Clinical characteristics of the smooth tubercle bacilli '*Mycobacterium canettii*' infection suggest the existence of an environmental reservoir

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Abstract

Over a 3-year follow-up, 30 out of the 318 unique *Mycobacterium tuberculosis* complex isolates recovered in the Republic of Djibouti had a smooth-type morphology and were Niacine-negative, the characteristics of '*Mycobacterium canettii*' strains. Unlike *M. tuberculosis*, '*M. canettii*' grew on nutrient-poor media at 30°C, and possessed characteristic lipids. They were isolated from respiratory and extra-respiratory sites from patients with typical forms of tuberculosis. Most cases resolved with antibiotic therapy but in two human immunodeficiency virus-positive patients '*M. canettii*' infection led to septicaemia and death. No cases of human-to-human transmission were observed. The proportion of tuberculosis cases caused by '*M. canettii*' was higher among French patients than among Djiboutian patients. Patients with '*M. canettii*' were significantly younger than those with tuberculosis caused by other *M. tuberculosis* complex strains. Smooth tubercle bacilli could be misidentified as non-tuberculous mycobacteria and appear to be limited to the Horn of Africa. Their characteristics are consistent with the existence of non-human sources of infection.

Keywords: Disease reservoirs, Djibouti, *Mycobacterium canettii*, *Mycobacterium tuberculosis* complex, trimethoprim–sulphamethoxazole combination, tuberculosis
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Introduction

Tuberculosis (TB) is a serious health problem in the Republic of Djibouti, with an estimated incidence of 620 per 100 000 persons in 2007 (ranked third in the world) [1]. This includes refugees from neighbour countries, Somalia and Ethiopia. TB infections are generally caused by *Mycobacterium tuberculosis* and occasionally by *Mycobacterium bovis*. Primary culture of *M. tuberculosis* on Löwenstein–Jensen (LJ) and Coletsos media usually results in characteristic colonies with a rough texture and a typical cord aspect upon microscopic examination. These features enable microbiologists to distinguish *M. tuberculosis* from other mycobacteria species. This is particularly important in developing countries, where biochemical or molecular techniques may not be available.

The Republic of Djibouti appears to be an exceptional place in terms of TB caused by '*Mycobacterium canettii*', a highly unusual tubercle bacillus related to the *M. tuberculosis* complex (MTBC). '*M. canettii*' was first isolated from a 20-year-old French farmer suffering from pulmonary tuberculosis by G. Canetti in 1969. Since then, this strain has been isolated on rare occasions from patients who lived or were presumably infected in East Africa [2–5] Molecular analysis of 43 smooth isolates, mostly originating from the Republic of Djibouti, by Multiple locus Variable Number of Tandem Repeats analysis and by analysis of the Direct Repeat structure showed that the taxon was genetically heterogeneous [6]. The RD12^{can} region first reported to be deleted in two '*M. canettii*' isolates was later shown to be intact in other smooth isolates [7] whereas the existence of mosaicism

suggested that '*M. canettii*' had exchanged genetic material with another mycobacteria species [8]. In this article we report clinical, epidemiological and microbiological data that confirm the existence of a single '*M. canettii*' taxon and that suggest the existence of a reservoir of '*M. canettii*' in the Republic of Djibouti.

Patients and Methods

Ethics statement

In the present retrospective study two categories of bacterial isolates were analysed. Some were collected from sputum as part of the patients' usual care and therefore approval by an ethics committee was not needed. The second category of strains was isolated from lymph nodes as part of a study for which approval of a local ethics committee (the Paul Faure Centre Ethics Committee) had been obtained [9]. A translator speaking multiple languages (Somalian, Arabic, Affar) informed the patients of the study and asked for their consent. The consent was given in writing, mainly by fingerprinting as most of the patients were illiterate. None of the patients refused the specimen collection. Diagnostic methods are usually very basic and discontinuous in Djibouti but, during the work on lymph nodes, state-of-the-art methods were used and the patients benefited from complete therapeutic treatment and follow-up. The patient information and clinical data reported in the present work were rendered anonymous (removing the possibility of tracing the actual patient). Consequently, written informed consent by the patients for the clinical information to be used for research was not asked. This is the accepted procedure for the Paul Faure Centre Ethics Committee in the Republic of Djibouti.

Patients

The majority of patients included in this study attended one of the two medical centres in Djibouti, Republic of Djibouti. The Paul Faure Anti-tuberculosis Centre (PFC) cares for Djiboutian patients with suspected TB. Within this centre, a study was performed between January and April 1999 to evaluate antibiotic resistance patterns [10] and to confirm the performance of the Amplified Mycobacterium Tuberculosis Direct Test for the rapid diagnosis of lymph node tuberculosis [9]. The study involved collecting mycobacteria culture-positive lymph nodes by fine-needle aspiration in patients suspected of having TB, as recommended by the National TB Programme of Djibouti. Therefore, no additional invasive examination was made because of the study. Biological tests that were performed on the bacterial strains resulted in more appropriate and faster implementation of the patient's treatment. When a case was diagnosed, an investigation of the close family members was quickly instituted and all the suspected cases were called for clinical examination. The French Bouffard Military Hospital (BMH) cares for French and Djiboutian soldiers and their families. All patients with a positive culture for mycobacteria between January 1998 and June 2001 were retrospectively included in the study. Eighty-five per cent of these patients were aged more than 15 years.

Sample processing and identification test

Direct smear examination was performed systematically. Acid-fast bacilli were detected in biological samples by auramine staining. Positive results were confirmed by Ziehl–Neelsen staining. The lymph node samples collected at PFC were cultured in Mycobacterial Growth Indicator Tubes (MGIT; Becton-Dickinson, Le Pont de Claix, France) and on solid medium (LJ and Coletsos slants). The strains from patients at BMH were isolated on solid medium only. All the isolates were sent to the Percy Military Hospital for further characterization. Bacteria belonging to the MTBC were identified by 16S ribosomal RNA analysis using the Amplified Mycobacterium Tuberculosis Direct Test (Gen-Probe-bioMerieux, Lyon, France) which does not allow differentiation of '*M. canettii*' from the other members of the MTBC. Conventional biochemical procedures were applied [11] and samples were also tested using the AccuProbe assay (Gen-Probe-bioMerieux). For selected strains, standardized dilutions of colonies were simultaneously cultured on both LJ and trypticase-soy plates (BioRad, Marnes la Coquette, France) at 37°C and 30°C, for each medium. Middlebrook 7H10 and 7H11 media were also used.

Drug susceptibility testing

Susceptibility of the isolates to isoniazid (0.2 g/L), rifampicin (40 g/L), streptomycin (4 g/L), ethambutol (2 g/L), cycloserine (10 g/L), capreomycin (10 g/L), kanamycin (10 g/L) and thiacetazone (2 g/L), was determined by the proportion method on LJ medium [12]. Resistance to pyrazynamide was measured in both liquid medium (BACTEC 960) and LJ medium. Susceptibility to trimethoprim–sulphamethoxazole, amikacin, ciprofloxacin and ofloxacin was tested by the disc diffusion method, using commercially available antibiotic discs (BioRad) [13]. An isolate was considered to be susceptible to one of these molecules when the inhibition diameter was larger than 19 mm [13].

Lipid analysis

Cellular lipids were extracted with chloroform/methanol and then analysed as previously described [14]. The fatty acid