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Smooth Tubercle Bacilli: Neglected Opportunistic Tropical Pathogens

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Abstract

Smooth tubercle bacilli (STB) including ‘‘*Mycobacterium canettii*’’ are members of the *Mycobacterium tuberculosis* complex (MTBC) which cause non-contagious tuberculosis in human. This group comprises less than one hundred isolates characterized by smooth colonies and cordless organisms. Most STB isolates have been obtained from patients exposed to the Republic of Djibouti but seven isolates, including the three seminal ones obtained by Georges Canetti between 1968 and 1970, were recovered from patients in France, Madagascar, Sub-Sahara East Africa and French Polynesia. STB form a genetically heterogeneous group of MTBC organisms with large 4.48 ± 0.05 Mb genomes which may link *Mycobacterium kansasii* to MTBC organisms. Lack of inter-human transmission suggested a yet unknown environmental reservoir. Clinical data indicate a respiratory tract route of contamination and the digestive tract as an alternative route of contamination. Further epidemiological and clinical studies are warranted to elucidate areas of uncertainty regarding these unusual mycobacteria and the tuberculosis they cause.

Keywords: *Mycobacterium tuberculosis* complex, *"Mycobacterium canettii", Canetti strain, Mycobacterium kansasii,* smooth tubercle bacilli, clinic, pathogenesis, evolution, Djibouti, Horn of Africa, experimental work, bioinformatics, lipids, lipolytic enzymes, amoebas, cellulases.

Résumé

Les souches lisses du bacilli tuberculeux y compris ''*Mycobacterium canettii*'' sont des membres du complexe *Mycobacterium tuberculosis* (CMTB) responsables d'une tuberculose non-contagieuse chez les êtres-humains. Ce groupe comprend moins d'une centaine d'isolats caractérisés par des colonies lisses et par l'absence de 'cord-factor'. La majorité de ces isolats ont été obtenus chez des patients ayant séjourné dans la République de Djibouti alors que sept autres isolats, en incluant les trois souches séminales isolées par Georges Canetti entre 1968 et 1970, ont été obtenus à partir des patients en France, Madagascar, l'Afrique de l'Est sub-saharienne et la Polynésie Française. Ces souches forment un groupe génétiquement hétérogène avec des grands génomes (4.48 ± 0.05 Mb) qui pourrait être le lien entre *Mycobacterium kansasii* et les membres du CMTB. L'absence d'une transmission inter-humaine a suggéré l'existence d'un réservoir environnemental encore inconnu. Les données cliniques indiquent une voie de contamination respiratoire et la voie digestive comme une voie alternative de contamination. Plus d'études épidémiologiques et cliniques sont nécessaires pour élucider les champs d'incertitude concernant ces mycobactéries rares et la tuberculose qu'elles causent.

Mots-clés: Complexe *Mycobacterium tuberculosis* , *"Mycobacterium canettii", souche Canetti*, *Mycobacterium kansasii,* souche lisse du bacilli tuberculeux, clinique, pathogenèse, évolution, Djibouti, Corne de l'Afrique, travail expérimental, bioinformatique, lipides, enzymes lipolytiques, amibes, cellulases.

Introduction

In 2013, nine million people developed tuberculosis (TB) and 1.5 million people infected with TB died *(1)*. The vast majority of cases were caused by *Mycobacterium tuberculosis stricto sensu*, a cord-forming organism exhibiting rough colonies *(2-4)* while a few cordless isolates, referred as 'smooth tubercle bacilli' (STB) were reported to form smooth colonies *(5)*. The first three STB isolates made by Georges Canetti in 1968-1970 *(6)* were further named '*Mycobacterium canettii*' following the isolation of an additional STB isolate from a tuberculous lymph node in a Somali child *(7)*. Then, a total of 93 STB have been isolated from patients exposed to tropical countries, mainly the Republic of Djibouti, which reports the highest prevalence and incidence of STB *(5, 7-17)*. The reason for this geographical specificity is not really understood. Despite its rarity, STB deserve special attention due to their epidemiological, clinical and microbiological characteristics, which are unique among the *M. tuberculosis* complex (MTBC).

Particularities of the STB infection

No environmental or animal STB isolates have been identified, contrary to that of *M. tuberculosis (18)*. Indeed, the three seminal STB isolates were not reported by Canetti himself, but were rather identified through two indirect sources *(6, 19)*. Accordingly, the precise history of these seminal isolates is poorly known, although it began prior to 1969, as deduced from a study on *M. tuberculosis* var. *hominis*, Canetti strain mycolic acids submitted for publication in 1968 *(20)*. This first isolate was obtained from a 20-year-old French farmer suffering from pulmonary TB although he had apparently never left France *(6,19)*. Canetti obtained a second isolate from a 54-year-old farmer also suffering from pulmonary TB in Madagascar, then a third isolate from a man suffering tuberculous adenitis in Papeete, Tahiti *(6, 19)*. Surprisingly, the first ever reported STB isolates were therefore from three patients with no reported contact with the Horn of Africa, where the vast majority of cases had been reported. In 1997, a fourth STB isolate (So93 strain) was reported as *'M. canettii' (7)*. The more general term 'STB' used here was quoted in a report on *M. tuberculosis* smooth variants in Djibouti *(5)*. Since 1997, a survey of the literature found a total of 93 STB isolates, mainly obtained from patients exposed to tropical countries (Figure 1A). With the notable exception of the three seminal isolates, all isolates were obtained between the 23° 26' 16" N and 23° 26' 16" S parallels in tropical countries with a coastline (Figure 1A). Furthermore, the unusual macroscopic phenotype of the STB strains may delay their diagnosis and may even result in them being under-reported. In Djibouti, no significant difference was found in the prevalence of the pulmonary form between STB [17/30 (56.6%)] and *M. tuberculosis stricto sensu* [2,188/3,772; (58%), P=0, 88>0, 1, X^2 test] (*Plan National de Lutte Anti Tuberculeuse*' 1997). However, the prevalence of enlarged lymph nodes in STB (12/30; 40%) was significantly higher than in *M. tuberculosis stricto sensu* (717/3,772; 19%) (P= 0.038, X^2 test). A recent epidemiological investigation in Djibouti too found that all STB lymph nodes were diagnosed in children and that all STB children had lymph nodes which were infected *(8)*. Indeed, the So93 strain was also obtained from lymphadenitis in a two-year-old Somali child *(7)*. Of note, the age of children with STB lymph nodes in the Horn of Africa shows a bimodal distribution with 7/14 children ≤ 4 years. This is the median age reported for *Mycobacterium avium hominissuis* lymph nodes *(21)*. This observation suggests that young children are infected by suction of contaminated fomites. These clinical observations suggest an oropharyngeal portal of entry for STB. Moreover, reports of STB-infected mesenteric lymph nodes *(15)*, as well one case of STB ascites *(19)* suggest a digestive tract route of infection in addition to the respiratory tract route. Interestingly, in contrast to classical TB infection, there is no evidence of human-to-human transmission of STB infection, suggesting the existence of an as yet unknown environmental reservoir *(5)*.

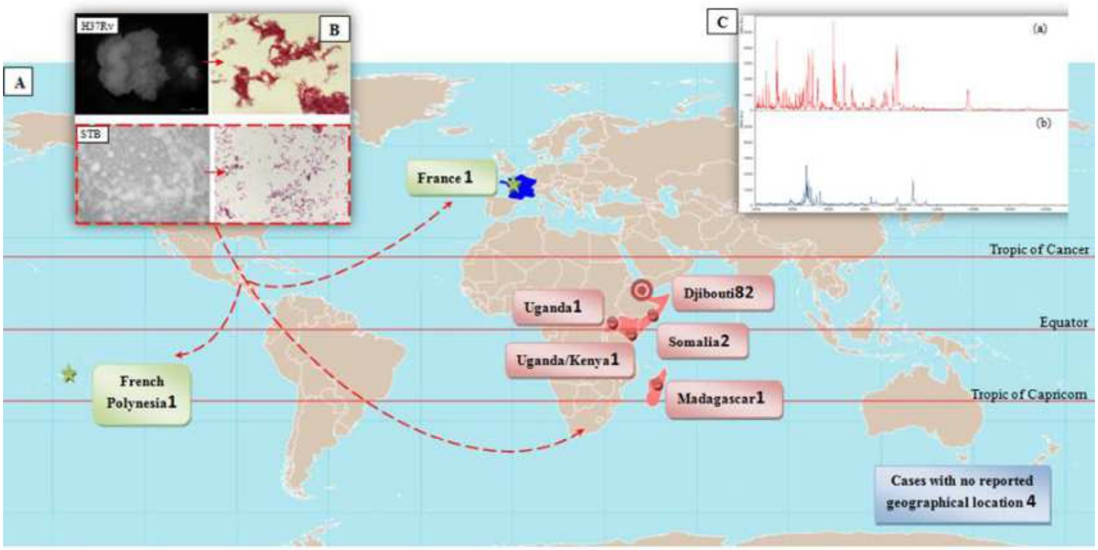

Figure 1: A) Geographical sources for STB infection in 93 patients. B) Aspects of STB and *M. tuberculosis* H37Rv colonies on 7H10 solid Middlebrook medium and Ziehl-Neelsen staining of mycobacteria. STB present smooth colonies and distribution of bacilli in singlets or aggregated small clumps instead of the cord-like aggregates usually seen with the rough H37Rv strains. C) MALDI-TOF spectrum for "*M. canettii*" (a) and *M. tuberculosis* H37Rv (b).

Particularities of the STB organisms

The generation time of STB is two-three times shorter than that of *M. tuberculosis* strains in both liquid media and solid media at 30°C and 37°C (three and eight days for STB and *M. tuberculosis* H37Rv, respectively, at 37°C as measured by BACTEC 460 System in numerical growth units), a feature also of *Mycobacterium microti* (*9*). By definition, STB present smooth colonies which are white to pale beige and glossy (Figure 1B) *(5)* correlating with the presence of a large amount of triglycosyl glycolipids *(7,23,24)*. Through electron microscopy scanning, colonies were observed to vary from small, singular, flat and cone-shaped to larger compound colonies formed by a homogeneous distribution of bacilli in singlets or aggregated in small clumps instead of the cord-like aggregates usually seen with rough MTBC strains *(7)* (Figure 1B). Specific biochemical traits, including antibiotic susceptibility patterns, are reported in Table 1. Matrix-Assisted Laser Desorption Ionisation-Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) fingerprinting *(25)* yields a distinctive peptide spectrum for '*M. canettii*' (Figure 1C).

Five available whole STB genomes indicate a 4.4202 Mb-4.52595 Mb chromosome larger than that of the other MTBC members. Whereas the evolutionary of *M. tuberculosis* is characterized by genome size reduction mostly linked to gene loss and host adaptation, STB carry traces of interactions with donor organisms suggesting environmental organisms which retain a broad spectrum adaptative capability *(17)*. No phages have been observed, but a controversial 55-kb prophage was identified in STB-I *(17, 26)*, nine spacers matching the *Mycobacterium marinum* strain M prophage and two spacers matching the Thibault or Redi *Mycobacterium* phages. These prophages may play a major role in the evolution of STB, as previously reported for *M.* abscessus *(27)*. Further study found that some STB isolates lacked the insertion element *IS*1081, while a new *ISMycA1* (GenBank accession number AJ619854) was discovered in the '*M. canettii*' CIPT140010059 genome *(12)* which is a distinctive characteristic of STB in comparison with the other MTBC members *(12)*. Indeed, the original ''*M. canettii*'' strain (CIPT 140010059) and So93 are indistinguishable from the other MTBC members as a result of sequencing of 16S rRNA and housekeeping genes (*rpo*B, *kat*G, *rps*L and *gyr*A*) (7)*. Nevertheless, further analysis of six housekeeping genes yielded 14 (A-N) STB clonal groups *(12, 17)*. Compared to *M. tuberculosis* H37Rv, investigations showed the presence of an intact region of deletion RD9 and the *M. tuberculosis* specific deletion (TbD1) *(11,29,30)*.

Table 1 : Classification of STB in comparison with *M. tuberculosis* H37Rv.

Characteristics	**STB**	***M. tuberculosis* H37Rv**	**References**
Morphological			
Colony appearance	Eugonic smooth	Eugonic rough	*(5)*
Depth of growth	Aerophilic	Micro-aerophilic	*(22)*
Doubling time in liquid Tween Albumin medium	17 hours	25 hours	*(7)*
Generation time			
- LJ medium	22 (16–25 days)	23 (18–25 days)	*(5) (17)*
- BACTEC 460 System	3 days	8 days	
Growth on minimum solid media (Trypticase-soy agar)	Positive	Negative	*(5)*
Biochemical			
Nitrate reductase	Present	Present	*(5)*
Niacin production	Absent	Present	*(5)*
Drug susceptibility			
Pyrazinamide **(PZA)** **100 mg/L**	Resistant	Sensitive	*(45)*
***Thiophen*-2-carboxylic acid hydrazine (TCH)** **2 mg/L**	Resistant	Resistant	*(15)* *(16)* *(44)*
Streptomycin **(SM)**	2 – 10 µg/mL	0.25–1.0 µg/mL	
Isoniazid **(INH)**	0.2–1 µg/mL	0.016–0.06 µg/mL	
Rifampin **(RIF)**	0.2 µg/mL	0.06–0.25 µg/mL	
Ethambutol **(EMB)**	2.5 – 7.5 µg/mL	0.06–0.25 µg/mL	
Molecular			
Genome size (**Mb**)	4.29797 – 4.52595	4.4115	*(17)*
GC%	65.40 – 65.60	65.50	*(17)*
TbD1 region	Present	Absent	*(29)*

Genomic analysis revealed that the precorrin gene *cob*F, preserved in many environmental mycobacteria, including *M. kansasii (31)*, is also present in all STB but is absent in all other MTBC members *(8, 17)*. STB strains show unprecedented high genetic heterogeneity with traces of intra-species horizontal gene transfer (HGT) compared to the worldwide population of MTBC strains, which represent one of the most extreme examples of a genetically homogeneous group *(8, 12, 17)*. Recently, distributive conjugal transfer was found to be a predominant mechanism for lateral gene transfer among STB, supporting the high heterogeneity observed in this group *(32, 33)*. This mechanism provides an incomparable means for generating rapidly remarkable genetic diversity in a single step, which makes each strain uniquely different from the others *(32)*. Thus a few STB isolates from a geographically restricted region, the Horn of Africa, show a larger genetic diversity than the world-wide population of MTBC strains. These observations led to a new evolutionary scenario for the emergence of pathogenic *M. tuberculosis* from an environmental organism, such as *Mycobacterium kansasii*, through transitional ‘‘smooth’’ tubercle bacilli *(34-36)*.

STB infection models

Only amoebas have been used as a cell model for ‘*M. canettii’* infection *(37)*. In this model, 89% of ‘*M. canettii’* organisms which were co-cultured with free-living *Acanthamoeba polyphaga* amoeba were ingested by trophozoites , a ratio which is significantly higher than for *M. tuberculosis*, *Mycobacterium bovis* and *Mycobacterium avium (37)*. This difference correlates with a 2.56 µm larger size for ‘*M. canettii*’ and smoothness reflecting the specific presence of glycolipid containing triglycosyl. In a *Mycobacterium marinum-Acanthamoeba* coculture model, it was shown that lipooligosaccharide modulates the phagocytosis of mycobacteria in *Acanthamoeba (38)*. In contrast to *M. tuberculosis* and *M. bovis*, ‘*M. canettii*’ survives into cytoplasmic vacuoles and escapes from encystment *(37)*. This specific behaviour could be related to the activation of cellulases Cel6, Cel12 and CBD2 to lyse the cellulose cell wall of the amoebal exocyst *(39, 40)*. In the absence of any known reservoir *(5)*, further studies presenting animal models with contradictory results may not be relevant to natural human infection.

Conclusions

With less than one hundred reported cases, STB infection remains a neglected infectious disease in tropical countries in East Africa. Indeed, their unique morphological features,which are unusual among the MTBC, with smooth, shiny luxuriant and rapidly growing colonies, may lower their presumptive identification as MTBC members. Their cordless appearance observed after Ziehl-Neelsen staining further complicates first-line identification in endemic countries. The reservoirs and mode of transmission remain unknown but comparing clinical data with scarce experimental data suggests contaminated drinking water and food as potential sources, with local replication in the oropharynx and cervical lymph nodes and further dissemination in the respiratory and digestive tracts. In terms of this hypothesis, looking for STB in the stools of patients would be of interest, as it has been observed in patients with *M. tuberculosis* pulmonary tuberculosis *(41, 42)*. Likewise, genetic and genomic data including large genome size and the abundance of phage sequences, suggest that STB form a heterogeneous group of tuberculosis organisms with intermediate features in between mammal-adapted *M. tuberculosis* organisms and environmental organisms such as *M. kansasii (36)*. By means of conclusion, the data reviewed here could form the foundation of efforts towards elucidating the reservoirs and sources of STB, along with the development of laboratory tests aimed at a point-of-care diagnosis of STB infection *(43)*.

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