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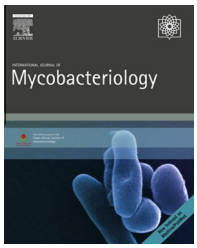

Full Length Article

Tuberculosis in Sardinia: An investigation into the relationship between natives and immigrants

Melania Ruggeri *, *Paola Molicotti*, *Marina Cubeddu*, *Sara Cannas*, *Alessandra Bua*, *Stefania Zanetti*

Department of Biomedical Sciences, University of Sassari, Sassari, Sardinia, Italy

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ABSTRACT

Objective/background: Tuberculosis (TB) has had a recrudescence in the last few decades in Italy as a result of many factors, among which migration from countries where TB is endemic is one of them. In Sardinia, a major island of Italy, there was no knowledge of the mechanisms of transmission of TB in the immigrant subpopulation and the impact it may have on the native subpopulation and on the community as a whole. Therefore, a molecular epidemiological study was carried out to get a clearer picture of the number and genetic features of *Mycobacterium tuberculosis* strains isolated from immigrants and from natives in Sardinia. *Methods:* Two groups of clinical isolates of *M. tuberculosis*, one collected from immigrants and the other one from Sardinians, were analyzed in this study. The genotyping was executed through the variable number tandem repeat-mycobacterial interspersed repetitive units technique and a first-line antimycobacterial drug-susceptibility test was also carried out. *Results:* Thirty-six clinical isolates from immigrants and 25 from Sardinians were analyzed. Variable number tandem repeat-mycobacterial interspersed repetitive units technique showed that all of them belonged to different strains and there was a quite high allelic diversity among them. Moreover, data collected allowed the finding of, with a good approximation, the phylogenetic relations among the strains isolated and the best-known phylogenetic groups. *Conclusion:* The study pointed out that since every strain is different, there was no TB transmission in any of the subpopulations and between immigrants and natives. This showed that the presence of immigrants was not a risk factor for contracting TB in the community.

* Corresponding author at: Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43 B, Sassari, Sardinia 07100, Italy.
E-mail address: melaruggeri@libero.it (M. Ruggeri).

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Introduction

Tuberculosis (TB) is the leading cause of adult death due to a single infectious agent worldwide [1]. The most recent data of the annual report "Global Tuberculosis Control" of the World Health Organization estimated that there were 9.6 million new cases and 1.5 million deaths in 2014. The most affected geographical areas are low-income developing countries in Africa (280 new cases/100,000 inhabitants) and Asia (210 new cases/100,000 inhabitants); percentages are decidedly lower in Europe (40 new cases/100,000 inhabitants) and Americas (30 new cases/100,000 inhabitants). In industrialized countries, after a significant decrease in TB incidence over the last century, there has been a recrudescence, due especially to the massive immigration from low-income and middle-income countries where the illness is endemic, to the appearance of multidrug resistant (MDR) strains and to the lack of prevention and control programs [1].

In Italy, the incidence of TB has registered a progressive increase in the last few decades; however, it has remained constant—under 10 new cases/100,000 inhabitants—therefore, Italy is considered a low incidence country. This increase has not involved all people in the same way, but it has affected some social classes, among which are immigrants. In 2008, the incidence rate was 3.8 cases/100,000 for people born in Italy and 50–60 cases/100,000 for people born abroad [2–4]. Foreign-born people coming from countries with a high incidence of TB are a population at high risk to become sick: (1) firstly, because they come from areas in which the disease is endemic and therefore is highly probable to contract the infection; and (2) secondly, their standard of living both in their country of origin and in Italy is not often compatible with the maintenance of good health. Consequently, the risk of developing TB is higher than the national average [4–7]. Some studies have shown that the presence of immigrants in low-incidence countries did not increase the risk for the native population of contracting the disease [8–10]. However, the same studies suggested that the situation may change depending on the degree of interaction between immigrants and natives, as well as among immigrants of different nationalities, which are related to social factors specific for each place [8–10].

Also, in Sardinia, one of the two major islands of Italy, the migration rate has progressively increased, and at the same time the percentage of immigrants among TB patients has also increased [3]. Sardinia is a low-density population island, with 68 inhabitants/km^2 and a percentage of resident immigrants of 2.5% [4]. They come from TB high-incidence countries, mainly from the African regions of Maghreb and Senegal, from East Asia, and from East Europe. Most of them are well integrated in the social fabric, where they work especially as care givers or shoppers, and they frequent the same places of natives (i.e., schools and supermarkets) [5]. In order to plan effective control programs, it is necessary to know the dynamics of transmission both in the population as a whole and in the subpopulations of which it is composed, especially those at high risk such as the immigrants [6,11]. To understand the mechanism of transmission of TB in the immigrants' subpopulation in Sardinia and the impact it may have on the native subpopulation and on the community as a whole, a molecular epidemiological study was carried out to get a clearer picture of the number and genetic features of *Mycobacterium tuberculosis* strains isolated from immigrants and from natives in Sardinia.

Materials and methods

M. tuberculosis strains

Two groups of clinical isolates of *M. tuberculosis* collected over a 10-year period were analyzed and compared in this study. The first group comprised of 36 isolates from immigrants coming from Africa, East Europe, and Asia. The second group was made up of 25 isolates from Sardinians coming from different districts of the land.

DNA extraction

Before starting the genotyping, every clinical isolate was inoculated in Middlebrook 7H9 added with ADC (Albumin Dextrose Catalase) Microbiol diagnostici (Z.I. Macchiareddu, Uta-Cagliari) until the log phase was reached. Also, the reference strain *H37Rv* was inoculated and used as a positive control in the following steps. DNA was then extracted and purified from each mycobacterial culture and used for the genotyping through the N-cetyl-N,N,N-trimethyl ammonium bromide procedure, following standard protocols [11].

Variable number tandem repeat-mycobacterial interspersed repetitive units genotyping

The isolates were genotyped by PCR amplification of a set of 15 loci MIRU with a discriminatory power among isolates of 96% of the total resolution, specific for epidemiological investigations (Table 1) [12,13]. PCR amplifications were performed using three different mixtures, characterized by different concentrations of magnesium. For each locus, the one that granted the best results was chosen. Mixes were prepared with 2 μL of 10× buffer, 4 μL of Betaine (Sigma-Aldrich Merck), 1.6 μL (mix 1) or 1.2 μL (mix 2) or 0.8 μL (mix 3) of $MgCl_2$, 0.2 μL of dATP, dCTP, dGTP and dTTP, 0.8 μL of each primer, 0.08 μL di DNA-polymerase and distilled water up to 18 μL. 2 μL of the mycobacterial DNA previously extracted and purified were added to the mix, for a final volume of 20 μL [14,15].

PCR were run in a thermal cycler, under the same condition for each locus: 95 °C for 15 min, 40 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C per 1:30 min, followed by 72 °C for 10 min. PCR products were analysed by 2% agarose gel electrophoresis, using 100-bp and 50-bp DNA ladders [14,15]. For each isolate and each locus, the number of the allelic repetitions was calculated through a comparison between the lenght of the amplicon obtained and the length of the amplicons of H37Rv.

In this way it was possible to associate to each isolate a numerical code formed by the allelic repetitions for each locus considered in the same order for all the isolates.

In the elaboration of the results, an index of allelic diversity D was calculated with the following formula: