From the Division of Global Health (IHCAR), Department of Public Health Sciences

Rapid detection of drug resistance and genetic characterisation of *Mycobacterium tuberculosis* isolates in Honduras

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Som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras in Gard Aulan, SMI, Nobels väg 18, Solna.

Fredagen den 28 januari, 2011, kl 09.00

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Stockholm 2011

ABSTRACT

Background: New challenges for tuberculosis (TB) control are emerging in the 21st century. The deadly combination TB/HIV as well as the development and spread of new forms of drug resistance are some examples. In this context, the timely detection of TB cases, especially of those with resistant TB is important. Improved laboratory methods can contribute to the early detection of such patients. Therefore, this thesis focuses on the evaluation of new diagnostic tools and the genetic characterisation of the disease in a low-middle income setting.

Aims: The purpose of this work was to increase the knowledge about effectiveness of new laboratory assays for early detection of drug-resistant TB. In addition, this thesis aimed to increase our understanding of the *M. tuberculosis* biodiversity and transmission patterns in Honduras.

Methods: The diagnostic accuracy of two low-cost techniques for rapid detection of drug-resistant TB was assessed. The microscopic-observation drug susceptibility assay (MODS) and the nitrate reductase assay (NRA) were compared to the proportion method on Lowenstein Jensen medium for detection of multidrug-resistant TB (MDR-TB) directly from sputum samples. Furthermore, the indirect NRA was evaluated for detection of resistance to selected second-line drugs using as reference standard the BACTEC-460TB. Additionally, the frequency and distribution of resistance-related mutations *M. tuberculosis* was explored. This characterisation was conducted in clinical MDR-TB isolates from different geographical settings. Finally, we described the genetic variability of clinical *M. tuberculosis* isolates from Honduran TB patients, using spoligotyping and restriction fragment length polymorphism (RFLP).

Results: Both NRA and MODS were highly sensitive and specific for direct detection of resistance against isoniazid and rifampicin. The indirect NRA had an acceptable performance for detection of resistance to ofloxacin, with less good results with kanamycin. The genotypic detection of drug-resistance in MDR-TB isolates confirmed the prevalence of the most common mutations associated with this phenotype. It also showed the differences in distribution depending on the geographic origin of the strains. In Honduras, the Latin American Mediterranean spoligotype is the most frequent in a M. *tuberculosis* population with high biodiversity.

Conclusions: MODS could be used for screening of suspected MDR-TB patients. However, NRA is the most reliable option for early detection of MDR-TB in resource-limited settings. NRA also has the potential to be further optimised for detection of extensively drug resistant TB. We also confirmed that knowledge about the frequency of geographic-specific mutations provides useful information for development and/or assessment of new genotypic tools for detection of drug resistance. The high level of genetic variability in the Honduran *M. tuberculosis* isolates suggests that the transmission of the disease in this setting is not caused by clonal spread of a specific strain. Further studies are needed to determine whether or not there is an association with TB clinical manifestations or HIV status with the genotypes prevalent in the country.

ISBN 978-91-7457-193-6