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Tuberculosis, a curable infectious disease, remains the leading cause of adult death. The HIV/AIDS epidemic has greatly exacerbate the already grave situation in the developing world by creating a deadly synergy each worsening the course of the other. *Mycobacterium africanum* is a subspecies of *Mycobacterium tuberculosis complex* (MTBC) and is isolated from tuberculous patients in certain parts of Africa. Genotypically, members of the MTBC are closely related, exhibiting 99.9% similarity at the nucleotide level and identical 16s RNA gene (rDNA) and 16s-23s rDNA spacer sequences. However, identification and discrimination between members of the MTBC are important for epidemiological purposes. This paper reviewed current knowledge about this subspecies.

INTRODUCTION

Tuberculosis (TB), a curable infectious disease, remains the leading cause of adult death. One third of the world's population is estimated to be infected by members of the *Mycobacterium tuberculosis complex* (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti* and *M. microti*) which are collectively responsible for about three million deaths each year, over 95% of which occur in developing countries (1, 2). The HIV/AIDS epidemic has greatly exacerbated the already grave situation in the developing world by creating a deadly synergy each worsening the course of the other (3).

Mycobacterium africanum is a subspecies of the *Mycobacterium tuberculosis complex* (MTBC) and is isolated from tuberculosis patients in certain parts of Africa. Castets and colleagues first described *M. africanum* in 1968 when it was isolated from sputum of a tuberculosis patient in Senegal (4). Genotypically, members of the MTBC are closely related, exhibiting 99.9%

similarity at the nucleotide level and identical 16S RNA gene (rDNA) and 16S-23S rDNA spacer sequences (5-8). They however differ widely in terms of their phenotypic characteristics (such as colony morphology, growth rate, and biochemical profile), pathogenicity, epidemiology, geographic range, and host preferences (9, 10). For example, *M. tuberculosis*, *M. africanum* and *M. canetti* are exclusively human, while *M. microti* is a rodent pathogen and *M. bovis* can infect a wide range of mammals (Table 1). The close genetic relatedness, overlapping phenotypes and slow growth make species assignment difficult (5, 11).

In recent times, insights from comparative genomics employing differential hybridization techniques have revealed significant differences in the gene content and genome organization of this group of closely related species, particularly as a number of large sequence polymorphisms (LSPs) (12-15). These differences can be used to more accurately identify subspecies within the MTB complex. Identification and discrimination between members of the

MTBC are important for epidemiological reasons. One of the main reasons for the correct identification of *M. bovis* is its natural resistance to pyrazinamide, a widely used antituberculous drug. In addition, transmission usually occurs through contaminated dairy products and *M. bovis* prevalence rates can be used to evaluate the effectiveness of a bovine TB control program. In contrast, the epidemiologic implication of identifying *M. africanum* is uncertain in view of the limited studies and awareness of this subspecies. However, it is important to correctly identify species within the MTBC to monitor transmission especially in developing countries and zoonotic implications of bovine tuberculosis, and the sporadic report of *M. africanum* in European herds.

Although a number of genetic markers have been described in the literature useful in the identification of *M. tuberculosis (stricti sensu)* and *M. bovis*, few have been described for *M. africanum*. Furthermore, there is no single marker available commercially to clearly differentiate individual members within the

in the presence of glycerol, and are sensitive to thiophene-2- carboxylic acid hydrazide (TCH). In contrast, *M. bovis* strains lack nicotinamidase function, do not produce niacin or reduce nitrate, are not stimulated by glycerol, and are resistant to TCH. However, strains of *M. africanum* exhibit a high degree of phenotypic heterogeneity having biochemical and morphologic characteristics that are intermediary between strains of *M. tuberculosis* and *M. bovis*, complicating laboratory identification.

All members of the MTBC have a doubling time close to 24h and visible growth on solid media takes 3-4 weeks to form colonies on Petri dishes (16, 17) (Table 1)

Difficulties in precisely defining or identifying *M. africanum* increase the potential to misclassification of clinical strains and complicating attempts to accurately determine the true prevalence of tuberculosis caused by *M. africanum* (18). Furthermore, data from pyrolysis mass spectrometry do not support a species

Table 1: Some properties of members of the *Mycobacterium tuberculosis complex* (MTBC)

Species	Host range	Colony form	Niacin	Nitrate reduction	TCH	PZA
<i>M. africanum</i>	Humans	Dysgonic	+/-	+/-	R/S	S
<i>M. bovis</i>	Cattle, goats, lions Elephants, humans etc.	Dysgonic	-	-	S	R
<i>M. bovis</i> BCG		Eugonic	-	-	S	R
<i>M. canetti</i>	Humans	Smooth	+	+	R	S
<i>M. microti</i>	Voles	Tiny	+	-	S	S
<i>M. tuberculosis</i>	Humans	Eugonic	+	+	R	S

Adapted from Cole ST (46)

MTBC or between the sub-species of *M. africanum*. This paper reviews current knowledge about this subspecies.

BACTERIOLOGY

M. tuberculosis strains have functional nicotinamidase, produce niacin, reduce nitrate, and have enhanced growth in

status for this group of strains, causing some authors to question the validity of this species (10, 16).

EPIDEMIOLOGY

M. africanum has been reported from several regions of sub-Saharan Africa and it is estimated to account for between

5% of patients with pulmonary TB in Ivory Coast and 60% of those in Guinea Bissau with variable proportion of subtypes (19-24). According to their biochemical characteristics, two major subgroups of *M. africanum* have been described, corresponding to their geographic origin. Subtype I or variant I, is a *M. bovis* like organism that is nitrate negative and predominantly clustered in West Africa. Subtype II or variant II is nitrate positive like *M. tuberculosis* and is clustered in East Africa (19, 25-29) though both subtypes have been reported in Guinea -Bissau (30).

In Uganda *M. africanum* subtype II is the main cause (67%) of human tuberculosis in Kampala (18) and transmission probably occurs at low rates on other continents as *M. africanum* has been isolated from patients for whom no link with Africa could be established. *M. africanum* was found to account for 1.25% of

cases in 1970 to just 9% in 2002. One possible explanation is the widespread use of BCG, which could be effective against less virulent strains of *M. africanum* as observed in experimental models (24, 32). However, whether this phenomenon is the same in other parts of Africa needs to be evaluated and is likely to shed light into the changing pattern of the population structure of the MTBC complex being driven by various selective pressures (Table 2).

In humans, the clinical manifestations of *M. africanum* are similar to those of other members in the group except that *M. africanum* is rarely isolated from patients with genitourinary tuberculosis (31). In experimental models, *M. africanum* appears to have reduced virulence compared to *M. tuberculosis* (33), but this laboratory observation is not supported by clinical evidence. *M. africanum* remains a highly

Table 2: Reported studies of *M. africanum*

Country (N)	Prevalence of <i>M. africanum</i> %	Type of Study	Author
Burkina Faso (300)	18.4	P	(20)
Cameroon	56	P	(32)
Cameroon (455)	9	P	(24)
Guinea-Bissau (229)	61	P	(30)
Ivory Coast (321)	5.3	P	(47)
SE England (16800)	1.3	H	(31)
Uganda	16	H	(22)
Uganda (234)	67.1	P	(18)

P* population based study, H* Hospital based study.

bacteriologically confirmed cases of tuberculosis in Southeast England. Over half of the patients infected with subtype I strain in South-East England were of Indian subcontinent origin, whereas patients of African ethnic origin predominated in the subtype II group, and a fifth of patients with either type were of European origin (31).

The prevalence of *M. africanum* in Cameroon dropped from 56% of all TB

pathogenic and transmissible tubercle bacillus rather than an opportunist or atypical mycobacterium. It can acquire mutations leading to isoniazid and rifampicin resistance similar to those seen in *M. tuberculosis* and *M. bovis* (17).

M. africanum can cause extra-pulmonary TB, like other members of the MTBC. For example, it was isolated from a patient with cutaneous tuberculosis with

bilateral nodular scleritis, nasal sinus invasion, and nasal septum perforation, who had concurrent pulmonary disease. In HIV positive patients enrolled in a trial substituting rifabutin for rifampicin in short course therapy for pulmonary tuberculosis, 49% had *M. africanum* isolated, indicating a high prevalence of *M. africanum* in human TB in Uganda (21).

M. africanum is not confined to the human population; an outbreak of tuberculosis due to this strain has been reported in pigs and cattle in Norway. The lesions in pigs were similar to those caused by *M. tuberculosis*, *M. bovis* and *M. avium*, with caseation in the lymph nodes of the head and jejunum. The source of the infection could not be established (34) and in Northern Bavaria, *M. africanum* was isolated from a mediastinal lymph node of a young bull from a herd of cattle, the source of infection was thought to be a member of the family of the farmer (35). Thorel reported the first isolation of *M. africanum* in monkeys and emphasized the potential public health hazard that animals may present for humans (36).

MOLECULAR INSIGHT

Recent advances in comparative genomic technologies include DNA arrays that can identify deletion events (13, 14, 37) and highly sensitive whole-genome sequence comparisons, which detect the full range of

polymorphisms from single nucleotide polymorphisms to gene rearrangement. Brosch *et al* (12) have uncovered several variable genomic regions in the members of the *M. tuberculosis* complex. Differential hybridization arrays identified 16 deleted regions (Region of difference (RD 1-16), ranging in size from 2 to 12.7 Kb, that were absent from BCG Pasteur relative to H37Rv. Based on the presence or absence of these regions, a degree of relatedness to the last common ancestor of the *M. tuberculosis* complex was proposed that showed the progressive loss of genes (deletions) and the lineages existing within members of the group (Fig.1). This opened new perspectives in tuberculosis epidemiologic and diagnostic research, as one of these deletions (RD1) is believed to have been the primary attenuation event in the derivation of *M. bovis* BCG from *M. bovis*, since all *M. bovis* BCG isolates bear this deletion (37) and reinsertion of this region into *M. bovis* BCG partially restored its virulence (38).

Relating these findings to previously suggested differences in virulence and other factors is providing better insights into the epidemiology and pathogenesis of tuberculosis in general. These data, which suggested a sequential accumulation of Large Sequence Polymorphism (LSPs), served to construct a phylogenetic scheme for the evolution of the MTBC (Fig.1).

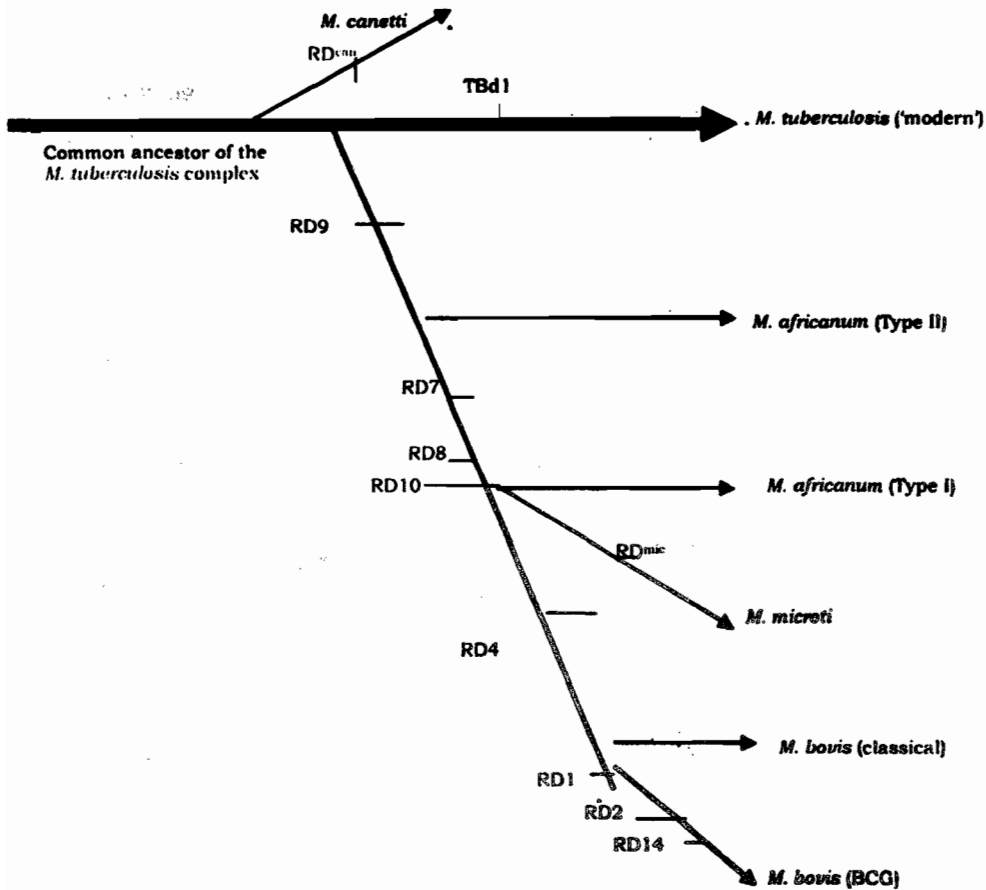


Fig. 1: Scheme of the proposed evolutionary pathway of the tubercle bacilli illustrating successive loss of DNA in certain lineages (RD). The scheme is based on the presence or absence of conserved deleted regions genes. Note that *M. africanum* has an intermediate position between *M. tuberculosis* and *M. bovis*. Adapted from Brosch et al (12)

In addition, these regions of difference (RD) can be used to identify subspecies within the MTBC. From the evolutionary pathway proposed (Fig 1), isolates characterized as *M. africanum* form two distinct genetic groups and are close to the common ancestor of the *M. tuberculosis* complex in that they are not lacking the TbD1 specific deletion common in 'modern' *M. tuberculosis*. What differentiate the genetic groups of *M. africanum* presently are RD7, RD8, RD9, and RD10. In the first group RD9 alone is deleted while RD7, RD8 and RD10 are present, while in the second group RD7, RD8, RD9 and RD10 are all deleted (12, 39-41).

There have been reported isolates of *M. africanum* which express genetic profile similar to those of 'modern' *M. tuberculosis* in that the TbD1 is missing while other regions of difference are present, however, some have suggested that these isolates should be called *M. tuberculosis* (41). 16S rRNA gene and internal transcribed spacer sequences, specific repetitive elements like IS610 and the direct repeat locus, in combination with phenotypic testing have shown to accurately differentiate several MTBC groupings and were used to evaluate large collections of clinical isolates (40, 42, 43). They are useful in identifying members of the MTBC as a group from other species

of mycobacteria but less efficiently for inter or intra species identifications. Table II shows some of the markers that have been described.

Spacer oligonucleotide typing (Spoligotyping) is the only DNA-based methodology for which most MTBC members are believed to have signature features (44, 45). Based on results with three independent markers (IS6110 RFLP analysis, spoligotyping and VNTR), Viana-Niero *et al.* have suggested that *M. africanum* has a specific spoligotyping signature with the absence of spacers 8,9, and 39 (18, 23) while another group (18) proposed a different set of criteria to define *M. africanum*. A combination of the geographic origins of the strains, susceptibility to TCH, hybridization to at least two of the *M. bovis* derived spoligotype spacers 33 to 36, and a specific *gyrB* DNA sequence define subtype I. Resistance to TCH and lack of hybridization to spacer 33 to 36 define subtype II (18).

Yet, Parsons and colleagues (40) proposed another classification of subspecies of the *Mycobacterium tuberculosis complex* based on sequences

that are highly conserved with respect to RD 1, RD 9, and RD 10. They suggested these three regions could be used to differentiate *M. tuberculosis* strains from the other members of the MTBC by screening isolates that lacked RD 9 for the presence of RD 10. If both are deleted, they subsequently tested these isolates for the presence of RD 3, RD 5, and RD 11. Isolates lacking only RD 9 were identified as *M. africanum*. Hence, RD9 is a common deletion found in *M. bovis*, *M. bovis* BCG, and *M. africanum* but not in *M. tuberculosis*. This finding supports the accepted view that *M. africanum* is evolutionarily intermediate between *M. tuberculosis* and *M. bovis* (12) but does not distinguish between subtypes I and II.

A large population based study in an area where *M. africanum* is endemic could compare these proposed classification methods and potentially achieve a consensus definition of the subspecies. This in turn will facilitate the identification of phenotypic associations and host preferences of the organism.

Table 2: Genetic differences among members of the TBC

Component evaluated	Difference	Reference(s)
Variable alleles		
<i>oxyR</i> nucleotide 285	A in <i>M. bovis</i> , G in other members of the TBC	(48)
<i>pncA</i> nucleotide 169	G in <i>M. bovis</i> and <i>M. bovis</i> BCG, C in other members of the TBC	(49)
<i>katG</i> codon 463	CTG (Leu) in group 1, CCG (Arg) in group 2, CCG (Arg) in group 3 ^a	(8)
<i>gyrA</i> codon 95	ACC (Thr) in group 1, ACC (Thr) in group 2, AGC (Ser) in group 3 ^a	
<i>GyrB</i>	Sequence differences among members of the TBC	(29) (42)
Variable sequences for spacers between direct repeats^b		
Spacers 33 to 36 (derived from BCG)	<i>M. tuberculosis</i> does not hybridize to the spacers	(45)
Spacers 39 to 43 (derived from <i>M. tuberculosis</i>)	<i>M. bovis</i> and BCG do not hybridize to the spacers	(45)
Spacers 37 and 38	<i>M. microti</i> has a very short direct repeat region; many strains only hybridize to spacers 37 and 38	(50)
Spacers 8, 9 and 39	<i>M. africanum</i> does not hybridize to the spacers	(23)

M. africanum subtype I can be determined by spoligotyping, note also other genetic makers used in the differentiation of the MTBC. Table II. Adapted from Parsons *et al.* (40)

CONCLUSION

M. africanum accounts for a sizeable proportion of tuberculosis in Sub-Sahara Africa, although its identification has been hampered by lack of clear criteria. Comparison of several proposed identifications methods will hopefully lead to a consensus on the identification of this sub-species, which in turn allows for a better understanding of its clinical characteristics and host predilections.

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