1. Publishable summary

Summary of project objectives

Tuberculosis (TB) remains as a big killer among infectious diseases with 1.3 million deaths attributed to the disease in 2007. The HIV/AIDS pandemic and the emergence of drug resistance are signalled as the two main reasons for the deterioration of the TB situation worldwide. HIV/TB co-infection constitutes a deadly combination each one accelerating the development of the other disease. Approximately one third of the HIV/AIDS patients worldwide are co-infected with TB and in 2007 there were an additional 456,000 deaths among TB patients co-infected with HIV.

The emergence of drug resistance and multidrug resistant (MDR)-TB, defined as strains of *Mycobacterium tuberculosis* resistant to at least isoniazid and rifampicin, constitute the second and most important factor for the persistence of the disease as a global public health problem. TB is the only disease ever declared as a Global Emergency by the WHO in 1993. A more recent finding is the recognition of extensively drug resistant (XDR)-TB, defined as strains of *M. tuberculosis* that in addition to being MDR have become resistant to any quinolone and at least one of the three injectable drugs: capreomycin, kanamycin or amikacin. Thus, XDR-TB strains are resistant to the main first- and second-line anti-TB drugs, leaving those patients harbouring these strains with very little options for being cured. HIV/AIDS patients affected by XDR-TB have a high mortality rate as was shown in an outbreak in South Africa. XDR-TB has already been reported in many countries including Europe. Thus, MDR-TB and XDR-TB constitute now the most dangerous threat for the proper control of the disease. New and improved methods for fast detection of drug resistance, and especially of MDR and XDR-TB are urgently needed.

The overall objective of this project is to develop a two-approach system for the simultaneous detection of MDR- and XDR-TB in *M. tuberculosis* isolates and directly on sputum samples. This will be attained through the following measurable and verifiable specific objectives:

1. Development and standardization of a fast phenotypic colorimetric plate system for the detection of MDR- and XDR-TB in *M. tuberculosis* isolates. Based on previous results obtained in the development of a colorimetric test, the consortium will develop a rapid plate assay for the simultaneous detection of MDR- and XDR-TB isolates.

2. Design and standardisation of an improved molecular platform for the detection of MDRTB. Supported on preliminary results generated by CorpoGen in a previous project, an oligocolor plate format will be further improved for the rapid and clear detection of MDR-TB isolates.

3. Development of a fast colorimetric plate system for the direct detection of MDR- and XDRTB in sputum samples. Based on the results obtained under objective 1, the system will be implemented in a new format adapted for sputum samples.

4. Design and standardisation of a molecular tool for XDR-TB detection. Based on an active search of the most prevalent mutations associated with drug resistance and a programme of high-throughput sequencing by SMI a new plate system will be developed to include simultaneous molecular detection of resistance to the drugs defining XDR-TB.

5. Validation of the colorimetric plate system in different settings to assess accuracy and robustness of the new technology.

6. Validation of the molecular tool in different settings for accuracy, robustness and ease of implementation.

7. Prospective evaluation of the colorimetric plate system and the molecular tool in target populations.

Description of the work performed

During the second reporting period (February 2009-July 2010) many activities presented in the work packages (WP) were continued or started in order to achieve the general project objectives. WP 1 "Development and standardization of a phenotypic colorimetric plate system for detection of MDR- and XDR-TB in *M. tuberculosis* isolates." with the participation of ITM (Belgium), SMI (Sweden), LIC (formerly SATLD), (Latvia), CorpoGen (Colombia) and Cetrángolo (Argentina). The main tasks of this WP were finished during the previous reporting period. In the current period the final analysis of data generated during the multicenter evaluation performed in several sites was finished. Final cut-off values and critical concentrations of all antibiotics were agreed and interpretation of results was based on these parameters. The final format of the phenotypic colorimetric plate for detection of MDR- and XDR-TB in *M. tuberculosis* isolates has been completed.

As mentioned in the first periodic report, the DIAPOPS platform was initially evaluated in WP 2. Unfortunately, the test did not reach enough sensitivity and specificity to justify further development. For this reason, we designed a new molecular platform based on the rifoligotyping methodology with the innovation that all probes hybridize at the same temperature. This new membrane format was optimized for detection of resistance to rifampicin and isoniazid with very good results and is currently being adapted for detection of resistance to fluoroquinolones, kanamycin and capreomycin. Also, a new slide microarray format has been developed for detection of multidrug resistance that is scheduled to be evaluated by other members of the consortium.

Based on the results obtained in WP1 and starting with a similar plate design, a new format was explored for application in the detection of MDR- XDR-TB directly in sputum smear-positive samples (WP3). Different possibilities were evaluated, including the development of the assay in a new format on a solid culture medium. This new format would also include the possibility for the rapid identification of *M. tuberculosis* in the same plate by the use of specific reagents. Being able to perform the assay directly on sputum samples will shorten the turnaround time by omitting the pre-isolation step needed in all indirect drug susceptibility testing methods currently in use. A new liquid medium-based approach and candidate new coloured indicators have also been explored.

The database of gene mutations associated with drug resistance in *M. tuberculosis* (WP4) developed during the first reporting period was updated and maintained. This WP was coordinated by the ITM (Beneficiary 1) in close collaboration with Beneficiary 8 (Cetrángolo) and participation of the other members of the consortium. Further analysis to assess the geographic distribution of the different mutations associated with drug resistance is under way.

Sequencing activities to look into new mutations responsible for drug resistance to first- and second-line drugs have continued during the present reporting period. Also, genome sequencing activities have been started to investigate new possible candidate genes that could be regulating the emergence of new forms of drug resistance.

Main results achieved so far

The main results obtained until now can be summarized as:

1. Development of the final format and evaluation of the colorimetric plate assay for the rapid detection of MDR-TB and XDR-TB in *M. tuberculosis* isolates.
2. Definition of critical concentrations of drugs responsible for MDR- and XDR-TB used to analyze results of the multicenter evaluation of the proposed test.
3. Validation of the new membrane-format for a new molecular assay for MDR-TB detection.
4. Design of oligonucleotides to detect mutations associated with resistance to ofloxacin, kanamycin and capreomycin.
5. Development of a new microarray slide format for detection of multidrug resistance in TB.
6. Development and validation of a new colorimetric plate format for rapid detection of MDR- and XDR-TB in clinical isolates.
7. Optimization of the new colorimetric plate format for detection of MDR- and XDR-TB directly from sputum samples.

Expected final results and impact

The final outcome of the project would be fast and improved assays for detection of MDR- and XDR-TB both as an indirect method in *M. tuberculosis* isolates, as well as directly in sputum samples. This project will go a step further by developing and testing a double-approach for the simultaneous detection of MDR-TB and XDR-TB in clinical isolates of *M. tuberculosis* in a first phase, and directly on sputum specimens once the fast phenotypic colorimetric assay and the molecular test are completely developed. Very few of the available tests have been designed for or applied in sputum samples. The project will also contribute to the current knowledge of the mechanisms of *M. tuberculosis* resistance to second-line drugs.

Project website

http://room.projectcoordinator.net/~oligocolor

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