Molecular Diagnosis of Multi-drug Resistant Tuberculosis (MDR-TB): Opportunities and Challenges

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* complex (MTBC) has been a global public health challenge for decades and the emergence of MDR-TB further complicates clinical management. Rapid diagnosis using Real time PCR (qPCR) provides explicit high diagnostic sensitive and specific detection of Rifampicin resistant TB (GenXpert™, USA) in sputum. Sanger DNA sequencings to identify MDR-TB are tedious and time consuming. Recent Next Generation Sequencing (NGS) proves to be a high throughput assay for rapid diagnosis of MDR-TB, facilitating early anti-tuberculosis therapy.

A total of 4,047 respiratory specimens were collected from patients in Hong Kong and Guangzhou, among which 501 were TB-positive as detected by qPCR assay with diagnostic sensitivity and specificity of 97.9 and 99.2%, respectively. Preliminary resistance screening identified 25 drug-resistant specimens including 10 MDR-TB patients. TB-NGS was performed using MiSeq™ on all drug-resistant specimens alongside 67 pan-susceptible specimens, demonstrating 100% concordance to phenotypic drug susceptibility test. Since 2010 in Hong Kong, MTBC harboring novel drug resistant mutations to Ofloxacin (Ala74Ser), Rifampicin (Ile572Phe), and Pyrazinamide (Thr-12Cys) were further verified by molecular cloning, transformation, overexpression and DNA supercoiling assay as well as virtual X-ray crystallography and drug susceptibility test. The interpretation of NGS data should be carefully referenced to the WHO Catalogue Mutations and novel drug resistance in local MTBC isolates.