PETTS Samples from the South African Medical Research Council for sequencing

BACKGROUND ON STRAINS

PETTS - "Preserving Effective TB Treatment Study" is a multi-national epidemiological study on risk factors for acquired resistance amongst MDR-TB patients. PETTS focuses on resistance to SLDs among MDR-TB subjects, specifically, the frequency, risk factors, and consequences of acquired resistance. Eight countries (Peru, The Republic of South Africa, Estonia, Latvia, Russia, the Philippines, South Korea, and Thailand) with 21 clinical sites volunteered to participate, from South African four provinces (Mpumalanga, Northwest, KwaZulu-Natal, Eastern Cape). WHO estimated the prevalence of MDR-TB among new cases to range from 1.7% to 18%, and among previously treated patients, from 6.7% to 46% in the 8 participating countries at the onset of PETTS. PETTS is the largest prospective cohort of MDR-TB subjects to date, generating comprehensive data regarding the prevalence of SLD resistance in MDR-TB subjects in eight countries: 43·7% of subjects starting treatment for MDR-TB had additional resistance to ≥1 SLD, including 20·0% to ≥1 second-line injectable drug, 10·5% to all three second-line injectable drugs, and 12·9% to fluoroquinolones. Overall, 6·7% had XDR-TB ranging from 15·2% in South Korea to 0·8% in the Philippines.

Sites enrolled consecutive, consenting adults with locally confirmed, pulmonary MDR-TB starting SLD treatment, from 2005 through 2008. Sputum cultures were sent to CDC for centralized drug susceptibility tests (DST) for 11 first-line and SLDs. DST results were compared with clinical and epidemiological data to determine principal factors associated with SLD resistance. Enrolment required a baseline mycobacterial culture from sputum collected within 30 days before or after starting SLD treatment, and ≥1 follow-up positive culture from sputum collected ≥30 days later. Both isolates had to be shipped to CDC, Atlanta, for centralized drug susceptibility testing (DST), while the sites retained original specimens for later use. Standardized demographic, socioeconomic, and clinical information was recorded for each subject by trained personnel, including details of previous and current treatment, surgery, hospitalization, comorbidities (emphasizing HIV infection), local microbiology results, baseline chest x-ray, and final treatment outcomes. Cases were classified according to previous treatment and previous treatment outcomes.

Baseline sputum specimens were cultured for M. tuberculosis complex and tested for susceptibility to at least INH and RMP locally. Follow-up sputum specimens were collected and cultured monthly for the duration of the patient’s MDR-TB treatment. A duplicate of positive baseline and follow-up cultures were shipped to CDC. The sites retained the original cultures, and MRC will use these cultures to isolate DNA for this sequencing effort.
Among 1,278 confirmed MDR-TB subjects, >40% of baseline isolates had resistance to ≥1 SLD, 20·0% to ≥1 second-line injectable drug and 12·9% to ≥1 fluoroquinolone; 6·7% had extensively drug-resistant (XDR) TB, ranging from 0·8% to 15·2% across study sites. Previous treatment with SLDs was consistently the strongest risk factor for baseline resistance to fluoroquinolones, second-line injectables, and other SLDs, increasing the risk of XDR-TB 4-fold. Fluoroquinolone resistance and XDR-TB were more frequent in female than males (RR 1·5 fluoroquinolone resistance, RR 2·2 XDR-TB). Unemployment, alcohol abuse, and smoking were associated with resistance to injectable SLDs. Other risk factors differed between drugs and countries.

**SOUTH AFRICAN PETTS STRAINS**

293 patients were enrolled in South Africa, and if including the base line and last positive culture from a patient, the maximum number of DNA samples will be less than 600. DNA will be isolated from colonies on the original LJ-media, without growing the cultures again. DNA will be extracted from culture specimen as per standard practice. Tubes will be labelled with a unique patient identifier “subject’s code”, a unique specimen code and date.

**Shipment**: The DNA samples will be shipped and kept frozen on dry ice. After the samples have been picked up by the courier, the name of the courier, tracking no. and sample information sheet will be sent to the recipient.

**Shipment conditions**

- Tubes have to be labelled with a unique patient identifier “subject’s code” and a unique specimen code.
- For immediate use or shipment they will be stored at 4°C. For long term storage (over a week) store at -20°C.
- The specimen will be sealed inside double bag, and any accompanied documentation is inserted into and outer pocket, separated from the specimen itself.
- Shipment is done preferably at 4°C; room temperature is acceptable providing transportation do not last longer than 2 days, from the time samples are taken away.