Reviewer's report

Title: Mycobacterium tuberculosis ecology in Venezuela: Epidemiologic correlates of common spoligotypes and a large clonal cluster defined by MIRU-VNTR-24

Version: 1 Date: 23 February 2009

Reviewer: M. Cristina GUTIERREZ

Reviewer's report:

This manuscript describes the spoligotypes of 1,298 Mycobacterium tuberculosis complex strains isolated from patients at different localities in Venezuela from 1997 to 2006, including urban and indigenous areas. A subgroup of strains representing the major spoligotype clusters were submitted to additional genotyping by analysis of MIRU-VNTR 24 loci. The authors associated the spoligotypes with epidemiological parameters in an attempt to track the dynamic of Mycobacterium tuberculosis strains with particular genotypes in Venezuela.

Major comments

1) This manuscript is an extension of previous reports by the same authors showing the population structure of M. tuberculosis strains in diverse areas of Venezuela. A significant part of data from the two Amerindian population have been already published by the authors (ref 24-in press-, Delta Amacuro; ref 25, Amazonas, 41 isolates), as well as data from the city of Valencia (ref 28, 317 strains) describing the predominant local clone. Although the present manuscript includes a larger collection of strains, the genotyping data do not represent a novelty as there are not new findings, and the population structure of M. tuberculosis strains in Venezuela has already been described in the frame of a nation wide study performed by others (reference 5).

2) Abstract, background, lines 1-2: could the authors modify this phrase. Please note that the situation they describe (endemic TB with few highly transmitted M. tuberculosis genotypes) is not the rule but just one of the multiple situations found in the world.

3) Table 1 shows that 70 out of 467 strains in Valencia had genotype SIT605. This corresponds to 15%, and not to 75% as indicated in the text Page 11, line 24.

4) The authors invested many efforts to determine the genetic family of the M. tuberculosis strains. However their classification relies only on spoligotypes, except for an indeterminate number of strains with SIT605 which were additionally analysed for the presence of 45 SNPs. Could I suggest to use their available MIRU-VNTR data to determine and/or confirm the assignment of strains to a particular family, as described in reference 3.
5) This paper has a clearly defined epidemiological objective, that makes its originality. However, it does not offer sufficient evidence for the conclusions:

5.1) It is confuse how the patients were selected for the study. Is it possible that the criteria differed between the different localities? For instances, did the collections comprised only smear positive, culture positive specimens, or all the culture positive specimens? There were only adults included in some local surveys, as the patients were > 15 year-old? If the selection criteria were not harmonized, it would not be valid to merge patients from different localities for correlation of genotypes with epidemiological data.

5.2) It is unclear which and how many epidemiological data were available for each local group of patients. These records should be presented in a general Table. Correlation of these data with particular spoligotypes should only be done if available data are representative.

5.3) The multivariate analyze indicated that none patient’s data was associated with any specific spoligotype.

6) The authors speculate on the less virulence of SIT53. Please note that strains with this spoligotype are not a compact group but may show high genetic heterogeneity when they are analyzed using other markers, due to the lower discriminatory power of spoligotyping. This is precisely the result obtained by the authors in this manuscript, as showed in figure 3.

Minor comments

1) Results, clustering analysis: please indicate “clustering analysis of spoligotypes”.

2) The authors classed strains with ST605 either in “LAM” or in “U” families in the Figures. Please review the Figures and check the corresponding percents in the text.

3) Methods, spoligotyping and MIRU-VNTR typing: please indicate in the text only modifications to standard methodology already described in the included references.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'