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Case Report

Epidemic of tuberculosis in a high school in Northern Sardinia

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ABSTRACT

The aim of this study was to investigate the *Mycobacterium tuberculosis* transmission among high school student and teacher populations in a high school in Northern Sardinia.

Tuberculin skin-test screening, chest-X-rays, QuantiFERON-TB Gold, microbiological examination, spoligotyping and variable numbers of tandem repeats (VNTR) analysis of *M. tuberculosis* isolates were performed.

This study indicates the effectiveness of the epidemiological investigation.

In schools there are special circumstances of living together that favor the emergence of tuberculosis (TB) outbreaks [1]. An outbreak of pulmonary TB in a high school was reported to the "Public Health Service" of Northern Sardinia. In December 2008 a young boy, attending the third class of a high school, was diagnosed with a case of pneumonia and was treated at home, but on New Year's Day he was admitted to hospital with hemoptysis and fever and was diagnosed with pulmonary TB. This study highlights the public health interventions that can be implemented to improve contact investigations and to readily identify the subjects who develop active disease in order to contain the transmission of tubercular infection.

Tuberculosis Skin Testing (TST) and chest-X-rays were carried out at the Public Health Service of Alghero. TST testing was carried out using Mantoux method with 5 IU of Protein Purified Derivative (PPD) (Biocine Test, Chiron Siena), and it was considered positive when the induration was $\geqslant$10 mm. TST was performed in two stages: at the time of the identification of the first case of pulmonary TB and 2 months later.

QuantiFERON TB Gold in tube (QFT-TB) (Cellestis Limited, Australia) was performed on the students with a negative TST and was carried out in the laboratory of Mycobacteriology, University of Sassari, following the kit's directions.

Microbiologic examinations on subjects with suspected active TB were carried out in the laboratory of Mycobacteriology, University of Sassari, according to standard protocols [2–4].

The drug sensitivity assay was performed on the isolated strains with the proportional method using the BACTEC MGIT 960 system.

Genotyping based on spoligotyping was executed by Genoscreen (Lille, France), 15-loci variable numbers of tandem

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Table 1 – Characteristics of the study population.

Patient characteristics	Number (percentage)
Contact	
Students	130
Teachers	51
Other contacts	393
Total	574
Race	
Italian	574 (100%)
Tuberculosis	
Active TB	5 (0.9%)
Latent TB	178 (99.1%)
TST results	
First screening	
TST Positive[1]	153 (27%)
TST Negative[2]	421 (73%)
Second screening (on 231 subjects)[3]	
TST positive	19 (8%)
TST negative	212 (92%)
Total TST positive	172 (30%)
QFT-IT results[4]	
QFT-IT negative	81
QFT-IT positive	2

1 TST was positive in 36 students, 36 teachers and 76 other contacts.
2 TST was negative in 94 students, 16 teachers and 317 other contacts.
3 The subjects negative to both tests and 107 people, who were not considered at risk, were not included in the second screening with the TST.
4 Performed on 83 students with negative TST.

repeats analysis (VNTR-MIRU) was performed on the clinical isolates of *Mycobacterium tuberculosis* in the laboratory of Mycobacteriology, University of Rome, following standard protocol [5].

The TST and QFT-TB results were indicated in Table 1. Among all the subjects with positive TST, 54 received the preventive therapy; five subjects developed active TB: a professor, three students of the school and a patient who attended the same sports club of a student with active TB. All of these subjects were tested positive for *M. tuberculosis* to all microbiological examinations performed and all had positive QFT-TB, TST and chest-X-ray. Two children, the son of the ill professor and another one who had had contact with a sick person, showed a radiological picture compatible with pulmonary TB, and they were immediately treated with anti-tubercular therapy. The microscopic and molecular tests were positive for the son of the teacher; however the culture assay was negative. The second child was negative to all microbiological examinations performed.

Each isolated strain of *M. tuberculosis* was assessed for sensitivity to streptomycin, isoniazid, rifampicin and ethambutol. The strains were susceptible to all drugs tested.

The microbiological examinations were also performed on two family members of the two students with active TB and the results were negative. Both close contacts were positive to QFT-TB and TST.

The teacher who developed active TB is a 49-year-old Italian man, who teaches both in the class attended by the students with active TB and in two other classes at the same school.

Spoligotyping indicated that the tubercular strains isolated from students and from a friend of one of them were identical, while those isolated from the teacher was different in the 15 spacer. VNTR-MIRU analysis indicated that all subjects were infected by the same strain of *M. tuberculosis* (Fig. 1).

Schools are settings with high concentrations of young people where conditions of crowding and age-related susceptibility favor the emergence of TB outbreaks [6].

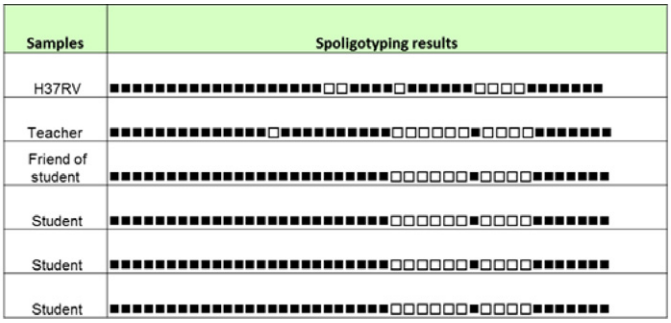
Several studies reported that environmental factors play an important role in the transmission of TB infection [7,8]. In this outbreak, the index case was a teacher who had begun to be symptomatic 9 months before he was diagnosed with TB. During the time that the professor was symptomatic none developed active TB, and this may be related to the period when his symptoms began to appear. The index case began to show signs in spring when the fine weather made it possible to keep the windows open, whereby the class had better ventilation and frequent airing out. Later, there were summer holidays, so for several months the contact between the symptomatic professor and the students was interrupted. At the beginning of the school year contact was resumed, and with the arrival of autumn and winter, conditions such as poor ventilation and air exchange facilitated the transmission of *M. tuberculosis* and consequently the development of active disease in some individuals more susceptible than others to the tubercular bacillus.

The cases of active TB occurred within the same class even if the index case was teaching in two other classes of the same school. It has been shown that the host factors may influence TB's epidemiology. It seems that there is genetic variability (ethnic and racial factors) in human TB susceptibility [9,10]. In this case, the researchers of this study were of the opinion that ethnic and racial factors should not be considered as possible risk factors since the examined population was homogeneous. Probably the professor would spend many hours in that class, and it is obvious that there is a relation between the hours spent together with the index case and the risk of infectivity [11].

It is interesting that in two children with a radiological picture compatible with pulmonary TB that the culture assay is negative; probably this was the result of the immediate treatment with anti-tubercular therapy.

In the present study spoligotyping and VNTR-MIRU techniques were used to establish the genetic relationship between the *M. tuberculosis* strains isolated from patients with active TB. All five isolates showed identical patterns with standard VNTR-MIRU typing; in contrast, a small variation was detected in the isolate of the teacher using the spoligotyping method. Despite this variation, both the above genotyping results and the TB patient's history strongly indicate that the five isolates are not independent, but represent the progeny of a single, original strain. It could be argued that observed change results from microevolution in a clonal population of *M. tuberculosis*.

In this outbreak of TB, thanks to the successful work of Public Health Services, the extension of the transmission of



VNTRs-MIRU	424	577	580	802	960	1644	1955	2163b	2165	2401	2996	3192	3690	4052	4156
H37Rv	2	4	3	1	3	2	2	5	3	2	3	3	6	5	3
Student	2	3	2	2	5	2	4	5	3	4	5	3	5	6	3
Student	2	3	2	2	5	2	4	5	3	4	5	3	5	6	3
Friend	2	3	2	2	5	2	4	5	3	4	5	3	5	6	3
Student	2	3	2	2	5	2	4	5	3	4	5	3	5	6	3
Teacher	2	3	2	2	5	2	4	5	3	4	5	3	5	6	3

Fig. 1 – Spoligotyping and VNTR-MIRU results of the tubercular strains isolated. In the spoligotyping the black and with square indicate the presence or absence of the specific spacer at positions 1–43 in the DR locus. In the 15-loci VNTR all *M. tuberculosis* isolated strains had the same genetic profile. H37Rv *M. tuberculosis* was added as a control.

the tubercular infection was limited. Primary care physicians and public health authorities have to assume a leading role in early detection and treatment of patients. The investigation of the contacts is a responsibility of the health department to ensure that all persons who are suspected of having TB are identified; emphasis is placed on the need for identification and prompt chemoprophylaxis, especially in susceptible adolescent populations.

Conflict of interest

The authors declare that there are no conflict of interest in this study.

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