberculosis in Ghana. J Infect 1999; 38: 171-175.

[43] Groenheit R, Ghebremichael S, Svensson J, Rabna P, Colombatti R, Riccardi F, Couvin D, Hill V, Rastogi N, Koivula T and Kallenius G. The Guinea-Bissau family of Mycobacterium tuberculosis complex revisited. PLoS One 2011; 6: e18601.

[44] Meyer CG, Scarisbrick G, Niemann S, Browne EN, Chinbuah MA, Gyapong J, Osei I, Owusu-Dabo E, Kubica T, Rusch-Gerdes S, Thye T and Horstmann RD. Pulmonary tuberculosis: virulence of Mycobacterium africanum and relevance in HIV co-infection. Tuberculosis (Edinb) 2008; 88: 482-489.

[45] Niemann S, Rusch-Gerdes S, Joloba ML, Whalen CC, Guwatudde D, Ellner JJ, Eisenach K, Fumokong N, Johnson JL, Aisu T, Mugerwa RD, Okwera A and Schwander SK. Mycobacterium africanum subtype II is associated with two distinct genotypes and is a major cause of human tuberculosis in Kampala, Uganda. J Clin Microbiol 2002; 40: 3398-3405.

[46] Yeboah-Manu D, Asante-Poku A, Bodmer T, Stucki D, Koram K, Bonsu F, Pluschke G and

Gagneux S. Genotypic diversity and drug susceptibility patterns among M. tuberculosis complex isolates from South-Western Ghana. PLoS One 2011; 6: e21906.
[47] Botstein D, White RL, Skolnick M and Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980; 32: 314-331.
[48] Foxman B and Riley L. Molecular epidemiology: focus on infection. Am J Epidemiol 2001; 153: 1135-1141.
[49] Hermans PW, van Soolingen D, Dale JW, Schuitema AR, McAdam RA, Catty D and van Embden JD. Insertion element IS986 from Mycobacterium tuberculosis: a useful tool for diagnosis and epidemiology of tuberculosis. J Clin Microbiol 1990; 28: 2051-2058.
[50] McAdam RA, Hermans PW, van Soolingen D, Zainuddin ZF, Catty D, van Embden JD and Dale JW. Characterization of a Mycobacterium tuberculosis insertion sequence belonging to the IS3 family. Mol Microbiol 1990; 4: 1607-1613.
[51] Thierry D, Brisson-Noel A, Vincent-Levy-Frebault V, Nguyen S, Guesdon JL and Gicquel B. Characterization of a Mycobacterium tuberculosis insertion sequence, IS6110, and its application in diagnosis. J Clin Microbiol 1990; 28: 2668-2673.
[52] van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993; 31: 406-409.
[53] Magdeldin S. Gel electrophoresis: principles and basics. INTECH 2012-04-04.
[54] Mathema B, Kurepina NE, Bifani PJ and Kreiswirth BN. Molecular epidemiology of tuberculosis: current insights. Clin Microbiol Rev 2006; 19: 658-685.
[55] Rozo-Anaya JC and Ribon W. Molecular tools for Mycobacterium tuberculosis genotyping. Rev Salud Publica (Bogota) 2010; 12: 510-521.
[56] Said HM, Krishnamani K, Omar SV, Dreyer AW, Sansom B, Fallows D and Ismail NA. Evaluation of semiautomated IS6110-based restriction fragment length polymorphism typing for mycobacterium tuberculosis in a high-burden setting. J Clin Microbiol 2016; 54: 2547-2552.
[57] Scott AN, Menzies D, Tannenbaum TN, Thibert L, Kozak R, Joseph L, Schwartzman K and Behr MA. Sensitivities and specificities of spoligotyping and mycobacterial interspersed repetitive unit-variable-number tandem repeat typing methods for studying molecular epidemiology of tuberculosis. J Clin Microbiol 2005; 43: 89-94.
[58] Ali S, Khan MT, Anwar Sheed K, Khan MM and Hasan F. Spoligotyping analysis of Mycobacterium tuberculosis in Khyber Pakhtunkhwa area, Pakistan. Infect Drug Resist 2019; 12: 1363-1369.
[59] Alame-Emane AK, Pierre-Audigier C, Aboumegone-Biyogo OC, Nzoghe-Mveang A, Cadet-Daniel V, Sola C, Djoba-Siawaya JF, Gicquel B and Takiff HE. Use of genexpert remnants for drug resistance profiling and molecular epidemiology of tuberculosis in Libreville, Gabon. J Clin Microbiol 2017; 55: 2105-2115.
[60] Asiimwe BB, Ghebremichael S, Kallenius G, Koivula T and Joloba ML. Mycobacterium tuberculosis spoligotypes and drug susceptibility pattern of isolates from tuberculosis patients in peri-urban Kampala, Uganda. BMC Infect Dis 2008; 8: 101.
[61] Coll F, McNerney R, Guerra-Assuncao JA, Glynn JR, Perdigao J, Viveiros M, Portugal I, Pain A, Martin N and Clark TG. A robust SNP barcode for typing Mycobacterium tuberculosis complex strains. Nat Commun 2014; 5: 4812.
[62] de Jong BC, Hill PC, Aiken A, Jeffries DJ, Onipede A, Small PM, Adegbola RA and Corrah TP. Clinical presentation and outcome of tuberculosis patients infected by M. africanum versus M. tuberculosis. Int J Tuberc Lung Dis 2007; 11: 450-456.
[63] Dekhil N, Skhairia MA, Mhenni B, Ben Fradj S, Warren R and Mardassi H. Automated IS6110-based fingerprinting of Mycobacterium tuberculosis: reaching unprecedented discriminatory power and versatility. PLoS One 2018; 13: e0197913.
[64] Deribew A, Abebe G, Apers L, Abdissa A, Deribe F, Woldemichael K, Jira C, Tesfaye M, Shiffa J, Aseffa A, Bezabih M, Abeje T and Colebunders R. Prevalence of pulmonary TB and spoligotype pattern of Mycobacterium tuberculosis among TB suspects in a rural community in Southwest Ethiopia. BMC Infect Dis 2012; 12: 54.
[65] Diab HM, Nakajima C, Kotb SA, Mokhtar A, Khder NF, Abdelaal AS, Hegazy A, Poudel A, Shah Y and Suzuki Y. First insight into the genetic population structure of Mycobacterium tuberculosis isolated from pulmonary tuberculosis patients in Egypt. Tuberculosis (Edinb) 2016; 96: 13-20.
[66] Gehre F, Antonio M, Otu JK, Sallah N, Secka O, Faal T, Owiafe P, Sutherland JS, Adetifa IM, Ota MO, Kampmann B, Corrah T and de Jong BC. Immunogenic Mycobacterium africanum strains associated with ongoing transmission in The Gambia. Emerg Infect Dis 2013; 19: 1598-1604.

[67] Godreuil S, Renaud F, Choisy M, Depina JJ, Garnotel E, Morillon M, Van de Perre P and Banuls AL. Highly structured genetic diversity of the Mycobacterium tuberculosis population in Djibouti. Clin Microbiol Infect 2010; 16: 1023-1026.

[68] Ratovonirina NH, Rakotosamimanana N, Razafimahatratra SL, Raherison MS, Refregier G, Sola C, Rakotomanana F and Rasolofo Razanamparany V. Assessment of tuberculosis spatial hotspot areas in Antananarivo, Madagascar, by combining spatial analysis and genotyping. BMC Infect Dis 2017; 17: 562.

[69] Said HM, Kock MM, Ismail NA, Mphahlele M, Baba K, Omar SV, Osman AG, Hoosen AA and Ehlers MM. Molecular characterization and second-line antituberculosis drug resistance patterns of multidrug-resistant Mycobacterium tuberculosis isolates from the northern region of South Africa. J Clin Microbiol 2012; 50: 2857-2862.

[70] Tessema B, Beer J, Merker M, Emmrich F, Sack U, Rodloff AC and Niemann S. Molecular epidemiology and transmission dynamics of Mycobacterium tuberculosis in Northwest Ethiopia: new phylogenetic lineages found in Northwest Ethiopia. BMC Infect Dis 2013; 13: 131.

[71] Tilahun M, Ameni G, Desta K, Zewude A, Yamuah L, Abebe M and Aseffa A. Molecular epidemiology and drug sensitivity pattern of Mycobacterium tuberculosis strains isolated from pulmonary tuberculosis patients in and around Ambo Town, Central Ethiopia. PLoS One 2018; 13: e0193083.

[72] Augusto CJ, Carvalho WDS, Almeida IN, Figueiredo LJA, Dantas NGT, Suffys PN and Miranda SS. Comparative study of RFLP-IS6110 and MIRU-VNTR from Mycobacterium tuberculosis isolated in the state of Minas Gerais, Brazil. Braz J Microbiol 2018; 49: 641-646.

[73] Garcia de Viedma D, Alonso Rodriguez N, Andres S, Ruiz Serrano MJ and Bouza E. Characterization of clonal complexity in tuberculosis by mycobacterial interspersed repetitive unit-variable-number tandem repeat typing. J Clin Microbiol 2005; 43: 5660-5664.

[74] Affolabi D, Sanoussi N, Codo S, Sogbo F, Wachinou P, Massou F, Kehinde A and Anagonou S. First insight into a nationwide genotypic diversity of mycobacterium tuberculosis among previously treated pulmonary tuberculosis cases in Benin, West Africa. Can J Infect Dis Med Microbiol 2017; 2017: 3276240.

[75] Bedewi Z, Worku A, Mekonnen Y, Yimer G, Medhin G, Mamo G, Pieper R and Ameni G. Molecular typing of Mycobacterium tuberculosis complex isolated from pulmonary tuberculosis patients in central Ethiopia. BMC Infect Dis 2017; 17: 184.

[76] Eldholm V, Matee M, Mfinanga SG, Heun M and Dahle UR. A first insight into the genetic diversity of Mycobacterium tuberculosis in Dar es Salaam, Tanzania, assessed by spoligotyping. BMC Microbiol 2006; 6: 76.

[77] Getahun M, Ameni G, Kebede A, Yaregal Z, Hailu E, Medihn G, Demssie D, Girmachew F, Fiseha Y, Meaza A, Dirse N, Agonafir M, Dana F, Tsegaye F, Alebachew Z, Abebe A, Kebede A and Lemma E. Molecular typing and drug sensitivity testing of Mycobacterium tuberculosis isolated by a community-based survey in Ethiopia. BMC Public Health 2015; 15: 751.

[78] Homolka S, Post E, Oberhauser B, George AG, Westman L, Dafae F, Rusch-Gerdes S and Niemann S. High genetic diversity among Mycobacterium tuberculosis complex strains from Sierra Leone. BMC Microbiol 2008; 8: 103.

[79] Koro Koro F, Kamdem Simo Y, Piam FF, Noeske J, Gutierrez C, Kuaban C and Eyangoh SI. Population dynamics of tuberculous Bacilli in Cameroon as assessed by spoligotyping. J Clin Microbiol 2013; 51: 299-302.

[80] Mulenga C, Shamputa IC, Mwakazanga D, Kapata N, Portaels F and Rigouts L. Diversity of Mycobacterium tuberculosis genotypes circulating in Ndola, Zambia. BMC Infect Dis 2010; 10: 177.

[81] Ouassa T, Borroni E, Loukou GY, Faye-Kette H, Kouakou J, Menan H and Cirillo DM. High prevalence of shared international type 53 among Mycobacterium tuberculosis complex strains in retreated patients from Cote d'Ivoire. PLoS One 2012; 7: e45363.

[82] Stucki D and Gagneux S. Single nucleotide polymorphisms in Mycobacterium tuberculosis and the need for a curated database. Tuberculosis (Edinb) 2013; 93: 30-39.

[83] Uzoewulu GN, Lawson L, Nnanna IS, Rastogi N and Goyal M. Genetic diversity of Mycobacterium tuberculosis complex strains isolated from patients with pulmonary tuberculosis in Anambra State, Nigeria. Int J Mycobacteriol 2016; 5: 74-79.

[84] Viegas SO, Machado A, Groenheit R, Ghebremichael S, Pennhag A, Gudo PS, Cuna Z, Miotto P, Hill V, Marrufo T, Cirillo DM, Rastogi N, Kallenius G and Koivula T. Molecular diversity of Mycobacterium tuberculosis isolates from patients with pulmonary tuberculosis in Mozambique. BMC Microbiol 2010; 10: 195.

[85] Nimmo C, Shaw LP, Doyle R, Williams R, Brien K, Burgess C, Breuer J, Balloux F and Pym AS. Whole genome sequencing Mycobacterium tuberculosis directly from sputum identifies more genetic diversity than sequencing from culture. BMC Genomics 2019; 20: 389.

[86] Coll F, McNerney R, Preston MD, Guerra-Assuncao JA, Warry A, Hill-Cawthorne G, Mallard

K, Nair M, Miranda A, Alves A, Perdigao J, Viveiros M, Portugal I, Hasan Z, Hasan R, Glynn JR, Martin N, Pain A and Clark TG. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med 2015; 7: 51.

[87] Guerra-Assuncao JA, Crampin AC, Houben RM, Mzembe T, Mallard K, Coll F, Khan P, Banda L, Chiwaya A, Pereira RP, McNerney R, Fine PE, Parkhill J, Clark TG and Glynn JR. Large-scale whole genome sequencing of M. tuberculosis provides insights into transmission in a high prevalence area. Elife 2015; 4: e05166.

[88] Affolabi D, Anyo G, Faihun F, Sanoussi N, Shamputa IC, Rigouts L, Kestens L, Anagonou S and Portaels F. First molecular epidemiological study of tuberculosis in Benin. Int J Tuberc Lung Dis 2009; 13: 317-322.

[89] Godreuil S, Torrea G, Terru D, Chevenet F, Diagbouga S, Supply P, Van de Perre P, Carriere C and Banuls AL. First molecular epidemiology study of Mycobacterium tuberculosis in Burkina Faso. J Clin Microbiol 2007; 45: 921-927.

[90] Assam Assam JP, Penlap Beng V, Cho-Ngwa F, Toukam M, Ngoh AA, Kitavi M, Nzuki I, Nyonka JN, Tata E, Tedom JC, Skilton RA, Pelle R and Titanji VP. Mycobacterium tuberculosis is the causative agent of tuberculosis in the southern ecological zones of Cameroon, as shown by genetic analysis. BMC Infect Dis 2013; 13: 431.

[91] Belay M, Ameni G, Bjune G, Couvin D, Rastogi N and Abebe F. Strain diversity of Mycobacterium tuberculosis isolates from pulmonary tuberculosis patients in Afar pastoral region of Ethiopia. Biomed Res Int 2014; 2014: 238532.

[92] Debebe T, Admassu A, Mamo G and Ameni G. Molecular characterization of Mycobacterium tuberculosis isolated from pulmonary tuberculosis patients in Felege Hiwot referral hospital, northwest ethiopia. J Microbiol Immunol Infect 2014; 47: 333-338.

[93] Diriba B, Berkessa T, Mamo G, Tedla Y and Ameni G. Spoligotyping of multidrug-resistant Mycobacterium tuberculosis isolates in Ethiopia. Int J Tuberc Lung Dis 2013; 17: 246-250.

[94] Yimer SA, Hailu E, Derese Y, Bjune GA and Holm-Hansen C. Spoligotyping of Mycobacterium tuberculosis isolates among pulmonary tuberculosis patients in Amhara Region, Ethiopia. APMIS 2013; 121: 878-885.

[95] Yimer SA, Norheim G, Namouchi A, Zegeye ED, Kinander W, Tonjum T, Bekele S, Mannsaker T, Bjune G, Aseffa A and Holm-Hansen C. Mycobacterium tuberculosis lineage 7 strains are associated with prolonged patient delay in seeking treatment for pulmonary tuberculosis in Amhara Region, Ethiopia. J Clin Microbiol 2015; 53: 1301-1309.

[96] de Jong BC, Antonio M, Awine T, Ogungbemi K, de Jong YP, Gagneux S, DeRiemer K, Zozio T, Rastogi N, Borgdorff M, Hill PC and Adegbola RA. Use of spoligotyping and large sequence polymorphisms to study the population structure of the Mycobacterium tuberculosis complex in a cohort study of consecutive smear-positive tuberculosis cases in The Gambia. J Clin Microbiol 2009; 47: 994-1001.

[97] Ejo M, Gehre F, Barry MD, Sow O, Bah NM, Camara M, Bah B, Uwizeye C, Nduwamahoro E, Fissette K, De Rijk P, Merle C, Olliaro P, Burgos M, Lienhardt C, Rigouts L and de Jong BC. First insights into circulating Mycobacterium tuberculosis complex lineages and drug resistance in Guinea. Infect Genet Evol 2015; 33: 314-319.

[98] Ferdinand S, Sola C, Chanteau S, Ramarokoto H, Rasolonavalona T, Rasolofo-Razanamparany V and Rastogi N. A study of spoligotyping-defined Mycobacterium tuberculosis clades in relation to the origin of peopling and the demographic history in Madagascar. Infect Genet Evol 2005; 5: 340-348.

[99] Lahlou O, Millet J, Chaoui I, Sabouni R, Filali-Maltouf A, Akrim M, El Mzibri M, Rastogi N and El Aouad R. The genotypic population structure of Mycobacterium tuberculosis complex from Moroccan patients reveals a predominance of Euro-American lineages. PLoS One 2012; 7: e47113.

[100] Cadmus S, Hill V, van Soolingen D and Rastogi N. Spoligotype profile of Mycobacterium tuberculosis complex strains from HIV-positive and -negative patients in Nigeria: a comparative analysis. J Clin Microbiol 2011; 49: 220-226.

[101] Lawson L, Zhang J, Gomgnimbou MK, Abdurrahman ST, Le Moullec S, Mohamed F, Uzoewulu GN, Sogaolu OM, Goh KS, Emenyonu N, Refregier G, Cuevas LE and Sola C. A molecular epidemiological and genetic diversity study of tuberculosis in Ibadan, Nnewi and Abuja, Nigeria. PLoS One 2012; 7: e38409.

[102] Thumamo BP, Asuquo AE, Abia-Bassey LN, Lawson L, Hill V, Zozio T, Emenyonu N, Eko FO and Rastogi N. Molecular epidemiology and genetic diversity of Mycobacterium tuberculosis complex in the Cross River State, Nigeria. Infect Genet Evol 2012; 12: 671-677.

[103] Kamudumuli PS, Beylis N, Blann L and Duse A. Molecular typing of drug-susceptible and -resistant Mycobacterium tuberculosis in Johannesburg, South Africa. Int J Tuberc Lung Dis 2015; 19: 834-840.

[104] Maguga-Phasha NTC, Munyai NS, Mashinya F, Makgatho ME and Mbajiorgu EF. Genetic diversity and distribution of Mycobacterium tuberculosis genotypes in Limpopo, South Africa. BMC Infect Dis 2017; 17: 764.

[105] Marais BJ, Mlambo CK, Rastogi N, Zozio T, Duse AG, Victor TC, Marais E and Warren RM. Epidemic spread of multidrug-resistant tuberculosis in Johannesburg, South Africa. J Clin Microbiol 2013; 51: 1818-1825.

[106] Mlambo CK, Warren RM, Poswa X, Victor TC, Duse AG and Marais E. Genotypic diversity of extensively drug-resistant tuberculosis (XDR-TB) in South Africa. Int J Tuberc Lung Dis 2008; 12: 99-104.

[107] Hoza AS, Mfinanga SG, Moser I and Konig B. Molecular characterization of Mycobacterium tuberculosis isolates from Tanga, Tanzania: first insight of MIRU-VNTR and microarray-based spoligotyping in a high burden country. Tuberculosis (Edinb) 2016; 98: 116-124.

[108] Mbugi EV, Katale BZ, Streicher EM, Keyyu JD, Kendall SL, Dockrell HM, Michel AL, Rweyemamu MM, Warren RM, Matee MI, van Helden PD, Couvin D and Rastogi N. Mapping of Mycobacterium tuberculosis complex genetic diversity profiles in Tanzania and other African countries. PLoS One 2016; 11: e0154571.

[109] Stavrum R, PrayGod G, Range N, Faurholt-Jepsen D, Jeremiah K, Faurholt-Jepsen M, Krarup H, Aabye MG, Changalucha J, Friis H, Andersen AB and Grewal HM. Increased level of acute phase reactants in patients infected with modern Mycobacterium tuberculosis genotypes in Mwanza, Tanzania. BMC Infect Dis 2014; 14: 309.

[110] Asiimwe BB, Koivula T, Kallenius G, Huard RC, Ghebremichael S, Asiimwe J and Joloba ML. Mycobacterium tuberculosis Uganda genotype is the predominant cause of TB in Kampala, Uganda. Int J Tuberc Lung Dis 2008; 12: 386-391.

[111] Bazira J, Asiimwe BB, Joloba ML, Bwanga F and Matee MI. Mycobacterium tuberculosis spoligotypes and drug susceptibility pattern of isolates from tuberculosis patients in South-Western Uganda. BMC Infect Dis 2011; 11: 81.

[112] Lukoye D, Katabazi FA, Musisi K, Kateete DP, Asiimwe BB, Okee M, Joloba ML and Cobelens FG. The T2 Mycobacterium tuberculosis genotype, predominant in Kampala, Uganda, shows negative correlation with antituberculosis drug resistance. Antimicrob Agents Chemother 2014; 58: 3853-3859.

[113] Easterbrook PJ, Gibson A, Murad S, Lamprecht D, Ives N, Ferguson A, Lowe O, Mason P, Ndudzo A, Taziwa A, Makombe R, Mbengeranwa L, Sola C, Rastogi N and Drobniewski F. High rates of clustering of strains causing tuberculosis in Harare, Zimbabwe: a molecular epidemiological study. J Clin Microbiol 2004; 42: 4536-4544.

[114] Jonsson J, Hoffner S, Berggren I, Bruchfeld J, Ghebremichael S, Pennhag A and Groenheit R. Comparison between RFLP and MIRU-VNTR genotyping of Mycobacterium tuberculosis strains isolated in Stockholm 2009 to 2011. PLoS One 2014; 9: e95159.

[115] Shi J, Zheng D, Zhu Y, Ma X, Wang S, Li H and Xing J. Role of MIRU-VNTR and spoligotyping in assessing the genetic diversity of Mycobacterium tuberculosis in Henan Province, China. BMC Infect Dis 2018; 18: 447.

[116] Sanoussi CN. Mycobacterium tuberculosis complex strain diversity may impact disease presentation, diagnosis and outcome. Antwerp: faculty of pharmaceutical, Biomedical and Veterinary Sciences 2019.

Supplementary Table 1. Overview of studies on molecular epidemiology of *Mycobacterium tuberculosis complex* in Africa

Authors	Country	Study period	Age (years)	sample Size	Type of patients	No of isolates	No of Strains	Clustering Rate (%)		HIV status n (%)
								%	n	
Affolabi D et al., 2012	Benin	Jul 2005-Oct 2006		201	New pulmonary TB patients	201	126	64.9		
Affolabi D et al., 2017	Benin	Jan-Dec 2014		100	Previously treated pulmonary TB patients	100	100	69		15.2
Alame-Eman AK et al., 2017	Gabon	NA		159	New pulmonary TB and previously treated patients	159	159	75.5		
Asiimwe et al., 2008	Uganda	Feb-Nov 2006	≥18	386	New Pulmonary TB and suspected patients	344	344		5	NA
Asiimwe et al., 2008	Uganda	Feb-Nov 2006	≥18	386	New Pulmonary TB, and suspected patients	344	344		33	92 (26.7)
Assam et al., 2013	Cameroon	Feb-July 2009	15-75	964	Suspected Pulmonary TB patients	169	169	83		NA
Bazira et al., 2010	Uganda	May 2007-Apr 2008	≥18	167	New pulmonary TB and previously treated patients	125	62		16	
Bazira et al., 2011	Uganda	May 2007-Apr 2009	≥19	167	New pulmonary TB and previously treated patients	125	62		16	28 (50.9)
Bedewi et al., 2017	Ethiopia	Oct 2012-Sep 2013	≥18	338	Suspected Pulmonary TB patients	281	281	79.3		NA
Belay M et al., 2014	Ethiopia	Sept 2009-Mar 2010	≥18	325	Suspected Pulmonary TB patients	105	105	76.1		95 (29.23)
Cadmus et al., 2011	Nigeria	Apr 2004-Oct 2005	NA	176 (1033)	Suspected Pulmonary TB patients	163	163		32	49 (30.6)
De Jong et al., 2009	Gambia	NA	≥15	386	Suspected Pulmonary TB patients	376	359	12.8		(8.40)
Debebe T et al., 2014	Ethiopia	Oct 2010-Jun 2011	15-80	123	Suspected Pulmonary TB patients	118	118		17	12 (10.2)
Deribew et al., 2012	Ethiopia	Feb-Mar 2009	≥15	482	Suspected Pulmonary TB patients	17			11	5 (5.5)
Diab HM et al., 2016	Egypt	2012-2014	NA	230	Pulmonary TB and previously treated patients	230	230		13	NA
Diriba B et al., 2013	Ethiopia	Sep 2009-Feb 2012	NA	183	Suspected MDR-TB patients	183	183	85.7		NA
Easterbrook et al., 2004	Zimbabwe	May-Oct 1997	27 to 40	516	Suspected Pulmonary TB patients	224	224	78.6		371 (74)
Ejo M et al., 2015	Guinea	2005-2010	NA	359	New pulmonary TB patients	359	184		28	NA
Eldholm et al., 2006	Tanzania	Oct-Nov 2005	NA	147	Suspected Pulmonary TB patients	147	147	52		NA
Ferdinand S et al., 2005	Madagascar	1994 and 2000	NA	333	New pulmonary TB and previously treated patients	301			60	NA
Gafirita J et al., 2012	Rwanda	Mar-Sep 2009	≥18	153	New recruitment pulmonary TB and previously treated	151	151	76.2		69 (45.7)
Gandhi J et al., 2013	South Africa	Jan 2005-Dec 2006	NA (34)	148	XDR-TB patients	243	86	96		126 (85.13)
Gehre et al., 2013	Gambia	Jun 2002-Dec 2009	≥15	1003	Suspected Pulmonary TB patients	884	884		70	NA
Getahun et al., 2015	Ethiopia	2010 and 2011	≥15	96	Suspected Pulmonary TB patients	92	91	70		NA
Homolka S et al., 2008	Sierra Leone	2003-2004	NA	103	Previously treated patients	97	97	13		NA
Hoza AS et al., 2016	Tanzania	Nov 2012-Jan 2013	Mean (33)	372	New pulmonary TB and previously treated patients	80	80	21.3		NA
Godreuil S et al., 2007	Burkina Faso	Jan-Dec 2001	15-75	120	Suspected Pulmonary TB patients	120	61	49.3		43 (35.8)
Godreuil et al., 2010	Djibouti	2 Month period in 2004	NA	62	Pulmonary TB patients	62	62		4	NA
Glynn JR et al., 2008	Malawi	1995-2003	25-45	1.248	Pulmonary TB patients	1194		37		(47)
Goyal M et al., 1999	Ghana	not indicated	average 37.1	175	Pulmonary TB patients	159	159	47.2		
Groenheit R et al., 2011	Guinea-Bissau	1989-2008	NA	414	Pulmonary TB patients	414	414	81.2		
Kamudumuli PS et al., 2015	South Africa	Jan 2009 and Dec 2010	all ages	500	New pulmonary TB and previously treated patients	500	500	68		
Mathema et al., 2015	South Africa	Jun 2006 Feb 2010	median 45	5.513	Pulmonary TB patients	1240	1240	45.2		(43.6)
Koro Koro et al., 2013	Cameroon	Feb 2004 to Mar 2005	>15	565	Pulmonary TB patients	565	565		186	
Lahlou O et al., 2012	Morocco	2004-2006	12 to 80	592	Pulmonary TB patients	592	592	88.3		NA
Lawson L et al., 2012	Nigeria	Aug 2009 to Jul 2010	>18	520	Pulmonary TB patients	423	423		36	
Maguga-Phasha et al., 2017	South Africa	Nov 2012 and Nov 2013	1 to 65	487	Pulmonary TB patients	215	215	13		

Marais BJ et al., 2013	South Africa	Mar 2004-Dec 2007	15-60	434	Pulmonary TB patients	434	56	87.1		NA
Marais E et al., 2014	South Africa	2004 and 2007	NA	351	Pulmonary TB patients	351	351	88.3		(62.7)
Mbugi EV et al., 2016	Tanzania	2016	35.1	293	Pulmonary TB patients	293	293	47.3		21 (9.81)
Meyer CG et al., 2008	Ghana	Sep 2001 to Jul 2004	6 and 60	2335	Pulmonary TB patients	1906	1906	63		(15)
Mlambo CK et al., 2008	South Africa	Jun 2005 and Dec 2006	NA	699	Pulmonary TB patients	845	845	37		NA
Mulenga C et al., 2010	Zambia	January and July 2006	14-79	499	Pulmonary TB patients	273	98	74.2		NA
Niemann S et al., 2002	Uganda	1995-1997	NA	234	Pulmonary TB patients	234	234	82		
Ouassa T et al., 2012	Côte d'Ivoire	Dec 2008 to Dec 2009	NA	194	Pulmonary TB patients	194	194	88.1		NA
Ratovonirina NH et al., 2017	Madagascar	Aug 2013 to May 2014	No age limitation	523	Pulmonary TB patients	467	394		41	NA
Said et al., 2012	South Africa	Jun 2007 to Jan 2008	6-69	336	Pulmonary TB patients	336	336	86.3		NA
Saifodine A et al., 2014	Mozambique	Nov-09	18-62	116	Pulmonary TB patients	67	67		7	(74.5)
Stavrum R et al., 2009	South Africa	2001 and 2002	15 to >59	5866	Pulmonary TB patients	252	252		28	(44.4)
Stavrum R et al., 2014	Tanzania	Apr 2006-Nov 2008	>15	842	Pulmonary TB patients	252	248		3	(38.3)
Tessema et al., 2013	Ethiopia	Mar 2009 and Jul 2009	31.6 mean	260	Pulmonary TB patients	244	244	45.1		62 (25.40)
Thumamo BP et al., 2012	Nigeria	Jun 2008 to May 2009	<15 to >64	137	Pulmonary TB patients	97	81	79		NA
Tilahun M et al., 2018	Ethiopia	May 2014 and Mar 2015	18-67	105	Pulmonary TB patients	86	86	76.7		(16)
Uzoewulu GN et al., 2016	Nigeria	2009-2011	10 to 82	550	Pulmonary TB patients	180	180	70.5		(19)
Viegas et al., 2010	Mozambique	2007-2008	15-82	445	Pulmonary TB patients	445	445	78.2		(22)
Yeboah-Manu D et al., 2011	Ghana	Oct 2007 to Mar 2009	2 to 90	232	Pulmonary TB patients	162	162	81.4		NA
Yimer et al., 2013	Ethiopia	2008 and 2010	15 to 75	375	Pulmonary TB patients	240	237	40		NA
Yimer et al., 2015	Ethiopia	2008 and 2010	average 30	240	Pulmonary TB patients	231	231	32		(22.10)
Lukoye D et al., 2014	Uganda	Aug to Dec 2008	>18	536	Pulmonary TB patients	533	497	10		(33.1)