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Mycobacterium tuberculosis complex detection in human remains: tuberculosis spread since the 17th century in Rio de Janeiro, Brazil

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ABSTRACT

Paleogenetic analysis for tuberculosis (TB) was conducted on bone and sediment samples dating from the 17th to 19th centuries from the archeological site of Nossa Senhora do Carmo Church in Rio de Janeiro, Brazil. Forty samples were analyzed, corresponding to 32 individuals from 28 burials, 22 of primary type and 6 of secondary type. The samples were collected following strict paleogenetic investigation guidelines and submitted to ancient DNA (aDNA) extraction. In order to detect TB infection, aDNA hybridizations with the molecular targets of *Mycobacterium tuberculosis* complex (MTC) IS6110 and IS1081 were applied. Additionally, the ancestry of individuals was assessed by human mitochondrial DNA (mtDNA) analysis of hypervariable segment I (HVS-I) sequence polymorphisms. The results of aDNA hybridizations demonstrated varying levels of MTC intensity in 17/32 individuals (53.1%), using the IS6110 target. The IS1081 MTC target showed lower sensitivity, confirming TB positivity in 10/32 (31.2%) individuals. The mtDNA analysis allowed the recovery of HVS-I sequences in 23/32 individuals (71.8%). The majority of these individuals (21/23, 91.3%) were of European ancestry, especially in primary burials. Haplogroups U, J, V, T, K, N, H and R, were identified with haplogroup U being the most frequent at 6/23 (26.1%). African and Amerindian mtDNA haplogroups were observed in two individuals in secondary burials. In spite of the ecclesiastic and aristocratic bias of the population of the study, human ancestry analysis revealed the prominent contribution of Europeans in the introduction or spread of TB in the New World.

1. Introduction

The mycobacterial disease tuberculosis (TB) has plagued humans, and probably our hominid ancestors, for millennia (Wilbur et al., 2008). The epidemiological history of mycobacterial infection has been supported by reports of ancient *Mycobacterium tuberculosis* (Donoghue et al., 2004). In spite of this, some issues remain obscure or have generated debate. Investigations of ancient DNA (aDNA) have been helpful in answering important questions regarding the origin, evolution, and spread of tuberculosis. For many years, it was thought that human tuberculosis evolved from the bovine disease by adaptation of *Mycobacterium bovis* to the human host during domestication, 10,000–15,000 years ago. It is now known that members of the *M. tuberculosis* complex (MTC) evolved from a common ancestor that existed about 3 million years ago (Brosch et al., 2002), and that the bacillus suffered a major bottleneck approximately 15,000–20,000 years ago (Sreevatsan et al., 1997). The recovery of *M. tuberculosis* aDNA from North American bison from the Pleistocene (17,500 years ago) and from Egyptian and South American human mummies have provided strong validation.

Historical evidence supports the epidemic character of TB in Europe from the 16th to the early 20th centuries (Daniel, 2000; Fletcher et al., 2003). Pulmonary tuberculosis is indicated as a major cause of death in Europe during the 18–19th centuries (Cole, 2002). Paleogenetic studies have shown the presence of MTC and *M. tuberculosis* aDNA in European populations. It is recognized that TB existed in America before the arrival of European settlers. Several studies have verified paleopathological evidence on human bones indicative of TB as well as the detection of MTC aDNA, in human remains (Buikstra and Cook, 1981; Gomez i Prat and de Souza, 2003; Salo et al., 1994). In South America, pulmonary and osteological TB have been assessed by MTC aDNA, paleopathology, and multidetector computed tomography (MDCT) analysis, mainly in pre-Columbian populations of Peru and Chile, with single records in Venezuela and Colombia (Allison et al., 1973; Arriaza et al., 1995; Klaus et al., 2010; Requena, 1945; Sotomayor et al., 2004;

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Spigelman and Lemma, 1993). These studies suggest that MTC was introduced by the first human migrations during the peopling of America, remaining at low endemic levels. An epidemic pattern was only reached in urban centers or when biocultural or/and social disruption took place (Gomez i Prat and de Souza, 2003). It has also been suggested that non-tuberculosis *Mycobacterium* species caused infection during this period, and that Europeans introduced new strains of MTC into the Americas. Whether TB emerged or was introduced into New World, the reality is that, clearly, TB became more widespread and potent after the arrival of Europeans, and a large number of cases and death among native Americans was related to intensification of contact with Europeans. There is currently no aDNA record of TB existence in prehistoric or even in historic times in Brazil. More likely than reflecting a lack of infection, this can be attributed to the climatic conditions of the country, which are not favorable to preservation, and therefore, there is a scarcity of mummies or well preserved skeletal remains.

Nossa Senhora do Carmo Church (1761) in Rio de Janeiro was the chapel of the Portuguese royal family during the Empire Colonial period and the Cathedral of the city until 1976. In 2007, during an architectural restoration, archeological excavation of the church identified numerous burials dating from the 17th to 19th centuries. Human remains were collected following paleogenetic investigation guidelines on the archeological site to avoid aDNA degradation and contamination by modern DNA of the skeletal series. The material provided an excellent opportunity to research the ancestral patterns of the human population and the presence of pathogens of parasitic and infectious diseases during the Brazilian historical period since the beginning of Portuguese colonization at the end of the Brazilian Empire. In the present study, we conducted genetic analysis of human remains from Nossa Senhora do Carmo Church in order to (i) detect the presence of TB infection by MTC aDNA analysis; (ii) examine human ancestry of the population buried at the archeological site through mitochondrial DNA (mtDNA) analysis; and (iii) generate new data on the epidemiology of tuberculosis in South America during early European contact.

2. Materials and methods

2.1. The archeological site Nossa Senhora do Carmo Church

The history of the Nossa Senhora do Carmo Church or Antiga Sé (1761) began with the construction of a chapel for the installation of the Carmelite order during the first years following the founding of the city of Rio de Janeiro, before the year 1600. After the collapse of the chapel during a celebration day, the church was rebuilt and officially inaugurated in 1761. In 1808, after the arrival of the Portuguese Royal Family in Brazil, the church was designated as the Royal Chapel. In the same year, the church was named the Cathedral of Rio de Janeiro and remained so until 1976. In 2007, as part of the commemoration of 200th anniversary of the arrival of the Portuguese Royal Family to Brazil, an architectural and artistic restoration was undertaken, and numerous burial sites were discovered under the floor of the church.

The archeological excavation of the Church Nossa Senhora do Carmo was conducted by the Institute of Brazilian Archaeology (Instituto de Arqueologia Brasileira – IAB) from January, 2007 through March, 2008. Twelve areas were excavated and three types of archeological structures were restored: combustion structures, occupied structures, and ceremonial structures (Dias, 2008). Combustion structures were identified as a fire, together with bones, tools, and Neo-Brazilian pottery, located in the more ancient level of the site, suggestive of a prehistoric period pre-dating to the chapel construction (Dias, 2008). Ceremonial structures were identified when artifacts of Catholic rituals where found in the exhumed burials, near or related to the body. Three structures of occupation, the Vermelha Chapel, the Nossa Senhora do Carmo Church complex, and the Senhor dos Passos Chapel were identified. Afro-Brazilian artifacts were found associated with Vermelha Chapel. Forty-three human burials and ossuaries were identified dating from 17th to 19th centuries. The bio-anthropological analysis was conducted according to Buikstra and Ubelaker (1994). Data on gender and age at time of the death were estimated by morphological and morphometric characteristics of the skeletal remains. Samples from 28 burials were analyzed in this study, 22 were classified as primary type and 6 as secondary type. A primary type was defined as the first placement of a totally or almost totally articulated body. Secondary burial is when the remains of a primary burial are exhumed, altered, or moved to an ossuary or other place, and therefore the burial is presented in two or more stages (Souza, 1997). Most primary burials were simple, with only one individual, with 4 being collective, containing more than one individual. Most subjects were young adults, under 30 years old (78%), and there were an equal number of men and women ($n = 8$) (Table 1). The archeological remains selected for TB research comprised a series of bones, teeth, and sediment-samples related to organs usually affected by pulmonary and osseous TB (Table 1). Sediment-samples were fragmented tissue contained in the surrounding matrix, collected *in situ* in the area where the organ would have been located. Pathological evidence of TB, as osseous lesions, was not observed on the rib or vertebrae samples.

2.2. Precautions to avoid contamination

Measures were taken to avoid aDNA degradation, contamination from modern DNA, and cross-contamination during the collection procedures in the archeological excavation and the aDNA analysis, including use of protective clothing, gloves, head covering, masks, and sterile instruments and equipment (Cooper and Poinar, 2000; Drancourt and Raoult, 2005). The excavated samples were immediately placed in sterile containers at 4 °C, protected from light and humidity, and transported to the laboratory. The samples were maintained at −20 °C until the aDNA analysis in the Unit of Paleogenetics, an isolated environment, exclusively dedicated to aDNA research, physically distant from the major laboratory [Laboratory of Molecular Genetics of Microorganisms (LGMM), Oswaldo Cruz Institute/Oswaldo Cruz Foundation (IOC/FIOCRUZ)]. The preparation of samples, aDNA extraction, and PCR were performed at the Unit of Paleogenetics. Electrophoresis, sequencing, and sequence analysis were conducted at the LGMM. Mycobacteria had not previously been introduced into either laboratory. All work surfaces and equipment were treated with sodium hypochlorite and exposed to UV irradiation. All reagents were separated in aliquots for single use. Cotton-filtered tips were always used in pre-PCR and PCR steps. To avoid cross-contamination, each sample was mechanically cleaned and crushed independently. Extraction blank controls were processed in parallel with samples (1 blank for each 6 samples). PCR negative controls, with no DNA template, were always included (1 blank for each 4 samples). The authenticity criteria include the absence of a detectable amplicon in the extraction blank and PCR negative controls; PCR positive controls were not included. More than one target was performed for MTC detection, and host DNA was analyzed in parallel with the MTC DNA. In addition, environmental control samples were collected around and between the burial sites and analyzed to monitor possible MTC contamination of soil.

2.3. aDNA extraction

All bone and teeth samples were decontaminated of exogenous DNA by exposing the surface to UV light for 15 min on all sides, and