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Molecular Characterization of *Mycobacterium bovis* and its Significance: Role for Control of Zoonotic Tuberculosis in Africa

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Abstract

Mycobacterium bovis is the main causal agent of bovine tuberculosis (BTB) that causes zoonotic tuberculosis (TB) in humans, even though *M. caprae*; contribute to a lesser extent, which is mostly acquired from domestic animals and their products, in which cattle are the major reservoir. This review paper discussed the importance of zoonotic tuberculosis in Africa and highlighted the uses of molecular methods in diagnosis, control and prevention of zoonotic tuberculosis. Despite the fact, there are various diagnostic methods available nowadays; the molecular techniques are proving more rapid and plausible sensitivity and specificity results that increase the diagnosis precision. In addition, molecular diagnostic methods used to indicate the epidemiological status of different strain distribution with regard to cluster distribution and reactivation of dormant or treated cases of tuberculosis indicating the drug resistance situation of the strain. Hence, applying molecular techniques for diagnosis of zoonotic tuberculosis in developing countries will help in decreasing the incidence, prevalence, mortality, morbidity and loss of economic due to carcass condemnation, milk drop and livestock trade banning in the continent. Advanced molecular methods truly play a key role in prevention and control program of tuberculosis in general and bovine tuberculosis in specific, which leads to the cooperation of various disciplines. Introducing cost effective and easy molecular diagnostic methods is recommended, implementing molecular diagnostic methods in research areas and intensive training of personnel in the use of equipment.

Keywords: Zoonotic tuberculosis; Molecular techniques; Africa; *M. bovis*

Abbreviations: AFS: Acid Fast Stain; AIDS: Acquired Immune Deficiency Virus; BCG: Bacillus Calmette–Guérin; BTB: Bovine Tuberculosis; CBPP: Contagious Bovine Pleuropnemonia; DNA: Deoxyribonucleic Acid; DRE: Double Repetitive Element; DST: Drug Susceptibility Test; ETR: Exact Tandem Repeats; FAFLP: Fluorescent Amplified Fragmented Length Polymorphism; Flip: Fast Ligation Mediates; GC: Gas Chromatography; HIV: Human Immune Deficiency Virus; LAM: Lipoarabinomannan; LF: Lateral Flow; LM: Ligation Mediated; LPA: Line Probe Assay; MIRU: Mycobacterial Interspersed Repetitive Unit; MTC: Mycobacterium tuberculosis Complex; OIE: World Organization for Animal Health (Office International des Epizooties); PCR: Polymerase Chain Reaction; PFGE: Pulsed-Field Gel Electrophoresis; PGRS: Polymorphic GC-Rich Sequences; PLHIV: People Living With HIV; QUB: Queen's University Belfast; RFLP: Restriction Fragment Length Polymorphism; RIF: Rifampin; SNP: Single Nucleotide Polymorphism; TB: Tuberculosis; VNTR: Variable Number Tandem Repeat; WHO: World Health Organization

Introduction

The worldwide in 2015, there were an estimated 10.4 million new TB cases worldwide. Six countries accounted for 60% of new cases: India, Indonesia, China, Nigeria, Pakistan, and South Africa. "Global progress depends on major advances in TB prevention and care in these countries.

From 2014 to 2015, the rate of decline in TB incidence worldwide remained at only 1.5%. This needs to accelerate to a 4% to 5% annual decline by 2020 to reach the first milestones of the End TB Strategy, the WHO notes.

Globally, an estimated 1.8 million people died of TB in 2015, at least 3 people per minute, of whom 0.4 million were co-infected with HIV. Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained 1 of the top 10 causes of death worldwide in 2015, responsible for more deaths than HIV and malaria, the WHO says.

Mycobacterium bovis is the main causal agent of bovine tuberculosis (BTB) that causes zoonotic tuberculosis (TB) in humans, even though *M. caprae*; contribute to a lesser extent, which is mostly acquired from domestic animals and their products, in which cattle are the major reservoir [1]. The disease also causes serious threat in endangering wildlife species. Animal TB is widely distributed in many developing countries. The disease results in huge economic loss, particularly the problem is intense in urban and per-urban cross breed dairy cattle due to low productivity, mortality and carcass condemnation as well as trade restrictions of live animals, products and by products of animals [2]. Based on WHO (2016) report, in 2015, there were an estimated 149 000 cases of zoonotic TB.

People with the habit of consuming raw/unpasteurized milk or untreated products of infected animals in general are risky. Rural communities where bovine TB is endemic and cattle herders, dairy workers and other workers that come in close contact with infected animals or their products in particular are at a higher risk of chance of contracting zoonotic TB [1,3].

Despite the fact zoonotic TB is controlled and even eliminated in most western hemisphere of the world, it is a major public health concern in developing parts of the world. It is considered as a "neglected zoonotic diseases" [1-9], where it, remains as a fundamental problem. Despite the wide distribution, it is uncommon to get control measures programs in animals or pasteurization of milk in most areas of developing countries, especially in pastorals community. It can be said that the bovine TB control program in developing country is null [2]. Studies indicates for example in most African countries, bovine TB is prevalent, but effective disease control including regular milk pasteurization and slaughterhouse meat inspection, is largely absent.

Regarding the impact until now, there was no holistic assessment of the global consequences of zoonotic TB has yet been done. The partial reason for this may have been caused by the difficulty of differentiating TB caused by *M. tuberculosis* or *M. bovis*, which requires mycobacterial culture and the subsequent use of biochemical or molecular (e.g., genotyping) diagnostic methods. Hence, in low-income countries (in most of the African countries), facilities to identify the causative agent of TB are extremely limited. For effective tuberculosis control and prevention, implementing of diagnostic methods reliable in terms of time, cost, place, sensitivity, specificity, discriminatory power and reproducibility is essential. Hence, in this review it has been tried to cover the role of molecular diagnostic methods in African countries with the following important objectives:

- Overviewing the importance of zoonotic tuberculosis in Africa
- Overview of the *Mycobacterium bovis* in African countries
- Highlighting the uses of molecular methods in diagnosis, control and prevention of zoonotic tuberculosis

Bovine tuberculosis in African countries

Regarding zoonotic *Mycobacterium bovis* induced TB in humans, about 46 African, 22 Eastern Mediterranean and 27 Western Pacific countries reported to World Health Organization regions of its presence [3]. This disease is a serious problem for livestock production in sub-Saharan Africa and it is a health risk for humans as most human populations live in close contact with domestic animals in which the disease is highly prevalent and imperfectly controlled. Bovine tuberculosis has a harmful economic burden, although this has not been quantified in Africa yet [10,11].

At present, the general scale/level of zoonotic diseases is unknown because of, inadequate diagnostic tests for the causative agent in particular and lack of routine surveillance measurements in general together with the insufficient presence of skilled personals in the area. The unknown rate of this is high in developing regions in which bovine TB is endemic and sociocultural practices increase the risk of transmission of agent transmission to humans. The situation may be fundamentally different in other regions of developed countries compared to developing countries.

The TB transmission situation in developing countries is exacerbated by the presence of multiple additional risk factors such as human behavior and the high prevalence of HIV infections. The behavior of human in consuming animal products and by products, the common sharing of animal and human accommodations and the close interaction of Wild-domestic-human beings makes the difference in the transmission of BTB in developing regions of the world. Another important risk factor is the HIV/AIDS which found widely distributed, is thought to facilitate transmission and progression to active disease of any form of TB. Some studies showed a significantly increased proportion of *M. bovis* infections among HIV–co-infected TB patients compared with HIV-negative TB patients [12-14] Center for Disease Control, 2005. Despite the transmission can be through milk is common, the transmission could be eliminated by the pasteurisation of milk [15,16].

Regarding the incidence of pulmonary tuberculosis caused by *M. bovis*, is higher in farm and slaughterhouse workers than in urban inhabitants based on OIE report.

The bovine tuberculosis besides in domestic animal, it is also frequently reported in many species of wildlife. In wildlife, it was first reported in 1929 in greater kudu (*Tragelaphus strepsiceros*) and common duiker (*Sylvicapra grimmi*) in South Africa and by the 1940s, the disease was found to be endemic in greater kudu. In 1982 a prevalence of 10% in African Buffalo in Uganda, and 9% in warthog (*Phacochoerus aethiopicus*) was found, and in Zambia. The disease has also been reported in Kafue lechwe (*Kobus leche kafuensis*) and in a single eland (*Traurotragus oryx*) in the same year mentioned above. A tuberculosis outbreak occurrence was also reported in Kenya, in wild olive baboons (*Papio cynocephalus anubis*). Infection of *Mycobacterium bovis* has also been diagnosed in African buffalo in the Kruger National Park in South Africa, and more recently spill over to other species such as chacma baboon (Papio ursinus), lion (*Panthera leo*) and cheetah (*Acynonyx jubatus*) as well as greater kudu has occurred. As the report goes back to 20 years go back as mentioned by Bengis et al., due to lack of proper screening, diagnosing and controlling system of the disease in developing world like African countries, the disease remained widely distributed in the wildlife. Due to these, several wild living species in Africa has shown to be capable of maintaining *M. bovis* infection [16].

Zoonotic TB in Africa

In African countries where many diseases are found widely, several chronic diseases do have similar clinical forms with zoonotic tuberculosis. Some of the similar diseases with zoonotic tuberculosis in these regions of the world include the African trypanosomiasis, chronic contagious bovine pleuro-pneumonia (CBPP) or chronic multiparasitism, that shows signs of emaciation, loss of appetite, chronic cough and other signs of pneumonia challenging to differentiate from bovine tuberculosis. [17-19].

As the impact of zoonotic TB increases, it is initiating further attention and is included in the Global Plan TB stop program of 2016 [20]. Nevertheless, the issue goes beyond the need to reach key affected populations, efforts to prevent and control zoonotic TB must be cross-sectorial and multidisciplinary, including both human health and veterinary sectors in controlling the disease in its animal reservoir, developing diagnostic tools for diagnosing *M. bovis*, strengthening surveillance systems and data quality, and assessing economic impact [20]. Various studies in Africa showed that a median of 2.8% of all TB cases in humans were caused by *M. bovis*. Specifically median proportion of TB cases for Ethiopia, Nigeria and Tanzania, respectively, caused by *M. bovis* estimated were 17.0% (range: 16.7%–31.4%), 15.4% and 26.1% (range 10.8%–37.7%) [21-24]. Percentages of ≈30% were reported in 4 regionally based studies in Tanzania and Ethiopia.

Epidemiology of bovine TB (BTB)

The main hosts of *M. bovis* are bovids particularly the cattle despite it can infect most mammalian species. The disease is characterized in having chronic but progressive formation of typical granulomatous

lesions with varying degrees of necrosis, calcification and encapsulation. Transmission between animals is thought to occur mainly by inhalation of contaminated aerosol and therefore, affects the lungs primarily [25]. However, the infection can also occur via the gastro-intestinal tract or become systemic and affect other organs, such as the urinary tract or the mammary lymph nodes when animals ingest contaminated food, water or milk [26,27]. A Study in Ethiopia reflected that gastro-intestinal form of tuberculosis is more common in free grazing animals compared to animals kept indoor, that represents most of the African husbandry system [28]. The number of severe cases of animals with clinical manifestation may be limited or absent in countries where active control measures are applied. Advanced disease and generalizations are usually more common in countries with insufficient or no control, adding to an increased risk for transmission to humans. Regarding cattle-animal transmission, the common and important routes of zoonotic tuberculosis is through consumption of contaminated milk [29,30].

Besides domestic cattle and human cases, at present, *M. bovis* infection have been reported in more than 40 free-ranging wild animal species. Despite significant variations in size, appearance and distribution of the TB lesions in different species, in the majority of affected wildlife species lesions closely resemble those in cattle [31,32]. A consistently different pattern of pathological changes however, has been described in lions where no histological evidence of necrosis was found. TB in wildlife can pose serious difficulties for BTB control and eradication, because they act as a reservoir. Particularly noteworthy is the case of the British Isles, where the European badger represents an important and well documented disease reservoir. Regarding *M. bovis* distribution across the rural and urban areas, it is more common in rural areas because of the presences of cattle, other wild animals together with the habit of raw milk consumption pattern in these areas is higher than urban areas [33,34].

Mycobacterium bovis prevalence studies

According to Griffith (1932), the prevalence studies and reports of tuberculosis in the developing countries looks quite similar to that found in the 1930's in most of the industrialized countries. Virtually speaking the disease present in all African country, however, very little accurate information on its distribution and prevalence is available. Evaluations of ante-mortem tests for the diagnosis of BTB in Africa are scarce currently, but a prerequisite to identify appropriate tools for future disease controlling program. The pre-disposing factors for human to acquire the disease in Africa mainly include consumption of raw milk and other dairy products. In some parts of Africa, there is also a tradition of sharing of human shelter with animals, which also play in disease transmission and dissemination [35,36].

TB is an old disease distributed worldwide confirmed through the molecular analysis study carried out on mummies from Egypt and South America and its presence in different periods [37,38]. Since 1985, many molecular typing methods developed for characterization of *Mycobacterium tuberculosis* complex. These techniques contributed for detection of epidemiological links among samples, and contribute for better understanding of BTB transmission dynamics between animal-human-wildlife [39].

There are two groups of molecular typing methods for *M. tuberculosis* complex invented so far. The first group is genomic methods for DNA includes, RFLP IS6110 [40-42], polymorphic GC-rich sequence (PGRS) analysis and pulsed-field gel electrophoresis (PFGE). The second method applies DNA-specific sequence amplification methods using polymerase chain reaction (PCR) [43,44]. The second method include, spoligotyping (spacer oligonucleotide typing) [22,45,46], ligation-mediated PCR (LM-PCR), double repetitive element PCR (DRE-PCR) [47,48]. Fast ligation mediated PCR (FliP), fluorescent amplified-fragment length polymorphism (FAFLP) [49-51], mycobacterial interspersed repetitive unit (MIRU) [52] analysis, amplification and sequencing of single nucleotide polymorphism (SNP), amplification of exact tandem repeats (ETR) and Queen's University Belfast (QUB) polymorphism of variable number tandem repeat are also included in the second group.

The genomic structure of *M. tuberculosis* complex is markedly homogeneous. The species of *M. turberculosis*, *M. africanum*, *M. bovis*, *M. bovis* (BCG), *M. caprae*, *M. pinnipedii*, *M. microti* and *M. canettii* are genetically related [53]. This fact gave way to the development of methods capable of differentiating among this genetically related species isolates. Various techniques were used for the differential identification of *M. tuberculosis* complex [54], being the latest one the standard method used until the late 1980's that presented however considerable disadvantages such as the low amount of identified phagotypes and its laboriousness.

Today, the development of new molecular methods for M. tuberculosis complex genetic characterization has greatly contributed to the understanding of the transmission dynamics and pathogenesis of the TB [55,56]. In addition, it increased the epidemiological knowledge of M. bovis population and structure, evolutionary steps which is advantageous for the study of TB outbreaks and for understanding the dynamics of the disease in the area [57-61]. This will help in the control of TB strains distribution and treatment regimen by indicating the active transmission and reactivation of a Mycobacterium strain.

Uses of molecular diagnostic methods

Since early period, several methods have been developed to diagnosis TB by various researchers and scientists. Some of the methods include acid-fast stain (AFS), Chest X-ray radiography, the Mantoux test (intradermal Tuberculin test) that replaced Pirquet's test, conventional fluorescence microscopy (auramine-rhodamine staining) (more advanced specialized), …etc.

The limitation for microscopy diagnosis is that it requires a high bacterial count in the specimen for a reliable result. While the culturing is a gold standard for TB diagnosis, however, it can take between six and eight weeks as TB bacilli and many other mycobacteria grow very slowly. Therefore, early and specific treatment of the patient is mostly delayed with standard biochemical and growth-based differentiation tests. This contributes for the spread of the pathogen within the population.

Recently, the molecular biology type of diagnosis method recently is becoming more important in the diagnosis of mycobacterial agents. This method assists culture either by serving as a rapid direct test on specimens or by enabling a rapid and unambiguous species differentiation from culture material, in which, this nucleic-acid-based methods have now largely displaced the classical diagnostic methods. This molecular genetic tests offer considerable time advantages in the identification of mycobacteria, enabling a more rapid initiation of resistance tests and specific treatment. They have high specificity and sensitivity, despite of the above truth, the standard methods should not be excluded or disregarded, rather should support them. The test

results always have to be confirmed with the help of the standard system/method.

Molecular characterization using the DNA fingerprinting currently supports routine contact tracing in many countries as well as studies on person-to-person transmission, early disease outbreak detection and identification, high transmission risk groups, laboratory cross-contamination [62,63], and the distinction between reinfection and reactivation [64,65]. In particular, DNA fingerprinting of *M. tuberculosis* has greatly improved the understanding of TB transmission. Moreover, the recognition of genotype families has facilitated studies on the population structure of the *M. tuberculosis* complex and its transmission dynamics and early warning system for the national health services of a particular area [66,67]. However, WHO recommends the microscopy (Conventional light microscopy and Light-emitting diode fluorescent microscopy), the culturing method (Culture on solid media, commercial liquid culture systems and rapid speciation, Drug-susceptibility testing for TB (DST first-line anti-TB agents, DST for second-line anti-TB agents and Non-commercial methods) and LF-LAM Urine test for PLHIV (http://www.unicef.org/supply/files/TB_DX.pdf).

Various DNA fingerprinting methods are currently available that serve different purposes and have variable characteristics for specific applications. From the many listed methods, the three most important and widely applied methods are spoligotyping, variable number tandem repeat [VNTR] typing, IS6110 restriction fragment length polymorphism [RFLP] typing) and their application. The commonly WHO recommended molecular methods are LPA and Xpert MTB/RIF assay (http://www.unicef.org/supply/files/TB_DX.pdf).

Indicate epidemiological status of a strain

In principle, molecular characterization /genotyping methods are based on the analysis of chromosomal DNA of *Mycobacterium tuberculosis* complex (MTC) isolates. Progressively, large numbers of different molecular methods have been developed to measure the genetic relationship between different MTC strains. In developing countries, despite the limitation of the availability in the use of molecular diagnostic technique, few studies conducted and published in various peer-reviewed journals worldwide (PubMed, Blackwell-synergy, ScienceDirect and EBSCO host electronic data search result) and showed the TB strains distribution. The techniques also can be helpful in offering general insights into the population structures of bacterial pathogens in certain geographical locations. Each molecular method provides specific genetic profiles referred to as fingerprints. When two or more strains have identical fingerprints they are referred to as, the same cluster and may be epidemiologically linked through various factors. Identification of M. bovis strains at molecular level gives insights about the sources of infection and identification helping the spread and maintenance of TB.

In African countries, few studies tried to isolate *M. bovis* strains molecularly. Biffa et al. [66] isolated 12 spoligotypes from 34 distinct strains; with SB1176 as a dominant spoligotype (41.2% of the isolates) followed by SB0133 (14.7%) from slaughtered animals in Ethiopia. Berg et al. tried to show for instance from Ethiopia about 120 isolates, in Tanzania 14 isolates, Burundi 10 isolates, and Uganda 9 isolates of *M. bovis* tried to identified. The same study by Berg et al. [7,67-69] identified a clonal complex of *Mycobacterium bovis* isolated at high frequency from cattle in Uganda, Burundi, Tanzania, and Ethiopia. Other studies showed *M. bovis* isolates in Ethiopia were also reported [70-74]. *M. bovis* and *M. tuberculosis* molecularly characterized and summarized by Brudey et al. [74,75].

In Zambia, *M. bovis* strains were isolated by [76,78]. More recently, different genotypes of strains of *M. bovis* isolated from cattle and Lechwe from the Kafue basin have been described as the M. bovis strains transfer between cattle and Lechwe, with the latter having developed into a sylvatic reservoir host.

In South Africa Hlokwe et al. [78] isolated *M. bovis* strains. In Tanzania Katale et al. [79]. *M. bovis* was isolated from human sputum, wildlife and domestic animals. Similarly Mwakapuja et al. [80] isolated *M. bovis* from cattle and wildlife.

In Madagascar [81-84] isolated *M. bovis* from isolated from cattle in different regions of Madagascar revealed a homogenous population structure and no geographical localization of strain types within Madagascar and also showed also a zoonotic importance of the isolates (Table 1).

Country	Hosts where the sample was taken	Reporter
Algeria	Cattle	(Sahraoui et al. [34])
Burkina Faso	Cattle and Human	Sanou et al.
Burundi	Cattle and human	(Rigouts et al. [76])
Cameroon	Cattle	Njanpop-Lafourcade et al. [120]; Awah-Ndukum et al. [100]
Ethiopia	Cattle, camel	(Ashenafi et al. [69]; Mamo et al. [70]; Ameni, et al. [28]; Ameni et al. [72]; Berg et al. [69]; Biffa et al. [66]; Tsegaye et al. [71])
Egypt	Cattle	Ramadan et al. [126], Mossad et al [117], Moussa et al. [118]
Kenya	Cattle	Gathogo et al. [109]
Madagascar	Cattle, cattle and human	(Rasolofo et al. [81]; Rasolofo et al. [82])
Mali	Cattle	Muller et al. [119]
Nigeria	Human and cattle	Cadmus et al. [32]

South Africa	Human, livestock, wildlife	Hlokwe et al. [78], Duarte et al. [105], Michel et al. [116]
Tanzania	Human, cattle and wildlife	Katale et al. [79], Mwakapuja et al. [80], Kazwala et al. [25]
Tunisia	Human	(Kahla et al. [111]
Uganda	Human	(Oloya et al. [122]; Oloya et al. [123]; Oloya et al. [124]
Zambia	Cattle, human, Lechwe	Malama et al. [74], Malama et al. [75], Munyeme et al. [76]

Table 1: Some molecular studies conducted in few of African countries on *M. bovis* of different sample sources.

Transmission status of *Mycobacterium bovis*

Molecular characterizations help to determine the source of infection and outbreaks, understanding the relationship between different outbreaks, and identify wild animal reservoir of *M. bovis.* The technique can provide insight into the risk factors for BTB transmission by allowing identification of the dynamics of hosts of this disease [85-87]. This method contributes in revealing the source of infection by identifying to the strain level and help in matching the strains isolated from various hosts in different areas and hosts. It is possible to detect through this method the genetic profiles of mycobacterium. Hence, comparing the genetic profiles between animal and human isolates of *M. bovis* enables to detect the source(s) of infection and the route(s) of transmission, and to predict the future picture of disease progression. The use of this technique is spreading in most areas of research, academic centers and TB specialized hospitals [88,89]. Therefore, using the molecular biology application, African countries where livestock and wildlife are living in close association with human, will help in detecting the source of infection as well as transmission dynamicity.

There has been an effort done in isolating *M. bovis* strain indicating the recent transmission or reactivation of tuberculosis by looking the homogeneity or heterogeneity of strains. Molecular method used as an epidemiological tools in identifying transmission chains between different hosts (transmission dynamicity), risk factors for TB infections, detect new outbreaks, verifying suspected false-positive cultures that may be due to laboratory contaminations and nosocomial transmission and detection of laboratory cross-contaminations [90,91]. Many studies in Africa showed the transmission dynamicity of *M. bovis* in human, cattle and wildlife (Table 2).

Country	Sample source	Sample size	Number of strain identified	Reported
Zambia	Cattle and human	150 (human sputum) and 288 (bovine tissues)	One *M. bovis* strain circulating in both human and cattle	Malama et al. [75]
Burkina Faso	Cattle and human	101(cattle carcasses) and 576 (Human sputum)	9 *M. bovis* isolates (2 from cattle and 7 from human)	Sanou et al.
Ethiopia	Cattle and human	260 (sputum), 32 cases of FNA samples from human and 207 cattle lesions	Three as *M. bovis*(from human) and 24 from cattle	(Gumi et al. [110])
	Human	40 FNA (human)	6 were positive for *M. bovis*	(Kidane et al. [112])
	Human	153 FNA (human)	1 *M. bovis* from human	(Seyoum et al. [128])
	Human	108 Sputum and FNA	7 *M. bovis*	Elias et al. [106])
	Human	299 Sputum and FNA	8 *M. bovis*	(Fetene et al. [108]; Beyene et al. [68])
Mozambique	Human	6 tuberculosis lymph adenitis isolates	1 *M. bovis*	Viegas et al. [130]
Uganda	human	98 isolates from human	1 *M. bovis*	(William [131]

Table 2: Molecularly identified *M. bovis* in human beings and cattle in some African countries.

Cluster transmission of tuberculosis strains in different geographical locations

Clusters are defined as groups of patients having isolates with fully identical RFLP patterns or, if strains have fewer than five IS6110 copies, with identical sub-typing determined by the Polymorphic GC-Rich Sequence probe. Clustering suggests a shared source of active transmission infection in the locality of the study. Here, if there are high cluster of TB strains found in various geographical locations, it indicates there is a high rate of active transmission. Due to the ability of molecular characterization at fingerprint level, it is easy to isolate the active strain in the area easily. The technique also will help to identify the reinfection/reactivations. In both cases, new infection and reactivation, molecular typing method is very crucial. This is because; the possibility of detecting the agent early is high and contributes for better control systems of tuberculosis [92].

Increases precisions

Ideally, all molecular detecting tools expected to be low-cost in price, highly discriminative power of disease causing agents, deliver quick results for the diagnosis, be straightforward to perform(not complicated), and produce easily and interpretable results that allow for accurate comparison between laboratories. In fact, bacterial discrimination between strains can be best done through whole genome sequencing for each strain, but, whole genome sequencing currently is too costly and time consuming hence, only parts of the genome are being examined. Considering the above fact, the conventional methods of TB diagnosis has been widely supported by the molecular techniques during the epidemiological investigations. Diagnostic techniques, using molecular biology methods are recently become more demanded by professionals and patients. Because, people need speedy response with high accuracy level diagnosis since the positive impact of test results for infection control decisions towards patient isolation and other therapeutic managements [93]. An accurate TB molecular diagnostic method can be obtained in fewer hours, with an increased sensitivity & specificity [94,95]. Consequently, results will be obtained very shortly than classical methods that favor a timely treatment of the disease that possibly decrease multi-drug resistance development. It will also promote the treatment of right patient with right therapies and tools [96]. Hence, the methods increase the diagnosis precision within short time.

Contributions for Control zoonotic tuberculosis

Molecular characterization became an important application tool for the control of bovine tuberculosis by “tracking” the epidemiology of the geographical location of agent and host type after the first *M. bovis* molecular characterization attempted by Collins and de Lisle [97]. With the help of this molecular method, it is easy to understand the source and mode of transmission of the bacilli, which help for more effective control measures to be implemented at the source. All these lead to fill the gap of controlling zoonotic tuberculosis in particular in developing countries where the zoonotic tuberculosis found widely distribute in various hosts. The methods will help also in speeding up the diagnosis and accessing patients for treatment regimen immediately before it reach complicated stages. Besides, the techniques help in indicating tracking the agent source from various hosts. It also indicates the transmission route between human, livestock and wildlife, in particular associated with animal movement and raw animal product consumption is commonly practiced [98]. In most bacteriological studies, genotyping of isolates via PCR is becoming a standard tool for more epidemiological diseases control and eradications. Besides, showing the epidemiological routes and sources of infection, the technique have proven to be useful in relating outbreaks of TB, which is an important characteristic for typing a strain diversity and ability of stability towards the control scheme.

Remarks for future works

The WHO recommends the need for strengthening the surveillance of zoonotic TB for better quantifying the burden of disease. In fact, there is one of the major barriers for diagnosis, as most commonly used laboratory procedures do not differentiate the *M. tuberculosis* complex into the species of *M. bovis* and *M. tuberculosis*, promote using of molecular systems. The other important challenge is that the zoonotic TB has treatment challenge, meaning; the disease occurs more often in extrapulmonary sites and is inherently resistant to pyrazinamide, one of the drugs in the standard first-line anti-TB treatment regimen. Hence, the main challenges in diagnosis and treatment of TB should also endorse the zoonotic tuberculosis through the holistic approach that links the human and animal health sectors to reduce the risk of TB transmission at the human–animal interface. Reports, findings and research works indicated greater global investment required, as currently mentioned by WHO (2016) report, the research for new diagnostics, drugs, and vaccines, TB research and development remain "severely underfunded,".

Using cost effective, reliable and less man power and easy molecular diagnostic methods are encourage to be available in many African countries, where huge livestock and wildlife are living close to human beings, that expose people for bovine tuberculosis. As many researches revealed the potential transmission of bovine tuberculosis to human beings, an early detection of active case and treatment of persons who have active TB disease, and the investigation of their contacts, is a priority in controlling the spread of the disease [99]. The upgrading and updating professional in using the molecular diagnostic methods is another gap that needs to address for future. Hence, professionals will contribute for TB prevention and control practices, develop and enhance tools for public health practitioners, and share best practices that will maximize current efforts [100-120].

Conclusion

Despite the fact tuberculosis seems decreasing worldwide where WHO works to end TB program, yet zoonotic TB remains one of the challenges in developing countries like Africa; Where there is a close interaction between human, livestock and wildlife. Some of the challenges to eradicate zoonotic TB in developing countries are absence of early diagnosis, having many hosts, presence of other acute diseases, economically unable to implement the stamping out techniques in the countries and other social and cultural issues. For many years since early period until now, the conventional diagnostic methods are contributing in diagnosis and treatment of tuberculosis even some being the gold standards in fact with their own limitations in diagnosis of tuberculosis [121-131]. Even currently in developing countries, the conventional methods are in intense use while in developed nations many other fast, high specific and sensitive diagnostic techniques are developed. For the current WHO motto “end TB” to be achieved it is expected an intense effort in identification, the source of infections and reservoirs and applying a timely treatment, control and prevention system. Hence, applying molecular base diagnosis and characterization of TB is a right tool for diagnosis and treatment regimen: As a result, it is possible to reduce the disease incidence, prevalence, mortality, morbidity, economical loss and development of drug resistance.

Recommendations

- Introducing cost effective and easy molecular diagnostic methods is recommended.
- Researchers in developing countries better to use molecular characterization for better understanding the source of infection and transmission dynamicity
- Molecular characterization technique is a tool to check whether there is an active transmission or reactivation of TB, hence better to adopt these methods for epidemiological survey
- Developing countries need to give attention to train personnel as the equipment need trained personnel

- At least reference laboratories should be present for molecular based diagnosis as part of the TB control programs in zonal level

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