Evidence of active transmission of drug resistant *Mycobacterium leprae* strain in Brazil

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The colony of Santo Antonio do Prata (Prata colony) was founded in the 1880’s and located in the Amazonic state of Pará, north of Brazil, has been recently reported one of the highest leprosy prevalence in the world.
Objective

To investigate the impact of drug resistance over disease relapse and transmission.
Methods

- Multibacillary (MB) cases: at least one complete MDT regimen and their contacts living in the Prata colony (including PB patients).
- Dermatological and neurological evaluation.
- Individuals presenting active leprosy (both relapse and new cases) were classified according to the Ridley & Jopling protocol.
- Skin biopsy for *M. leprae* drug susceptibility test (Shepard).
Methods

- Skin biopsy for *M. leprae* genotyping:
  - Drug resistance was investigated by direct sequencing of polymorphic sites of the *rpoB*, *folP1* and *gyrA* genes.
  - *M. leprae* strain typing was performed by Multiple-locus VNTR Analysis (MLVA).
Results

Clinical evaluation of 185 individuals

117 individuals treated for leprosy up to 2005

Sex
♂ = 61 (52.1%)
♀ = 56 (47.9%)

Mean age
♂ = 26.7
♀ = 24.7

Clinical form
♂ = MB 90 (80.8%)
♀ = PB 27 (19.2%)

68 intra and/or extra household contacts

10 New leprosy cases (14.7%)

Sex
♂ = 3 (30%)
♀ = 7 (70%)

Mean age
♂ = 26.4
♀ = 26.4

Clinical form
♂ = MB 64 (80.8%)
♀ = PB 20 (19.2%)

12 relapse cases (11.53%)

Sex
♂ = 7 (58.3%)
♀ = 5 (41.7%)

Mean age
♂ = 24.7
♀ = 24.7

Clinical form
♂ = MB 11 (91.7%)
♀ = PB 1 (8.3%)

9 resistance cases (75%)

6 MDR
3 DDS

4 primary resistance cases (40%)

2 MDR
1 DDS
1 Rifampicin

Hidden challenges
Results

- *rpoB* mutations were in codon 456, TCG to ATG (Ser to Met);
- *folP1* mutations occurred at codon 55:
  - 10 cases presented CCC to CGC (Pro to Arg),
  - one CCC to GCC (Pro to Ala),
  - one CCC to CTC (Pro to Leu).
<table>
<thead>
<tr>
<th>ID</th>
<th>Status</th>
<th>Clinical form at diagnosis</th>
<th>Year of diagnosis and relapses</th>
<th>Treatment schemes at diagnosis and relapses</th>
<th>Duration of treatment (DDS years, MDT months)</th>
<th>Clinical form in 2009</th>
<th>Patient rpoB</th>
<th>Patient folp1</th>
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<td>PA012</td>
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*PB*= Paucibacillary
*MB*= Multibacillary
*WT*= Wild type
*a*= Discrepancy: reference laboratory detected mutation in codon 55
*b*= Inconclusive because only 1/10 animal of the control group presented positive growth
*M*= Missing
*NA*= Not applicable
*AF*= No PCR amplification
**= Only control group of mice inoculated in mouse footpad assay, sequencing of bacilli recovered from mice and patient was compatible
Results - MLVA

UPGMA

Maximum parsimony

SNP type
Results

- Upon MLVA typing, clustering level was as high as 70%, forming 3 clades and 5 cluster with 2 individuals;
- Results suggest little variation in genetic profile of this population over time;
- We observed a considerable number of family links between reactivation cases (mostly multibacillary) and new cases;
Results

Análise de SNPs e resistência
Conclusion

- Disease control policies should focus on pockets of high endemicity of leprosy, often subjected to poor epidemiological surveillance due to logistic problems such as limited access to health services and poor adhesion to treatment due to cultural behavior.
- More importantly, our findings document active emergence and transmission of *M. leprae* resistant strains under specific, favorable conditions.
- The Prata colony could be a model for studying molecular epidemiology of resistance in leprosy.