Review Article

Genetic diversity and geographical distribution of strains of mycobacterium tuberculosis complex in Ethiopia: Review

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Abstract

In Ethiopia, tuberculosis (TB) is one of the major infectious diseases with wide spread geographic distribution and endemic nature, which has been well documented both in human and livestock of the country. TB in livestock has an important economic and public health significance although the actual prevalence of animal tuberculosis at the national level is yet unknown. Identification of the etiology of tuberculosis in human and different species of livestock has a paramount significance in order to understand the transmission pattern, the pathogenesis, and control of the diseases. So far, detection of animal TB in Ethiopia has been carried out most commonly on the basis of tuberculin skin testing, abattoir meat inspection and very rarely on bacteriological techniques. These diagnostic methods can not differentiate the specific species of Mycobacterium tuberculosis complex or other species /strains of Mycobacterium, hence utilization of advanced molecular techniques to characterize and genotype the causative agents is important. Genotyping of Mycobacterium species allows understanding the genetic diversity, transmission dynamics and evolutionary/phylogenetic relationships of the isolates in different hosts of animal and human. The aim of this paper is, therefore, to review molecular genetic diversity and distribution of mycobacterial infection due to M. tuberculosis complex (mainly M. bovis, M. tuberculosis) and non-tuberculous mycobacterium in Ethiopia in addition to highlighting the zoonotic significance of mycobacterial species originated from livestock to show the feasible prevention and control options of tuberculosis in cattle and other animals.

Introduction

Tuberculosis (TB) is a bacterial disease caused by Mycobacterium tuberculosis complex (MTBC), which is a leading cause of death worldwide. The major species of MTBC are M. africanum, M. canetti, M. microti, M. bovis, M. tuberculosis [1]. The members of M. tuberculosis complex are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences but differ widely in terms of their host tropisms, phenotypes, and pathogenicity.

Ethiopia is one of the 22 high burden countries with high annual TB incidence of 247 cases/100,000 population in 2012 [2]. M. tuberculosis is the most common cause of human TB, but an unknown proportion of cases are due to M. bovis. Human TB caused by M. bovis (bovine tuberculosis; BTB) is clinically indistinguishable from TB caused by M. tuberculosis and can only be differentiated by laboratory methods [3]. Specific data on zoonotic BTB transmission is very scarce in the developing world because the diagnosis of TB most often relays on sputum microscopy only. However, with recent advancement in molecular methods for characterization of M. tuberculosis complex including spoligotyping [4], deletion typing [5], MIRU-VNTR methods [6] identification of members of M. tuberculosis complex at species strain level has been possible. One of the members of M. tuberculosis complex, M. bovis has been considered as the classical causative agent of BTB in cattle; however, the organism has also been isolated from different species of livestock, wildlife and also from human which become a serious public health problem [1,7].

Molecular strain typing (genotyping) has contributed significantly to the understanding of TB epidemiology and has helped to improve TB control by providing information
Mycobacteria are resistant to decolorization due to its thick cell wall and high lipid content [12]. This property is termed acid fastness, so that Mycobacterium is commonly referred to as acid-fast bacilli. In contrast, these microorganisms are not readily stained with the Gram stain method and are considered weakly Gram-positive. The rods tend to stain irregularly and often have a beaded appearance. They are slow growers, i.e. they require more than 7 days forming colonies when sub-cultured on Lowenstein–Jensen media. *M. tuberculosis* forms rough colonies on Lowenstein–Jensen solid media. Colonies formed by *M. bovis* are smooth with irregular edges on egg based media [13]. The genus *Mycobacterium* is most closely related to the genera *Rhodococcus* and *Nocardia* and all three genera have a similar cell wall type and are acid fast but comparatively *Mycobacterium* have characteristics of slow growth rate [14].

**Morphology and biochemical composition**

*Mycobacterium* is a fastidious, slow growing, lipid-rich, hydrophobic and acid fast bacterial rod shape which resists decolorization with acid alcohol. It has no outer membrane; rather it has a cell wall made up of different macromolecules, namely peptidoglycans, arabinogalactan, mycoclic acid and lipopolysaccharide or lipoarabinomannan (LAM) which is anchored plasma membrane (Figure 2). Mycoclic acid is the major component of the cell wall envelope, greater than 50% by weight, a significant number which are responsible for their resistance to humoral defense mechanism, disinfectants, acids and alkalis. The staining characteristic of *M. tuberculosis* is due to the mycoclic acid which resists decolorization by acid alcohol Figure 2.

**Genotyping of mycobacterium tuberculosis Complex**

The genomics of *Mycobacterium tuberculosis* complex have proved considerably more difficult to elucidate than those of many other bacteria. This is due to the difficulties presented by extremely slow growth, the unique cell wall composition of the organism, the need for protection of laboratory personnel and a paucity of cloning vehicles [16]. For many years, it was thought that human tuberculosis evolved from the bovine disease by adaptation of an animal pathogen to the human host. This hypothesis is based on the property of *M. tuberculosis* to be almost exclusively a human pathogen, whereas *M. bovis* has a much broader host range.

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However, studies based on comparative molecular analysis of the genome of *M. tuberculosis* and *M. bovis* unambiguously showed that *M. bovis* has undergone numerous deletions relative to *M. tuberculosis* and also revealed that no new gene clusters that were confined specifically to *M. bovis*. This result indicates that the genome of *M. bovis* is smaller than that of *M. tuberculosis*. Hence, it is now agreed that *M. bovis* is the final member of a separate lineage that branched from the progenitor of *M. tuberculosis* isolates. Successive loss of DNA may have contributed to clonal expansion and the appearance of more successful pathogens in certain new hosts [5] (Figure 3).

Molecular genotyping has advanced the understanding of MTBC transmission and is helpful in identifying transmission links in a livestock. IS6110-based restriction fragment-length polymorphism (RFLP) analysis is often used for genotyping of MTBC. However, molecular characterization using RFLP method is laborious and requires culture for several weeks to obtain large quantities of genomic DNA. In addition, RFLP has poor discriminatory power for isolates with low numbers of insertion sites such as *M. bovis*.

![Figure 2: The structure of the M. tuberculosis complex cell wall](image)  
**Figure 2:** The structure of the *M. tuberculosis* complex cell wall [15].

![Figure 3: Evolutionary scenario of the MTBC Phylogenetically informative large genomic deletions and single nucleotide polymorphisms (SNP)](image)  
**Figure 3:** Evolutionary scenario of the MTBC Phylogenetically informative large genomic deletions and single nucleotide polymorphisms (SNP) [5].

Spoligotyping is a rapid, polymerase chain reaction (PCR)-based method for genotyping strains of the MTBC. It is easier to perform and requires smaller amounts of DNA than RFLP analysis but its discriminatory capacity is inferior to RFLP. Spoligotyping is useful in discriminating strains with low IS6110 copy numbers such as *M. bovis* and it can be performed on nonviable organisms. The clinical usefulness of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities [4].

Mycobacterial interspersed repetitive units–variable number tandem repeats (MIRU–VNTR) is another PCR-based method that is easily reproducible and does not require extensive DNA purification. MIRU–VNTR has become a major method for rapid, high-resolution genotyping of *M. tuberculosis* complex isolates. The method relies on PCR amplification of multiple loci (12, 15 or 24 loci) using primers specific for the flanking regions of each repeat locus and on the determination of the sizes of the amplicons, which reflect the numbers of the targeted MIRU–VNTR copies. Moreover, the results are expressed as numerical codes and are therefore easy to compare and exchange between laboratories [8,9].

**Bovine Tuberculosis (Btb) in Ethiopia**

Btb is a widespread and endemic disease of cattle in Ethiopia. *M. bovis* is the causative agent of tuberculosis in cattle. *M. bovis* is a member of the *M. tuberculosis* complex, which also includes *M. tuberculosis*, *M. caprae*, *M. microti*, *M. africanum*, *M. canetti*, *M. pinnipedii*, and *M. bovis* Bacillus Calmette–Guérin (BCG) [1]. Unlike most of the organisms in this group, *M. bovis* has a broad host range including; cattle, cervids, badgers, humans, and many other animals [1,18].

Several prevalence studies have been performed recently showed that Btb is endemic in cattle; however, prevalence varies depending on the geographical areas, breeds and husbandry practices. Abattoir and dairy farm studies from central Ethiopia have reported prevalence between 3.5 and 13.5% and locally in peri-urban Addis Ababa up to 50% [19-21]. In contrast, lower prevalence of 0.9% was reported in traditionally kept zebu cattle [22]. Other livestock than cattle have also been investigated. Based on gross pathology, prevalence of 5–10% was reported in camels slaughtered at Dire Dawa abattoir in eastern Ethiopia and in Addis Ababa abattoir. The observed variability of Btb disease frequency in Ethiopia might well be influenced by different livestock production systems (rural/pastoral/peri-urban) and different geographic and climatic contexts. Transmission of Btb seems to be higher in intensive peri-urban settings when compared to extensive rural and pastoral areas [23,24].

**Genetic diversity and geographical distribution**

In Ethiopia a number of molecular epidemiological studies on Btb in livestock were carried out in different regions of the country [19,20,24,25]. According to Berg, et al. [20] a total of seven different spoligotypes were identified, of which SB0133, SB1476, and SB1176 were more prevalent. SB0133
predominates in Jinka but it also represented in Addis Ababa, Gimbi and Woldia. SB1476 is the most common pattern in both Gimbi and Gonder but it was found in Jinka and Butajira. SB1176 is the most prevalent among the samples from Addis Ababa but can also be seen Butajira, Gonder and Woldiya. The remaining four spoligotypes patterns, SB0134, SB1488, SB1489 and SB1477, are all highly related to SB0133, and isolates with these patterns were mostly collected from Addis Ababa or Jinka abattoirs. The largest diversity of M. bovis strains was found in Addis Ababa abattoir with five different spoligotypes, likely reflecting the wide geographical area (Figure 4) from which cattle were sourced [20].

Sheep and goat tuberculosis in Ethiopia

TB in goats and sheep is caused by members of M. tuberculosis complex predominantly by M. bovis and M. caprae and few caused by M. tuberculosis. The overall animal prevalence of TB in small ruminants was 0.5% (95% CI: 0.2% – 0.7%) at ≥2mm and 3.8% (95% CI: 3% – 4.7%) at cutoff ≥4mm [26]. Caprine tuberculosis (TB) caused mainly by M. bovis and M. caprae poses a risk to goat health and production in developing world. Goats may become infected with M. bovis when sharing pastures with infected cattle, at watering points, and SB1476 is the most common pattern in Butajira, Gonder and Woldiya. SB1176 is the most prevalent among the samples from Addis Ababa, Gimbi and Woldia. The majority of camel TB lesions were caused by NTM [24]. On the other hand, the isolation of SB0133 M. bovis strains in the present study from camel of pastoral area of Ethiopia is in line with the isolation of this strain from cattle of southern Ethiopia [20]. The majority of camel TB lesions were caused by NTM [24]. On the other hand, Gumi [25] have characterized the isolation of SB0133 M. bovis strain SIT149 using spoligotype from disseminated generalized TB cases of camel and NTM as a causative agents of camel TB in south east pastoral camels of Ethiopia [25].

Genetic diversity and geographical distribution

Mycobacteriological culture and molecular characterization of isolates from goats resulted in isolation of M. tuberculosis strain SIT149 [26]; SIT53 [27] and non tuberculosis mycobacteria as causative agents of tuberculosis and tuberculosis like diseases in goats, respectively. The isolation of Mycobacterium tuberculosis in goats suggests a potential transmission of the causative agent from human. The SIT149 strain of M. tuberculosis is a dominant strain in Ethiopia and it was a common isolate in human pulmonary TB patients from the same Afar Pastoral Region. The isolation of the SIT53 strain of M. tuberculosis from goats suggests transmission from humans [27].

Camel tuberculosis in Ethiopia

Tuberculosis caused by M. bovis is the most common form of tuberculosis in domestic camels. The prevalence of camel TB was 10.04% (91/906) on the basis of pathology [24]. Tuberculosis as a zoonosis from camel to human also plays an important role among nomadic people where milk and milk products are consumed raw [28]. The occurrences of TB lesions in camels were relatively higher in the younger and older camels than other age groups. Older animals are affected by TB which could be due to the fact that older animals have weaker immune system. The higher frequency of lesion in younger camels could be due to the less developed immunity [29] Young camels can also be easily infected with higher doses of mycobacteria via colostrums from infected camel in a similar way, as it occurs in cattle.

Genetic diversity and geographical distribution

Molecular epidemiological studies on Camel tuberculosis indicated that one of the strains which caused a generalized disseminated TB in camel was SB0133 [24], whereas the other strain was SB1953 which has been recently reported to the database. In Ethiopia, a number of studies reported new strains with specific spoligotype pattern in cattle. On the other hand, the isolation of SB0133 M. bovis strains in the present study from camel of pastoral area of Ethiopia is in line with the isolation of this strain from cattle of southern Ethiopia [20]. The proportion of which BTB contributes to the total of tuberculosis cases in humans. It depends on the prevalence of the disease in animals, socioeconomic conditions, consumer habits, practiced food hygiene and medical prophylaxis measures. The primary bacterium that causes TB in humans is M. tuberculosis. In countries where BTB in cattle is still highly prevalent, pasteurization is not widely practiced.
and milk hygiene is insufficient. About 10% to 15% of human tuberculosis is considered to be caused by BTB. *M. bovis* can enter human hosts through ingestion, inhalation or direct contact with mucous membranes or broken skin. Milk is still regarded as the principal vehicle for transmission to humans in countries where bovine tuberculosis is not controlled. Ingestion of contaminated milk or other dairy products is more often associated with scrofula, abdominal tuberculosis and other extra-pulmonary forms of the disease [31]. Because of the ability of BTB to infect humans and animals, its control is more difficult. Human immunodeficiency virus (*HIV*) infection has created a special niche for *M. tuberculosis* complex in humans as a result of the defect/reduction in cell mediated immunity. *M. tuberculosis* still responsible for most cases of death due to infectious diseases after HIV. The incidence of pulmonary tuberculosis caused by *M. bovis* is higher in farm workers than in urban inhabitants [32]. In rural areas of Ethiopia most people drink raw milk and do have extremely close attachment with cattle (such as sharing shelter) that intensifies the transmission and spread of BTB [10].

**Prevention and control**

The basic strategies required for control and elimination of bovine tuberculosis are well known and well defined. However, because of financial constraints, scarcity of trained professionals, lack of political will, as well as the underestimation of the importance of zoonotic tuberculosis in both the animal and public health sectors by national governments and donor agencies, control measures are not applied or are applied inadequately in most developing countries [3]. Cattle should not be treated at all and as such farm animals with tuberculosis must be slaughtered (culled) [33]. This is because the risk of shedding the organisms, hazards to humans and potential for drug resistance make treatment controversial. As this disease is primarily transmitted from cattle to humans in milk, control of human infection can be achieved by pasteurization and control of bovine tuberculosis. Testing of cattle with an intradermal tuberculosis test (or by inspection at slaughter), combined with removal or quarantine of infected herds and pasteurization of milk, has proven very effective in reducing the incidence of *M. bovis* infection in humans. Elimination is complicated by the several wildlife reservoirs of *M. bovis* present in most countries of the world. However, practical elimination of human infection can be achieved with a control program targeting only domestic animals [34]. Milk should be pasteurized or effectively treated with heat prior to human consumption or further processing, as this is the generally agreed critical and effective control measure to prevent transmission of zoonotic tuberculosis through milk.

There should be an increased enlightenment of at-risk individuals and the public on the possible risks of *M. bovis* infection in man. Farmers and other occupationally at-risk individuals should be required to adopt appropriate measures to minimize exposure of employees and farm visitors to infections that can be transmitted to humans from animals. Also, the role of wild fauna in the epidemiology of tuberculosis in livestock and humans need not be ignored, as they have been reported to serve as a reservoir of the pathogen. Animal husbandry practices should be improved upon to reduce contact between domestic livestock and wild ruminants especially during grazing. Vaccination is practiced in human medicine, but it is not widely used as a preventive measure in animals [35-37].

**Conclusion and recommendations**

Tuberculosis (TB) is an infectious, granulomatous disease caused by acid-fast bacilli of the genus *Mycobacterium*. Bovine TB is still a significant zoonosis in many parts of the world. Genotypic diversity of *M. tuberculosis* complex (MTBC) is important to understand its epidemiology, host adaptation and clinical phenotypes. The development of molecular tools has added a new dimension to the classical epidemiology of tuberculosis and greatly enhanced understanding of the complex transmission dynamics within populations and between hosts. Spoligotyping appears to have the specific characteristics needed to satisfy these issues of epidemics and tuberculosis transmission. This method permits the concomitant identification and differentiation of MTBC strains and avoids the timing problems associated with the slow growth of these bacteria. There is molecular evidence for the widespread distribution of *M. bovis* in the cattle population in Ethiopia. It also demonstrated a relatively high degree of genetic polymorphism of the isolates. Clustering of specific strains of isolates suggests circulation of the strains in the different part of the country. The existence of potential inter-species transmission of the strain among livestock of pastoral area, isolation of *M. tuberculosis* in goat suggests transmission from human to animal. Molecular genetic studies on *Mycobacteria* species isolated from TB patients in Ethiopia, human zoonotic tuberculosis is more common in pastoral regions of the country.

Based on the above conclusion, the following points are recommended:

- Further research in identifying the circulating strains of MTBC in various hosts and their distribution in geographical area of the country should be undertaken.
- Future molecular mapping of the isolates to elucidate strain diversity in detail within Ethiopia is recommended.
- Understanding of the transmission dynamics of the different strains of mycobacteria should also be emphasized to design and assess the BTB control intervention both in livestock and human population.
- Creating awareness among the people, to meet the standard hygienic requirement and to improve husbandry practices is of paramount importance.
- Auditing of the ante-and post-mortem inspection of carcasses at abattoirs regarding the control and removal, from the food chain, of carcasses or parts thereof, considered unfit for human consumption because of the presence of tuberculosis, or for other reasons, is recommended.
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