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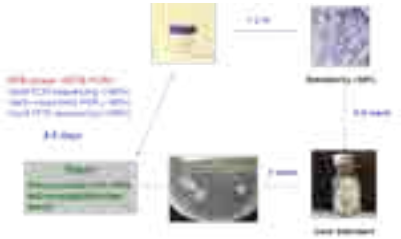
Rapid diagnosis of drug-resistant tuberculosis

Tuberculosis (TB) has reemerged as a global public health concern with an annual mortality of 3 millions. Coincident with the resurgence of tuberculosis, there is also an alarming increase of infections, due to multiple drug resistant tuberculosis (MDR-TB) organisms which are resistant to two or more of the first line anti-tuberculosis drugs including isoniazid and rifampicin. Recent threat has included extensively drug resistant tuberculosis (XDR-TB) defined as MDR-TB resistant to any fluoroquinolone and at least one second-line injectable drug. For rapid diagnosis of *Mycobacterium tuberculosis (M. tb)*, Nucleic Acid Amplification assays such as PCR facilitates the adequate and timely management of antituberculosis therapy. Conventional antimycobacterial susceptibility testing remains the standard protocol to monitor drug resistant strains. More than 90% of rifampicin resistant *M. tb* has been shown to be caused by mutations inside the 81-bp rifampicin resistance determining region (RRDR) located in the center of the *rpoB* (encodes for β–subunit of the RNA polymerase) gene. In Hong Kong, PCR-sequencing of *rpoB* gene of *M. tb* isolates revealed mutations in codons D516V, H526D and S531L inside RRDR accounted for most rifampicin-resistant *M. tb*. PCR-sequencing also identified hotspot mutations at positions 90, 91 and 94 of *gyrase A* (*gyrA*) gene accounted for over 85% of Ofloxacin-resistant *M. tb* in Hong Kong. For isoniazid resistance, multiple allele-specific PCRs (MAS-PCRs) assays targeting the mutations in codon 315 of *katG* gene and the 15th nucleotide preceding the operon successfully identified 60-75% isoniazid-resistant M. tb in clinical specimens. Using PCR-sequencing, novel mutations associated with rifampicin and Ofloxacin resistance were also identified among treatment experienced patients. Current study on massive parallel targeted sequencing (MPTS) for simultaneous prediction of drug susceptibility in *Mycobacterium tuberculosis* from respiratory specimens shows promising results. The cost-effectiveness of development, introduction and availability of these methods for rapid diagnostics improves public health control and early initiation of anti-tuberculosis therapy.



Speaker Biography

WC Yam is currently an Associate Professor in the Department of Microbiology, Faculty of Medicine from the University of Hong Kong. As a Clinical Scientist and Fellow Member of Royal College of Pathologists, he aims at rapid diagnosis of emerging infectious diseases including tuberculosis, drug resistant HIV-1, and SARS Corona virus which he had achieved major advancement for clinical application. More recently, he has been using molecular method to study drug resistant *Mycobacterium tuberculosis* and HIV. The studies on multi-drug resistant *Mycobacterium tuberculosis* and HIV-1 have included the development of Next Generation Sequencing.

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