

Molecular epidemiology

Advances in the molecular epidemiology of Tuberculosis

Dick van Soolingen and Kristin Kremer

Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

Ongoing studies have pointed out the significance of different predominant genotypes of *Mycobacterium tuberculosis* in various geographic areas. In South-East Asia, the former USSR Republics, and South Africa, Beijing strains are emerging and associated with (multi-drug) resistance.

Recent research in China pointed out the average proportion of Beijing genotype strains in 10 provinces amounts 73 %, but varies significantly by region. The highest density was observed in the Beijing region; 93 %. Currently, research is focused on the evolutionary development of Beijing strains. Two evolutionary lineages were distinguished and a "modern" lineage is associated with BCG vaccination in Vietnam. A project of the European Union is focused on disclosure of evolutionary adaptation of *M. tuberculosis* in response to vaccination and exposure to anti-tuberculosis drugs.

It seems important to have more studies on changes in the population structure of *M. tuberculosis* in Latin America and Africa and this will be discussed. Since 1993, IS6110 restriction fragment length polymorphism typing has been the gold standard in the molecular epidemiology of tuberculosis. However, this method is technically demanding and time consuming. Therefore, recently a new standard typing method, the variable number of tandem repeat (VNTR) typing, has been proposed. In the Netherlands, a large part of the collection of nearly 15,000 *M. tuberculosis* isolates is currently being re-typed with VNTR typing. This is not only meant to evaluate the utility of the new 24 loci VNTR typing, but also to conserve the epidemiological trace of tuberculosis transmission in the past.

An overview of molecular methods and tools to control, understand and track the Tuberculosis epidemic worldwide

Nalin Rastogi, Christophe Demay, Véronique Hill, Julie Millet, and Thierry Zozio

Unité de la Tuberculose et des Mycobactéries, Institut Pasteur de la Guadeloupe - Morne Jolivière, BP 484, 97183 Abymes Cedex, Guadeloupe.

The monitoring and reporting constitute key points of a global strategy in fight against tuberculosis (TB). The ability to identify and analyse the circulating clones of *Mycobacterium tuberculosis* is essential in this regard. We will focus on the determinants of cluster distribution and major advances in TB molecular typing, e.g., the use of PCR-based methods. Spoligotyping and MIRU-VNTRs (using a predefined selection of 12, 15 or 24-loci formats) are now adopted for large-scale, highthroughput genotyping of *M. tuberculosis*, and shown to be more reliable and faster methods for TB transmission analysis than the IS6110-RFLP method. Both spoligotyping and

MIRU-VNTRs patterns are perfectly adapted for easy creation and maintenance of databases. A recently published SpolDB4 database by our laboratory was representative of 39295 TB strains from 122 isolation countries (<http://www.pasteur-guadeloupe.fr:8081/SITVITDemo>). An updated version SITVIT2 is expanded to integrate both spoligotyping and MIRU-VNTRs data from 233 contributors. It describes spoligotyping patterns for 62592 isolates from 151 countries of origin, VNTRs (Exact Tandem Repeats or ETRs, A to E) on 4626 clinical isolates from 59 countries of origin, and 12-loci MIRUs for 8161 clinical isolates from 87 countries of origin. It also groups identical patterns shared by 2 or more patients (shared-types or clusters), and characterizes a total of 2747 SITs (spoligotyping international type), 245 VITs (VNTR International Types), and 847 MITs (MIRU International Types). Simultaneous spoligotyping and MIRU information is available for 4285 clinical isolates which represents over 50 % of the MIRU-typed strains, and >7 % of spoligotyped strains, permitting to draw new conclusions regarding the worldwide prevalence of specific SIT/MIT clones, and new distribution maps for major genotypic lineages. SITVIT2 allows to interpret worldwide genotyping information for molecular epidemiology, and evolutionary and population genetics of tubercle bacilli.

Molecular epidemiology of multidrug-resistant Tuberculosis in Spain

Sofia Samper, Patricia Gavin and María José Iglesias

Instituto Aragonés de Ciencias de la Salud, Hospital Universitario Miguel Server. Centro Investigacion Biomedica en Red, Enfermedades Respiratorias, Isabel La Católica 1-3, Zaragoza, Spain.

Tuberculosis (TB) remains one of the infectious diseases responsible for the most adult deaths in the world. The emergence of multi-drug resistant strains (MDR), as well as extensively drug-resistant (XDR), poses a serious challenge to TB control today. Spain has one of the highest incidences of TB in Western Europe; nevertheless, the incidence of MDR TB is considered low.

During the nineties, a particular MDR *Mycobacterium bovis* strain (B strain), now recognized XDR, was responsible for an outbreak, affecting mainly to HIV-infected patients. Non effective medical treatment was available for these patients, median survival was less than 50 days after diagnosis and re-infection rate was 45 %. More than 100 cases were registered from 1993 to 2002 in Spain.

Since 1998, the MDR *M. tuberculosis* strains isolated in Spain have been systematically molecular characterized (RFLP-IS6110, 12 and 15 MIRU–VNTR, and spoligotyping) with the purpose of increase the knowledge of MDR-TB in Spain.

Molecular analysis revealed that 33 % of isolates were clustered, suggesting recent transmission. There was a progressive increase in the number of MR-TB isolates from foreign patients during the 10-year period of the study. Five percent of patients were immigrants in 1998 against 75 % in 2007. All isolates from immigrants patients since the last ten years in Spain, except those of the Beijing family and *M. africanum*, belong to the Euro-American lineage. The participation in networks sharing the molecular patterns of these bacilli would follow up the chain of transmission, even across borders.

The spectrum of mycobacterioses in argentina in the light of PCR restriction analysis of the *hsp65* gene

Viviana Ritacco

CONICET, INEI ANLIS Malbrán, Argentina.

In recent years, PCR restriction analysis (PRA) of the *hsp65* gene was incorporated into the algorithm of mycobacterial species identification at the national TB reference laboratory. This affordable molecular method allowed typing of clinically relevant mycobacteria to the species and subspecies level. In the period 2003-2007, a total of 356 confirmed cases of non tuberculous mycobacterial diseases were due to slow growers. The species most frequently associated to these conditions were *M. avium* (n=149), *M. intracellulare* (127), *M. kansasii* (30) and *M. simiae* (12); in all these species, the predominant subspecies was type I. The two leading non tuberculous mycobacteria had different preferred hosts and presentations: disseminated *M. avium* disease was highly frequent in HIV+ patients living in large urban areas while lung *M. intracellulare* disease prevailed in HIV- persons living elsewhere in the country. Slow growers less commonly associated to disease were *M. marinum*, *M. xenopi*, *M. gordonae*, *M. terrae*, *M. lentiflavum* and *M. szulgai*. Rapid growers were responsible of 81 confirmed cases of mycobacteriosis, of which 31 were respiratory disease, 28 were secondary to invasive medical or cosmetic procedures; 6 were localized in lymph nodes, 3 produced disseminated disease, and 13 had miscellaneous localizations (digestive tract, joint, bone, soft tissue, peritoneum). The non tuberculous mycobacteria involved in these conditions were *M. fortuitum* type I (n=26), *M. chelonae* type I (20), *M. abscessus* type I (13) and II (5), *M. peregrinum* types I to III (5), *M. immunogenum* type II (3) and, occasionally, *M. flavescens* and *M. smegmatis* type I. A wide variety of mycobacterial species were isolated from clinical specimens without substantial evidence of pathological involvement. Inconclusive results due to amplification or restriction failure, mixed and unknown patterns were observed in a small but significant proportion of cases. Systematic PRA typing will eventually shed light on the hardly understood epidemiology of non tuberculous mycobacterial diseases in Argentina.

Genetic diversity of *Mycobacterium tuberculosis* strains prevalent in Colombia and their association with drug resistance

Gloria Puerto^{1,5}, Nidia Correa^{2,5} Claudia Castro^{1,5}, Maria Consuelo Garzon^{1,5}, Daylin Angeel,⁵ Claudia Llerena^{1,5}, Dora Orjuela^{1,5}, Juan Carlos Rozo^{1,5}, Verónica Gomez^{2,5}, Elsa Zapata^{2,5}, Oriana Rivera^{3,5}, Isabel Dulcey^{3,5}, Magnolia Carvajal^{3,5}, Beatriz Eugenia Ferro^{4,5}, Edna Lorena Hernández^{4,5}, Sonia Lorena Villegas^{4,5}, Maria Lilia Díaz^{3,5}, Jaime Robledo^{2,5} Wellman Ribon^{1,5}

1 Instituto Nacional de Salud, Grupo de Micobacterias.

2 Unidad de Bacteriología y Micobacterias. Corporación para Investigaciones Biológicas-CIB.

3 Grupo de Inmunología y Enfermedades Infecciosas (GINIIN). Universidad del Cauca.

4 Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM).

5 Centro Colombiano de Investigación en Tuberculosis (CCITB).

Knowledge on *Mycobacterium tuberculosis* isolates genetic characteristics is useful in understanding disease transmission mechanisms, in differentiating among complex members, in determining their geographic distribution and in

distinguishing relapses from reinfections. Objective: To characterize by spoligotyping *M. tuberculosis* isolates circulating in Colombia to establish their genotype, distribution and association with susceptibility patterns aimed at strengthening the National Tuberculosis Program. Methods: Nine hundred *M. tuberculosis* isolates (384 from the Colombian National Institute of Health collection and 516 from the Colombian Center of Research on Tuberculosis -CCITB) were characterized by spoligotyping. From these, 135 were MDR, 523 were sensitive and 242 showed other resistances. Results: The most prevalent family was the Latin American-Mediterranean LAM with 348 isolates (38.6 %), of which 46.5 % had some type of resistance and 15.8 % were MDR, followed by the Haarlem family with 283 isolates (31.4 %), out of which 32.8 % had some type of resistance and 12.7 % were MDR. Five isolates (0.5 %) belonged to the Beijing family, all of them MDR. One hundred forty nine isolates (16.5 %) were not found in SpolDB4 data base, 41.6 % of which had some resistance. Conclusions: The predominant genotype corresponded to LAM family, present in 50 % of Latin America. The detection of Beijing genotype isolates has important consequences for disease surveillance in the country. It must be established if patterns not reported internationally are limited to the country. No association was found between genotype and resistance except in Beijing family isolates.

Prevention and control of Tuberculosis in Colombia: a successful case with indigenous populations

Pier Palo Balladelli

PAHO/WHO Representative in Colombia.

Since 2002, the government of Colombia with the support of PAHO/WHO and ACDI has made a significant effort to prevent and control tuberculosis in indigenous communities. As of 2008, the DOTS Strategy has been adapted and implemented in eight territorial entities and seven ethnic groups. A key catalyst for the application of the strategy was the continuous dialogue with indigenous leaders.

Main results of this project: 1) The rate of successful treatment for indigenous populations has improved. For example, the percentage of successful treatment has increased from 73.6 % in 2002 to 87.2 % in 2006 in Amazonas and from 88.7 % to 93 % in Cesar. 2) The investment of sub-national governments on prevention and control programs has increased. For each dollar investment by ACDI, the sub-national levels have invested between eight and ten dollars. 3) Specific actions for indigenous communities have been included in the National Strategic Plan "Colombia Free of Tuberculosis". 4) National prevention and control tools have been adapted for indigenous populations. 5) Has been expanded the program coverage to other indigenous communities; and 6) other strategies such as IMCI, integrated management of vector borne diseases and Healthy Settings have been integrated.

The main challenges are to: 1) Keep the local political commitment. 2) Address co-infection problems. 3) Strengthen the training of health workers to incorporate intercultural aspects in the clinical attention. 4) Ensure sustainable and reliable monitoring activities in order to maintain the quality of the program. 5) Shape community-based networks to assist the monitoring and adherence to the treatment, and 6) incorporate the lessons learned of this project in other regions with indigenous populations.