

Molecular epidemiology

Genotyping of *Mycobacterium tuberculosis* strains in a colombian area of high incidence of tuberculosis

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In this study, 67 clinical isolates of *Mycobacterium tuberculosis* obtained over a 3-year period (2003–2005) from Quindío region (a high-incidence area), were characterized by spoligotyping and 26 strains by IS6110-RFLP respectively.

These techniques were performed according to international standard protocols recommended for *Mycobacterium tuberculosis* complex typing. Obtained patterns were compared with genotypes described at international spoligotyping database (SpolDB4) and IS6110-RFLP database (RIVM-Bionumerics).

Spoligotyping results showed that *M. tuberculosis* population in Quindío was similar with other Latin American and Mediterranean groups, among those T1, Haarlem 3 - LAM families were the most prevalent genotypes accounting 46.7 and 21.4 % respectively. Although these genotypes have been described previously in similar studies from different regions in Colombia the transmission mechanism seems to be different. According to IS6110-RFLP patterns, 10 different clusters were identified, evidencing a high diversity of *M. tuberculosis* strains with potential spread to neighboring areas, furthermore these findings suggested the persistence of an endemic focus what could explain the high incidence of tuberculosis disease in Quindío. Microepidemics of Tuberculosis were detected by the presence of two genotypes of short circulation periods, well managed by the local TB program. On the other hand a *M. africanum* isolate was identified corroborating the accuracy and suitability of spoligotyping for *Mycobacterium* complex species typing.

Genotyping of *M. tuberculosis* strains by RFLP and Spoligotyping have proved to be a powerful tool for understanding the transmission and infection spread, our results showed the need to improve early diagnosis and treatment control.

Key Words: Tuberculosis, Molecular epidemiology, Spoligotyping, RFLP-IS6110, Diagnosis, Genotyping.

An outbreak of *Mycobacterium abscessus* infection following liposuction

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Forty patients who underwent cosmetic surgery (liposuction) from May to August 2007 presented with signs of inflammation of the skin and abscesses or drainage at the original wound site. From 7 of these patients *Mycobacterium abscessus* was isolated. Interrogation revealed that the intervention was performed by two surgeons who used the same operating room and equipment. To determine the infection, interviews with the surgeons and the operation room technicians were conducted. Environmental samples were taken for the isolation of atypical mycobacteria and the ERIC-PCR, BOXA1R-PCR and Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD) were performed to characterize the isolated strains. Interviews with the surgeons revealed that the introduction of an ammonium quaternary solution to "sterilize" and "reuse" a disposable tube which connects the cannula with the vacuum pump initiated the first cases. When this procedure was abandoned no more cases were generated. No Mycobacteria were isolated from the environment or from the disposable tube. The three PCR techniques showed the same pattern for 6 of the 7 *M. abscessus* strains isolated from the patients. We concluded that outbreak was caused by at least two different *M. abscessus* strains. The introduction of an ammonium quaternary compound to sterilize the disposable tube was the most probable cause of the soft tissue infections. Mycobacteria generally are resistant to these solutions. We underline the importance of the control of the disinfection procedures in operating rooms.

Key Words: Molecular epidemiology, cosmetic surgery, ammonium quaternary compounds.

Genotyping of *M. tuberculosis* strains isolated in Rio Grande, Brazil

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Tuberculosis (TB) remains a serious public health problem in Latin America. Brazil belongs to the group of 22 countries responsible for 80 % of the world's TB cases. Rio Grande, city located in the southern extreme of Brazil, presents an incidence 40 % higher than the observed in this country. The knowledge of dynamic of strains transmission is an important goal for TB control. In this study the clonal diversity of 50 clinical isolates was performed by MIRU-VNTR (12 *loci*) and Spoligotyping methods, the results was analyzed by Bionumerics 4.1[®] and classified according to the SpolDB4.

MIRU-VNTR showed 42 distinct patterns with 15 strains distributed in seven different clusters and 35 strains with unique patterns. The Hunter-Gaston discriminatory index (HGI) was 0.992. According to the allelic diversities, seven of the 12 MIRU-VNTR loci were moderately or highly discriminative. Spoligotyping was performed on 48 strains identifying 26 different patterns, with 32 strains grouped in ten clusters and 16 had unique patterns. The HGI was 0.959. When the two methods were analyzed in combination, the HGI was 0.995 whereas 44 of the 50 different patterns were discriminated. These results showed a high discriminatory force these methods and a high clonal diversity in the strains studied.

Key Words: *Mycobacterium tuberculosis*, genotyping, Spoligotyping, MIRU-VNTR.

Oral presentation

A first insight on the population structure of *Mycobacterium tuberculosis* clinical isolates in Bogotá- Colombia

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Tuberculosis (TB) remains an important public health problem in Colombia. In 2006 the incidence was 24.6 per 100.000 population. The present study focused on molecular typing of *M. tuberculosis* strains (n=137) isolated from as many TB patients in Bogotá-Colombia during 1995 to 2006. Our primary aim was to have a first insight on the population structure of tubercle bacilli in Bogotá using spoligotyping in order to characterize the major circulating genotypic lineages.

Spoligotyping resulted in 40 different patterns, that were entered in an updated version of the SpolDB4 database (the initial version is available at <http://www.pasteur-guadeloupe.fr:8081/SITVITDemo>). The in-house updated version SITVIT2 maintained at the Institut Pasteur de Guadeloupe contained spoligotypes of 68000 clinical isolates from 160 countries of origin. Database comparison showed that a total of 5/40 patterns representing 137 isolates, corresponded to orphan strains. The overall repartition of strains according to major *M. tuberculosis* families ranked in the following order : Latin-America & Mediterranean or LAM, 69/137 or 50.4 %; Haarlem, 33/137 or 24.1 %; ill-defined T superfamily (not a clade sensu stricto as it is defined by default), 16/137 or 11.7 %; S clade, 4/137 or 2.9 %; X clade, 1/137 or 0.7 %. Lastly, the three largest STs that stand out from other are ST42 of LAM9 clade with 39/137 (28.5 %) of the isolates, ST53 of ill-defined T clade with 15/137 (10.9 %) of strains and ST62 of Haarlem clade with 14/137 (10.9 %) of strains. This study is currently ongoing using MIRU-VNTRs to establish the molecular clonality of the clustered isolates to identify genetic links between isolates from different TB patients in order to study transmission.

Key Words: Tuberculosis, *M. tuberculosis*, DNA Fingerprinting, Molecular Epidemiology, Databases.

Contribution of molecular epidemiology to the understanding of tuberculosis transmission in Colombia

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INTRODUCTION: Tuberculosis is a major health problem in Colombia with 12.000 new cases per year. Genotyping methods for *M. tuberculosis* are useful as molecular epidemiology tools that contribute to a better understanding of transmission in aspects such as relapses-reinfections, identification of outbreaks and ultimately are useful for studying phylogeny and co-evolution. **OBJECTIVES:** Characterize *M. tuberculosis* isolates from index and incident cases in household contacts cohorts in three different cities in Colombia using molecular epidemiology methods. **METHODS:** 473 *M. tuberculosis* isolates from index cases in three cohorts in Colombia (Medellin, Cali, Popayan cities) were genotyped using Spoligotyping and MIRUs methods. Isolates from the major cohort (361 isolates) were also characterized by RFLP as well as pair isolates from index and the correspondent incident cases and isolates from relapsing/reinfection cases. Analysis of similitude was performed using Bionumerics®. **RESULTS:** Haarlem and LAM families were predominant in isolates from two cohorts, Medellín 40.6 % and 34.0 %, Cali 37 % and 39.4 % respectively, while in the Popayan cohort the predominant was the T family, 32.2 %. Eighty one (17.1 %) of spoligotypes did not have match in SpolDB4 database. Isolates belonging to families X and Beijing were infrequent. Of the incident cases studied, 73 % had the same index case genotype. Most of repeated isolates (87.5 %) from index cases studied match the initial isolate. **CONCLUSIONS:** LAM and Haarlem families were predominant in the isolates studied but with differences according to the city. Genotyping demonstrate to be useful to define transmission in household contacts and relapsing in index cases.

Key Words: Molecular epidemiology, RFLP, spoligotyping, MIRU.

Poster Presentation

Molecular epidemiology of tuberculosis in a mexican population with high index of migration to USA

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INTRODUCTION: Worldwide, Tuberculosis (TB) remains as one of the leading causes of death. The higher incidence of TB in México and the increase of cases associated to

Mexican migrants, suggest that TB has the potential to become a binational health crisis. Zacatecas is one of the Mexican states with the highest rates of migration to the United States, and could represent a hot spot for TB. Thus, molecular epidemiology analysis to identify genotypic clusters of patients represents a good strategy to identify ongoing TB transmission in Zacatecas. **OBJECTIVE:** To identify by genotyping clusters of patients representing ongoing TB transmission in Zacatecas, Mexico. **MATERIALS AND METHODS:** A prospective molecular epidemiologic study of TB in Zacatecas-Mexico was performed from 2005 to 2008. Micobacteria culture-positive cases were subjected to Spoligotyping and IS6110-genotyping methods using standardized protocols. Cluster analysis of *M. tuberculosis* strains and epidemiological links among patients was performed according guidelines of the Centers for Disease Control, USA. **RESULTS:** Thirty six isolates of *Mycobacterium tuberculosis* were genotyped; Spoligotyping identified seven clusters, but only 2 were confirmed by RFLP analysis. Epidemiological information helps to identify two chains of TB transmission involving strains with high rates of transmission. Intrafamily and labor-link contacts were defined. **CONCLUSIONS:** Outbreaks of tuberculosis are present in Mexican population with high index of migration to USA. These outbreaks involve strains with high capacity for transmission in humans.

Key Words: Molecular Epidemiology, Chains of transmission, Genotyping.