

# Impact of immigration on tuberculosis in a low-incidence area of Italy: a molecular epidemiological approach

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## Abstract

The effects that immigration might have on the epidemiology of tuberculosis (TB) in a low-incidence area of Italy was investigated by determining, in autochthonous and immigrant TB patients, the molecular characteristics of the *Mycobacterium tuberculosis* complex (MTBC) isolates, which may provide information on their phylogeographical origin. A total of 1080 MTBC strains, collected during a 4-year period in Tuscany from 614 Italian-born and 466 foreign-born patients, were genotyped by spoligotyping and assigned to the different phylogeographical lineages that constitute the MTBC. The autochthonous Euro-American phylogeographical lineage, which includes the spoligotype families T, Haarlem, Latin American–Mediterranean (LAM), S and X, was highly prevalent among Italian-born patients, with a total of 477 cases (77.7%), and foreign-born TB patients, with a total of 270 cases (57.9%); 24 Italian-born (3.9%) and 141 foreign-born (30.3%) TB cases were due to MTBC genotypic families associated with distant geographical areas, i.e. East African–Indian (EAI), Beijing, Central Asian (CAS), and *Mycobacterium africanum*. Strains of *Mycobacterium bovis* and strains of undefined genotype, which are all considered together, as it is not possible to assign a specific geographical origin, accounted for 113 (18.4%) Italian cases and 55 (11.8%) foreign-born cases. A total of 79 Italian TB cases (12.9%) have been attributed to transmission from immigrants to the local population. No significant contribution to drug resistance appeared to be associated with imported MTBC strains. It is concluded that, at present, the overall impact of imported TB on public health in the low-incidence study area is relatively modest and of the same order as in other western countries.

**Keywords:** Molecular typing, *Mycobacterium tuberculosis*, spoligotyping

**Original Submission:** 4 September 2009; **Revised Submission:** 10 November 2009; **Accepted:** 10 December 2009

Editor: M. Drancourt

**Article published online:** 23 December 2009

*Clin Microbiol Infect* 2010; **16**: 1691–1697

10.1111/j.1469-0691.2010.03149.x

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## Introduction

Tuberculosis (TB) is the world's second most common cause of death from an infectious disease, after human immunodeficiency virus/AIDS, causing 50–100 million infections, 8–9 million new cases and nearly 2 million deaths every year [1]. As the global burden of TB falls principally on developing countries, accurate estimates of the role of immigration on the epidemiology of TB in low-prevalence countries are essential for the design of appropriate strategies for disease control.

In the present study, we investigated the impact that immigration might have on TB in a low-incidence area of

Italy by determining the molecular characteristics of *Mycobacterium tuberculosis* complex (MTBC) isolates, which may provide information on their origin. The MTBC consists, in fact, of different phylogeographical lineages [2–4], which are currently defined by at least two major types of genomic polymorphism, i.e. polymorphisms of the DR locus, assessed with the spoligotyping assay [5], and polymorphisms due to deletions of large chromosomal regions, termed large-sequence polymorphisms (LSPs) [3]. Spoligotyping classifies the MTBC strains into at least 15 families, including some heterogeneous 'ill-defined' families [6]; LSP analysis, on the other hand, classifies MTBC strains into six major phylogeographical lineages [3,7]. In both cases, certain spoligotype families or LSP-defined lineages are associated with specific human populations and geographical areas, whereas others are practically ubiquitous or, at least, widespread. If the two classification systems are combined, the MTBC lineages consist of: (i) the Indo-Oceanic lineage, corresponding to the spoligo-

type-defined East African Indian (EAI) family, occurring all around the Indian Ocean; (ii) the East Asian lineage, which includes the spoligotype-defined Beijing family, and is highly prevalent in many countries of East and Southeast Asia and is rapidly spreading in eastern Europe; (iii) the East African–Indian lineage, which is prevalent in East Africa, North India and Pakistan, and includes the spoligotype-defined Central Asian (CAS) family; (iv) the evolutionarily more recent Euro-American lineage, which is the most frequent lineage in Europe and the Americas, and has also spread across different regions of Africa and the Middle East, and includes the spoligotype families T, Haarlem, Latin American–Mediterranean (LAM), S and X; and (v) the West African I and the West African II lineages (from which *Mycobacterium bovis* originated), corresponding to strains traditionally identified as *Mycobacterium africanum* [3].

In this investigation, MTBC strains isolated from Italian-born and foreign-born patients during a 4-year period in Tuscany, a region of central Italy with a low incidence of TB, were genotyped by spoligotyping and assigned to the different phylogeographical lineages, in order to quantitate autochthonous and imported MTBC strains, to monitor incoming and expanding genotypes, to estimate the extent of transmission of imported strains to the local population and, finally, to evaluate the contribution, if any, of immigration to drug resistance in circulating MTBC strains.

## Materials and Methods

### Clinical isolates

A total of 1080 MTBC isolates were collected during a 4-year period, from 1 January 2002 to 31 December 2005, from the same number of TB patients hospitalized in Tuscany, Italy. The study isolates represent the vast majority (>90%) of the isolates in the region during the study period. All isolates were identified using DNA molecular probes (Accuprobe (Gen-Probe, San Diego, USA) and/or InnoLipa (Innogenetics, Ghent, Belgium)). The drug susceptibilities of isolates were determined with the radiometric BACTEC 460 TB system or the fluorimetric MGIT 960 system (Becton Dickinson, Towson, MD, USA), in accordance with the manufacturers' recommendations.

### Spoligotyping and phylogeographical lineages

Spoligotyping analysis of the isolates was performed basically as described by Kamerbeek *et al.* [5]. MTBC isolates were assigned to one of the 15 genotypic families defined in the international spoligotype database SpolDB4 of the Pasteur Institute of Guadeloupe [6], according to specific rules defin-

ing the major phylogenetic families for *M. tuberculosis sensu stricto*, i.e. EAI, Beijing, CAS, T, Haarlem, LAM, S and X, as well as signatures for the species *M. africanum* and *M. bovis*. In a second step, the spoligotype families were assigned to the LSP-based phylogeographical lineages defined by Gagneaux *et al.* [3,4]; in particular, isolates of the T, Haarlem, LAM, S and X families were assigned to the Euro-American lineage; the EAI, Beijing and CAS families were assigned to the Indo-Oceanic, East Asian and East African–Indian lineages, respectively; isolates with spoligotype signatures for *M. africanum* and *M. bovis* were considered to be distinct genotypic lineages.

## Results and Discussion

### Study patients

Tuscany is a region of central Italy, a low-incidence country with notification rates of bacteriologically confirmed TB cases ranging from six to ten per 100 000 population in the last 25 years. During the study years, the population of Tuscany was  $3.5\text{--}3.6 \times 10^6$  persons, of whom approximately 5–6% were foreign-born. The epidemiological features of study TB patients are summarized in Table 1. The country of birth of the 1080 study patients was known for 94.5% patients; 614 (56.9%) were Italian-born, and 466 (43.1%) were foreign-born. The Italian-born/foreign-born ratio decreased from 1.62 in 2002 to 1.15 in 2005, indicating a steady increase in foreign-born TB cases. Of the foreign-born patients, 27.9% were from Africa, 23.6% from Asia, 22.3% from Eastern Europe, and 11.6% from Central and South America.

### Distribution of MTBC clinical isolates among phylogeographical lineages

The MTBC clinical isolates were genotyped by spoligotyping and assigned to the spoligotype families and LSP

**TABLE 1.** The tuberculosis (TB) patients in this study

TB patients	Year				Total
	2002	2003	2004	2005	
No. of TB patients					
Total	254	268	306	252	1080
No. of Italian-born patients	157	152	170	135	614
No. of foreign-born patients	97	116	136	117	466
Italian-born/foreign-born ratio	1.62	1.31	1.25	1.15	1.32
No. of foreign-born patients from:					
Africa	36	35	30	29	130
Asia	19	25	33	33	110
Eastern Europe	11	24	40	29	104
Central and South America	11	13	14	16	54
Other countries <sup>a</sup>	20	19	19	10	68

<sup>a</sup>Includes patients from western Europe, North America, and Oceania, and patients whose country of birth is unknown.

**TABLE 2.** Distribution of *Mycobacterium tuberculosis* complex (MTBC) clinical isolates among genotypic lineages

MTBC genotypic lineage <sup>a</sup> and spoligotype-based families <sup>b</sup>	TB patients			Geographical area of birth of foreign-born patients				
	Italian-born	Foreign-born	Total (%)	Africa	Asia	Eastern Europe	Central and South America	Other countries <sup>c</sup>
Euro-American	477	270	747 (69.2)	90	17	80	35	48
T	213	98		29	9	39	7	14
Haarlem	137	93		21	4	32	17	19
LAM	69	69		37	3	5	10	14
S	50	4		2	—	2	—	—
X	8	6		1	1	2	1	1
Indo-Oceanic, EAI	8	37	45 (4.2)	2	26	1	1	7
East Asian, Beijing	16	59	75 (6.9)	2	36	7	9	5
East African-Indian, CAS	0	29	29 (2.7)	5	21	—	—	3
<i>Mycobacterium africanum</i> , AFRI 2 and I	0	16	16 (1.5)	16	—	—	—	—
<i>Mycobacterium bovis</i> , BOVIS	15	6	21 (1.9)	3	2	—	1	—
Undefined <sup>d</sup>	98	49	147 (13.6)	12	8	16	8	5

<sup>a</sup>According to Gagneux et al. [3], with modifications (see text).

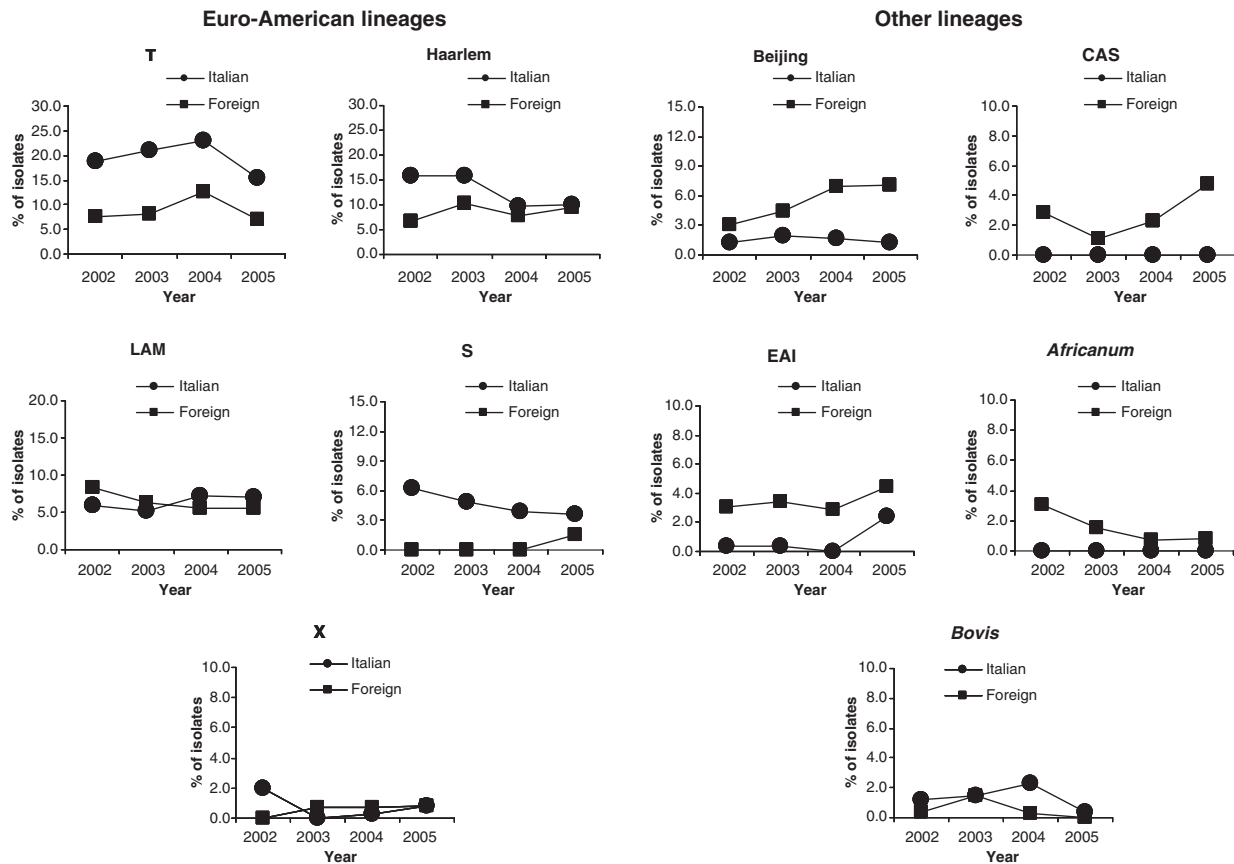
<sup>b</sup>Based on spoligotyping according to Brudey et al. [6].

<sup>c</sup>Includes western Europe, North America, and Oceania, and patients whose country of birth is unknown.

<sup>d</sup>Includes isolates with a signature that is as yet undefined or with uncertain classification in SpoIDB4.

phylogeographical lineages according to the criteria reported in Materials and Methods. The distribution of MTBC isolates from Italian-born and foreign-born patients among the genotypic lineages and the geographical origins

of foreign-born patients are given in Table 2; the time courses for the different genotypic lineages for Italian-born and foreign-born patients are reported in Fig. 1. The particular lineages were as follows.



**FIG. 1.** Distribution of *Mycobacterium tuberculosis* complex clinical isolates among genotypic lineages in Italian-born and foreign-born tuberculosis patients during the years 2002–2005 in Tuscany, Italy. The spoligotype-defined family of isolates is given above each panel. CAS, Central Asian; EAI, East African-Indian; LAM, Latin American-Mediterranean.

**Euro-American lineage.** As expected, MTBC isolates of the widespread Euro-American lineage were highly prevalent and represented 69.2% of all isolates. Within this lineage, the spoligotype families were ranked in the following order: T (41.6% of all Euro-American isolates), Haarlem (30.8%), LAM (18.5%), S (7.2%) and X (1.9%). The distribution of isolates of the ubiquitous T family in general reflected the proportions of Italian-born and foreign-born patients (Fig. 1). For foreign-born patients, eastern Europe and Africa accounted for the large majority of cases. Isolates of the Haarlem family were also proportionally distributed between Italian-born and foreign-born patients; however, Haarlem isolates from Italian-born patients showed a decrease in frequency, whereas they increased in frequency among foreign-born patients (Fig. 1); eastern Europe, Africa and the Americas were the major geographical areas of birth of Haarlem TB patients. The LAM isolates were, on the whole, equally distributed between Italian-born and foreign-born patients, with a small, if any, decrease in frequency among Italian-born patients and a moderate increase in frequency in foreign-born patients; as expected, Africa (particularly the Mediterranean countries) and South America accounted for the origins of most patients. The autochthonous S family was highly prevalent among Italian-born patients (50 of 54 patients, 92.6%); these strains showed a decrease in frequency over the years. Interestingly, no TB case due to S strains was found among immigrants in the first 3 years of the survey; all foreign-born cases (two from North Africa and two from eastern Europe) occurred in 2005. X strains, of Anglo-Saxon origin, were rarely found (1.3% of the total).

**Indo-Oceanic lineage.** A total of 45 isolates (4.2%) were of the Indo-Oceanic phylogeographical lineage (EAI family, according to spoligotyping), which is prevalent all around the Indian Ocean. Most isolates were from Asian patients; only three Italian patients were infected by this genotype in the years 2002–2004, but five Italian TB cases with EAI isolates were recorded in 2005, all of them sharing the rare SIT2852 spoligotype; these isolates clustered with a sixth SIT2852 isolate from a patient from the Philippines, thus suggesting the possibility of a recent TB outbreak.

**East Asian lineage.** A relatively high number of isolates (75, 6.9% of the total) belonged to the East Asian phylogeographical lineage, in particular to the Beijing family which included 68 'true' Beijing strains (SIT1) and seven Beijing-like strains. Most patients with Beijing strains (78.7%) were foreign-born; Asia, and China in particular, accounted for the origin of most patients (36 of 59); this was expected, as Asia has been

the main source of importation of Beijing strains to European countries [8,9]; there was also a consistent proportion from eastern Europe and South America (Peru in particular); this finding seems not to be casual, as the South American route of importation of Beijing strains to Europe has already been reported from Spain [10].

Importantly, the proportion of foreign-born Beijing patients increased during the years of our survey (Fig. 1). Also, the number of Italian-born Beijing patients was relatively high (16 patients, 21.3%), consisting of three to five patients each year, which might indicate ongoing active transmission of Beijing TB involving autochthonous residents.

**East African–Indian lineage.** Strains of the East African–Indian phylogeographical lineage (CAS family, according to spoligotyping) were exclusively found in foreign-born patients, mostly (21 of 29, 72.4%) from Asia (Pakistan in particular). Starting from 2003, the frequency of this genotype seems to be increasing in the region (Fig. 1).

***M. africanum.*** *M. africanum* strains were similarly isolated only from foreign-born patients, all from Africa, but their number showed a steady decrease (Fig. 1).

***M. bovis.*** *M. bovis* strains (21 isolates) accounted for just under 2% of all TB cases. Most *M. bovis* TB cases (15 cases, three of which were caused by *M. bovis* bacille Calmette–Guérin) occurred in Italian-born patients aged 33–93 years (mean, 69 years), which probably indicates, at least in most cases, a reactivation of a remote infection; the six foreign-born patients with *M. bovis* TB were young people (mean age, 28 years; range, 21–38 years), indicating a recently acquired infection.

#### **Considerations concerning the impact of immigration on the spread of TB**

From the data in Table 2, it appears that only 24 Italian TB cases of 614 (3.9%) were due to MTBC strains of genotypic families (EAI and Beijing) typical of distant geographical areas. These strains, which were very probably transmitted from immigrants to the local population, constitute approximately 17% of the 141 strains, isolated from immigrants, that are prevalent in distant geographical areas, i.e. strains of the Indo-Oceanic (EAI), East-Asian (Beijing), East African–Indian (CAS) and *M. africanum* genotypes. Assuming that strains of the Euro-American lineage (270 in foreign-born patients) and strains of *M. bovis* or undefined genotypes (55 altogether) were also transmitted from immigrants to the local population to the same extent (i.e. 17%), it can be calculated that in Italian-born patients a further 46 and nine cases could be,

at least theoretically, attributed to imported Euro-American and *M. bovis*/undefined strains, respectively. This gives a total of 79 cases of 614 (12.9%) TB cases that were potentially transmitted from immigrants to the local population. Obviously, this proportion is theoretical, and its estimation is perhaps too simplistic. It probably overestimates the proportion of imported strains transmitted to the local population because of at least two factors: (i) not all of the TB cases in foreign-born patients may be due to infections acquired in their native countries, as indicated by the TB cases in immigrants caused by the strains of the local S genotype—in this regard, extensive transmission between the local and foreign-born populations, caused mainly by autochthonous strains, has been reported from Madrid, Spain [11]; and (ii) our estimate is based on the assumption that all MTBC strains are transmitted with the same efficiency, and does not take into consideration that the MTBC genotypic families may be endowed with different transmissibility properties—for example, direct human-to-human transmission seems to be rare for *M. bovis*, but, on the other hand, highly efficient for the *M. tuberculosis* Beijing family [12,13].

Thus, at present, it appears that in our low-incidence area the overall impact of imported TB on public health is relatively modest and of the same order as in other western areas. In fact, the estimated proportion of native-born TB cases that can be attributed to transmission from the foreign-born individuals has been reported to be of the order 11–17% in Dutch, Canadian and US cities [14–18]. Several

other reports from western countries generally agree that immigration has little impact on transmission of TB to local populations, although it may help to maintain or increase the total number of cases of disease [19–23]. Hirsh et al. [7] have proposed a stable association between the TB pathogen and host that restricts the spread of the organism among different populations. This view, however, is only partly confirmed in our setting, as, considering the phylogeographical lineages other than the Euro-American lineage, only the East African–Indian (CAS) and the *M. africanum* strains remained associated with their host populations, without any transmission to the local population, whereas several cases of TB caused by Indo-Oceanic (EAI) and East Asian (Beijing) strains did occur among Italian born-patients. In this regard, the Beijing family of strains raises concern, as their prevalence (6.9%), although markedly lower than that reported in East and Southeast Asia and in eastern Europe, is higher than that reported in other western European countries [12]; moreover, Beijing strains are rapidly spreading worldwide [12,24,25], are, in general, considered to be hypervirulent [26,27], and have a propensity to develop drug resistance [12].

#### Drug resistance of MTBC isolates

As shown in Table 3, of a total of 1051 MTBC clinical isolates from Italian-born and foreign-born patients for which drug resistance data were available, 86.0% were susceptible to all first-line anti-TB drugs tested, i.e. rifampin, isoniazid,

**TABLE 3.** Drug resistance of *Mycobacterium tuberculosis* complex (MTBC) clinical isolates in Italian-born and foreign-born tuberculosis (TB) patients

TB patients	Pan-susceptible, no. (%)	Any drug resistance <sup>a</sup>				MDR			
		MTBC genotypic lineage				MTBC genotypic lineage			
		All lineages, no. (%)	Euro-American, no.	Other than Euro-American, no.	Undefined and BOVIS, no.	All lineages, no. (%)	Euro-American, no.	Other than Euro-American, no.	Undefined and BOVIS, no.
Italian-born, n = 597	521 (87.3)	68 (11.4)	Total 44 17 T 13 Haarlem 8 LAM 6 S	Total 3 3 Beijing	Total 21 14 undefined 7 BOVIS	8 (1.3)	Total 7 3 T 2 Haarlem 1 LAM 1 S	–	Total 1 1 undefined
Foreign-born, n = 454	383 (84.4)	58 (12.8)	Total 35 14 T 9 Haarlem 10 LAM 1 S 1 X	Total 15 7 Beijing 4 CAS 2 EAI 2 AFRI	Total 8 6 undefined 2 BOVIS	13 (2.9)	Total 9 4 T 4 Haarlem 1 LAM	Total 4 3 Beijing 1 EAI	–
Total, n = 1051	904 (86.0)	126 (12.0)	Total 79 31 T 22 Haarlem 18 LAM 7 S 1 X	Total 18 10 Beijing 4 CAS 2 EAI 2 AFRI	Total 29 20 undefined 9 BOVIS	21 (2.0)	Total 16 7 T 6 Haarlem 2 LAM 1 S	Total 4 3 Beijing 1 EAI	Total 1 1 undefined

CAS, Central Asian; EAI, East African–Indian; LAM, Latin American–Mediterranean; MDR, multidrug-resistant, i.e. resistant to at least rifampin and isoniazid.

<sup>a</sup>Includes isolates, other than MDR isolates, that are resistant to one or more first-line anti-TB drugs (rifampin, isoniazid, pyrazinamide, ethambutol and streptomycin).

pyrazinamide and ethambutol (data for streptomycin susceptibility were available for 346 isolates); 12.0% were resistant to one or more first-line drugs; and 2.0% were multidrug-resistant (MDR), i.e. resistant to at least rifampin and isoniazid. No extensively drug-resistant isolate was found. The proportions of isolates resistant to any drug did not differ statistically between Italian-born (11.4%) and foreign-born (12.8%) patients. The proportion of MDR isolates from foreign-born patients (13 of 454, 2.9%) was more than twice as high as that from Italian-born patients (8 of 597, 1.3%), but the difference did not reach statistical significance.

With regard to the phylogeographical lineages, most isolates resistant to one or more anti-TB drugs belonged to the Euro-American lineage (44 of 68 from Italian-born and 35 of 58 from foreign-born patients), with no particular prevalence of a particular spoligotype family being seen. Among the other phylogenetic lineages, drug resistance was restricted to three Beijing isolates from Italian-born patients and 15 isolates from foreign-born patients; in these patients, Beijing isolates accounted for approximately half (seven of 15) of the drug-resistant isolates. Most MDR isolates belonged to families of the Euro-American lineage, particularly T and Haarlem, in both Italian-born and foreign-born patients; no MDR isolate of genotypic lineage other than Euro-American was found among Italian patients, whereas one EAI isolate and three Beijing MDR isolates were detected among foreign-born patients.

In conclusion, in Tuscany, Italy, despite the high and increasing proportion of TB cases in immigrants, transmission of imported MTBC strains to the local population appears to be relatively modest at present; moreover, immigration does not seem to have a significant impact on the drug resistance of circulating strains. It should be underscored, however, that the impact of the imported TB on the spread of the disease to the local population is difficult to measure or forecast, owing to the chronic nature of TB, as infected individuals may stay latently infected for many years before they develop active TB. This highlights the need for continued and improved surveillance for TB among the local-born population and newly arrived immigrants, especially those from countries with high endemicity.

## Transparency Declaration

This work was financially supported by the University of Pisa and, partly, by the Italian 'Istituto Superiore di Sanità' (National Research Program on AIDS-2006, ISS Grant no. 50G.18).

The authors declare that they have no conflicting interests.

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