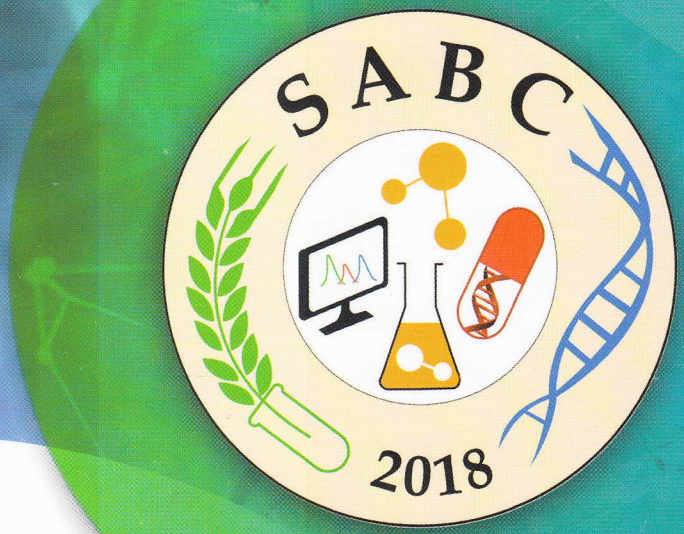
SOUTH ASIAN BIOTECHNOLOGY CONFERENCE

28-30 MARCH 2018 | COLOMBO, SRI LANKA
BIOTECHNOLOGY : ROLE IN REGIONAL DEVELOPMENT



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A process and a potential diagnostic kit for Drug-resistant *Mycobacterium tuberculosis* complex bacteria

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TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent. The six month drug regimen intended to completely eradicate the pathogen is threatened by drug-resistance. In 2016, there were 600000 new cases with resistance to rifampin (RIF), the most effective first-line drug, with 490 000 having multidrug-resistant TB (MDR-TB) i.e. resistance to the two main first line anti-TB drugs; RIF and Isoniazid (INH).

The objective was to develop a simple multiplex diagnostic assay to rapidly detect RIF and INH sensitivity or resistance simultaneously in *Mycobacterium tuberculosis* complexes present in a biological sample.

Patients with acid fast bacilli (AFB) positive sputum visiting the Chest Clinic, Kandy were recruited (n=250) with a control group of 25 patients confirmed to be of AFB negative. Specimens were processed and cultured on Lowenstein-Jensen medium and incubated at 37 ^{o}C for 8 weeks. RIF and INH resistance was determined by drug susceptibility testing (DST). Multiplex PCR with self-designed primers (*inhA* + *rpoB*, *katG* + *rpoB*) was carried out using H37Rv as a standard strain. The process was validated with 100 sputum samples. Denaturing gradient gel electrophoresis (DGGE) was performed using an 8% acrylamide gel in 1× TAE buffer. DGGE were carried out with 20-90% gradient gels at 60 ^{o}C for 10 hrs at 120V. Silver stained and photographed gels were analyzed with Genetools software.

Molecularly, 161 of 176 isolates were identified as MTB complex. Results of Multiplex PCR correlated with RIF mono-resistant (n=32) and MDR strains (n=6) of DST. The assay was validated further as four RIF mono-resistant and one MDR strain were identified.

The multiplex PCR process with DGGE is a rapid method that can be utilized to detect drug-resistance of MTB and the process and the primer sets have been patented.