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Tuberculosis and Detection of Drug Resistance by Combined Simultaneous Amplification Testing.

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Abstract:
To evaluate the efficacy of the combination of simultaneous amplification testing (SAT) and liquied culture testing (LCT) for the rapid detection of Mycobacterium tuberculosis (MTB) and drug-resistant mutants in respiratory samples. Suspected PTB and non-TB pulmonary disease samples were collected. All sputum samples were sent for acid-fast bacilli smear, SAT, culture and drug susceptibility testing (DST. LCT samples were tested by both RDB and DNA sequencing to identify drug resistance genes and mutated sites. The SAT positive rate was higher than the culture positive rate, with a coincidence rate. The sensitivity and specificity of SAT for diagnosing PTB were respectively, while those for culture were respectively. LC has high sensitivity and specificity in identifying drug resistance genes and mutated sites. The results of RDB correlated well with those of DST and DNA sequencing, with coincidence rates respectively. The combination of DST and Liquid Culture is promising for rapidly detecting PTB and monitoring drug resistance in clinical laboratories.

Keywords*: Tuberculosis, Liquid Culture, Drug Susceptibility Test (DST).*

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I. INTRODUCTION:

The Mycobacterium tuberculosis (MTB) epidemic is a major health concern worldwide. The new global tuberculosis (TB) report from the WHO stated that approximately one-third of the world's population was infected with MTB. Furthermore, in 2019 there were an estimated 10.4million new TB cases worldwide and approximately 1.4million deaths, with China having the third highest number of TB patients in the world? [1] With the widespread use of anti-TB drugs, multidrug-resistant tuberculosis (MDR-TB) and even extensively drug-resistant tuberculosis (XDR-TB) are now emerging and pose a considerable challenge to current TB prevention and control programs.2–4 Moreover, the number of inaccurate diagnoses and inappropriate treatments for TB patients are increasing, which encourages continued transmission of TB.[2]

II. MATERIAL AND METHODS:

The project study groups comprised of 181 TB positive individuals, 19 TB negativeindividuals confirmed by chest x-ray and 42 extrapulmonary tuberculosis individualsconfirmed by culture positivity/ histological evidence. All individuals included in thestudy were briefed about the project's aims and objectives, methodology to be followed,expected outcomes as well as their rights with regard to the use of their samples.[3]

Extra Pulmonary Tuberculosis (EPTB):

A total of 42 EPTB positive samplesconfirmed by culture positivity/ histological evidence were included in this group.Two sputum samples were collected within 24 hours of the interval (one on-spot andsecond early morning sample) from pulmonary tuberculosis patients & pooled togetherfor further research, while in case of extrapulmonary tuberculosis patients' samples werecollected based on the affected/involved organ. Example Cerebral Spinal Fluid (CSF) sample in case of Tuberculosis Meningitis. [4] Individuals in all the patient groups weregreater than 18 years of age, belonging to both sexes and attending the outpatientdepartment of a tertiary care hospital. All the samples were transported in the tertiarycontainment system, as per WHO guidelines [5].

Liquid Culture (Bact/ALERT 3D SYSTEM Biomérieux):

BacT/ALERT MP bottle of the bioMerieux company consisting Middlebrook 7H9 liquid medium (10 mL) supplemented with casein, bovine serum albumin and catalase were inoculated with 0.5 ml of the decontaminated specimen as per manufacturer instruction.

Reconstitution fluid (0.5 ml) containing glycerol, purified water, tween-80 was supplemented with an antibiotic solution (vancomycin, amphotericin B, trimethoprim, azlocillin, polymyxin B, nalidixic acid) was

added to the bottle before inoculation (109, 110). Bottles were incubated at 37 °C for 7 weeks. The results were available automatically after each 10 min on the computer screen of the BacT/ALERT instrument. [6]

Drug Susceptibility Test (DST)

Lowenstein Jenson (LJ) Medium DST:

The number of colonies obtained is multiplied by the dilution factor of inoculum to determine an actual number of colonies present on control medium and medium containing drug concentrations. This method is excellent and easy compared to the other method available for drug susceptibility testing [7].

III. RESULTS AND DISCUSSION:

If the antibiotic-containing bottle shows the presence of growth then the strain is considered as resistant. If no growth was observed at the same time of proportional control then the strain was called susceptible. If the growth was observed in control bottles within 2 days or 15 days later the test was considered as invalid and repeated again. [8] All invalid tests were repeated.

Drug Susceptibility profile	No of samples	Percentage
Susceptible to isoniazid	84	46.92%
Resistant to isoniazid	10	5.58%
Susceptible to rifampicin	86	48.04%
Resistant to rifampicin	8	4.46%
Susceptible to both drug	76	42.45%
Resistance to both drug (MDR)	85	47.48%

Table-1 Drug Susceptibility Profile of PTB Samples

Drug Susceptibility profile	No of samples	Percentage
Susceptible to isoniazid	16	100%
Resistant to isoniazid	0	0%
Susceptible to rifampicin	16	100%
Resistant to rifampicin	0	0%
Susceptible to both drug	16	100%
Resistance to both drug (MDR)	0	0%

Table-2 Drug Susceptibility Profile of EPTB Samples

Out of total 179 culture positive samples of PTB, 76 (42.45%) samples were found to be susceptible to both the drugs, 85 (47.48%) samples were found to be resistant to both the drugs showing MDR. Both L.J culture DST & liquid culture DST showed that 10 (5.58%) samples were resistant to only isoniazid drug and 8 (4%) samples showed resistance to only rifampicin drug. Out of total 16 culture positive samples of EPTB included in the study, all 16 (100%) samples were found to be susceptible to both the drugs showing no resistance to any drug, by both L.J medium DST & liquid culture DST (BacT/ALERT 3D SYSTEM bioMérieux).[9] The results of DST were considered as 'true results' when comparing with molecular methods since DST is considered the gold standard for determination of drug resistance (table 1-2).

The presence of growth on drug-containing LJ medium was considered as resistant to that particular drug whereas the absence of growth was considered as susceptible. Confirmed Multi-Drug Resistant sample and H37rv was used as a control for resistance and susceptibility respectively (figure).

Drug susceptibility profile of sample TB/32 showing the resistance to isoniazid and rifampicin drug containing LJ medium.

Results showing resistance to rifampicin have not shown any mutations in rpoB gene RRDR region. LC and DST assay from Tuberculosis Research Centre (TRC), Chennai. Since most of the INH resistant isolates were resistant to RIF as well so we concluded strains under study are MDR-MTB isolates[10]. Thus, chances that Mumbai may carry an extensive transmission rate of MDR-TB with rpoB and katG gene mutation cannot be ignored. The advantage of ASD-PCR method selected in the current study is the time between sampling and availability of the test results for clinical decision making. In general, Conventional methods of culture and DST take 6-8 weeks for definitive diagnosis [11]. Consequently, clinical decisions cannot be taken until then. In comparison, the molecular detection results of susceptibility to rifampicin and isoniazid are available within 8 hours due to changes occurred in rpoB and katG gene. This period is based on the time required for DNA extraction from clinical sample followed by PCR and amplification [12].

IV. CONCLUSION:

To conclude, the present study has standardized two new methods – Liquid Culture assay for detection of tuberculosis and DST assay for detection of drug resistance in M.tuberculosis. It has demonstrated that a LAMP assay with few modifications that do not compromise on its sensitivity and specificity is equal to or more than that of the currently available conventional methods. Research and optimization of the LC and DST assay indicate that it is very effective in the diagnosis of drug resistance in patient samples.

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