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USE OF LINE PROBE ASSAYS IN RESPIRATORY SAMPLES OF CONFIRMED PULMONARY TUBERCULOSIS CHILDREN

Yasha Mukim
MD, Assistant Professor,
Department of Microbiology,
Santosh Medical College,
Ghaziabad,

Arnab Ghosh
MD, Assistant Professor, Department of Virology,
PGIMER Chandigarh,

Dhulika Dhingra
MD,Assistant Professor, Department of Paediatrics ,
Chacha Nehru Bal Chikitsalaya, Delhi

***Abstract*—**In a tuberculosis high-burden country like India, pediatric tuberculosis constitutes approximately 10% of the incident total adult cases with approximately 9.5% children diagnosed as rifampicin-resistant tuberculosis in certain areas. Paucibacillary nature of pediatric tuberculosis causes numerous diagnostic challenges. National Tuberculosis Elimination Program(NTEP) has advocated the usage of molecular test Xpert MTB/RIF assay for early diagnosis and detection of rifampicin resistance, a surrogate marker of multidrug-resistance tuberculosis (MDR-Tb).(1) However, isoniazid-monoresistance remains undetected by Xpert MTB/RIF. There are doubts regarding isoniazid-monoresistance as precursor of MDR-Tb and confusion persists regarding treating isoniazid-monoresistance cases similar to drug-susceptible ones.(2) Line-probe assays, in-house or commercial, have the capability of rapidly detecting both isoniazid and rifampicin resistance directly from samples in contrast to time consuming based drug susceptibility testing methods but require proper standardization and stringent conditions.

The descriptive observational study was done in our hospital, a tertiary-care pediatric centre in Delhi over a 6-month period (July 2018-December 2018) with the aim of detecting isoniazid monoresistance directly from respiratory samples in Xpert MTB/RIF positive pediatric pulmonary cases by a commercial line probe assay, Geno Type MTBDR plus ver 2.0 **(Hains Life science, Nehren, Germany). Isoniazid susceptibility was confirmed for the culture positive samples by** Bactec MGIT 960 system (Becton Dickinson Diagnostic Systems, Sparks, MD).M. tuberculosis H37Rv strain (ATCC 27294) was used for quality control. A total of 35 children of age ≤ 12 years with clinical suspicion of pulmonary tuberculosis tested positive by GeneXpert MTB/RIF assay in gastric aspirate samples. Ziehl-Neelsen stained smear positivity was observed in only 14.2 % cases (5/35). GeneXpert MTB/RIF assay showed rifampicin resistance in one case (2.8%). Rest (34/35, 97.2%) were rifampicin sensitive. All smear-positive cases gave valid results in Geno Type MTBDR plus ver2.0assay while among smear negative cases five samples gave invalid results on repeated testing in the form of absence of amplification control and/or TUB band. Retrospectively we observed that these samples were stored for more than 4 weeks due to non-supply of kits. The problem of faint intensity of rpoB, katG, inhA bands was ameliorated by doubling volume of amplified sample during hybridization. Remaining procedure was performed as per manufacturer's instructions. Rifampicin susceptibility results corroborated with GeneXpert MTB/RIF results in 100% cases. Isoniazid monoresistance was observed in 11.5% samples (4/35) in the form of katG mutation at codon 315 (S315T1). The single rifampicin resistant isolate being also isoniazid resistant was an MDR-isolate . Twenty-three samples were Bactec MGIT 960 culture positive which included isolates of all 5 isoniazid resistant cases. Isoniazid resistance was observed in these 5 isolates only with MICs > 2 μg/ml.

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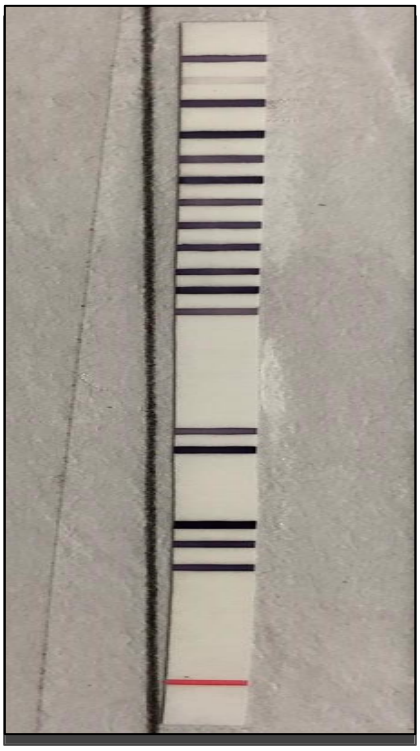
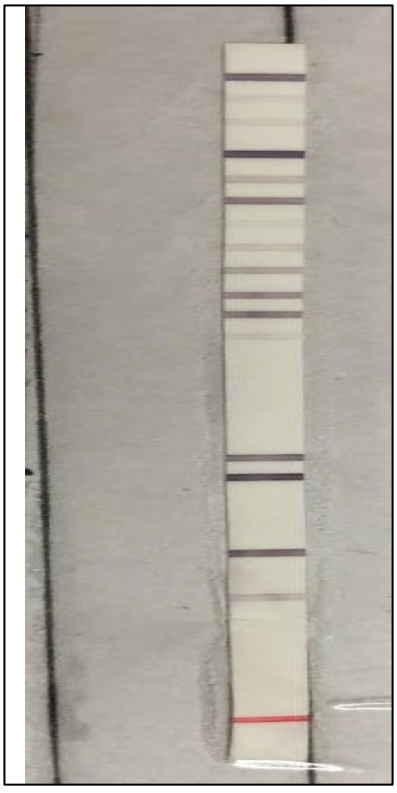
Picture 1,2 & 3 shows TUB complex and sensitivity to both Rifampicin and Isoniazid,TUB band detected and absence of INH WT1 band and no TUB complex detected, depicting invalid result.





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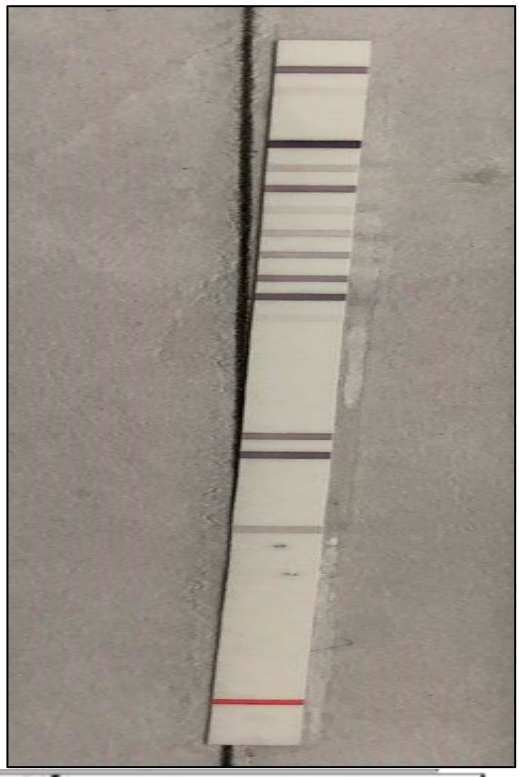

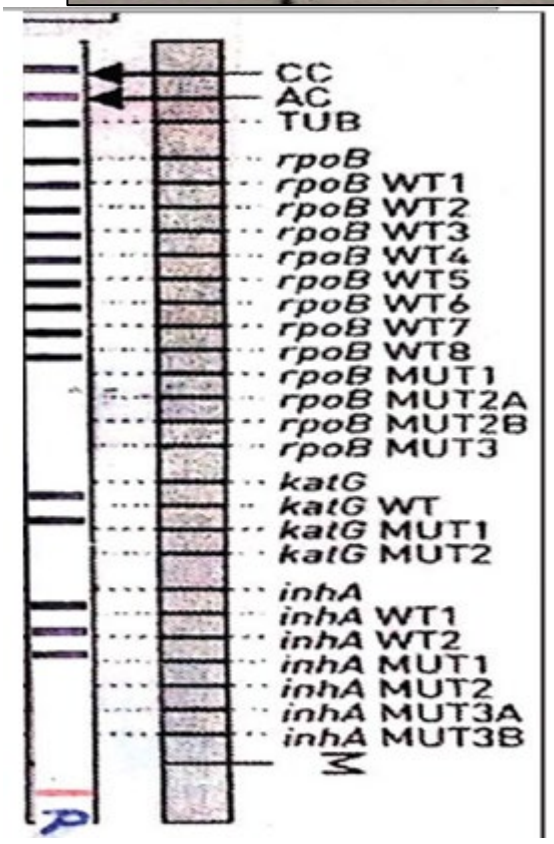

Picture 1 2 3

Similar to our findings, approximately 10.5% isoniazid-monoresistance has been reported by Rufai et al. in north Indian population. (3) Clinical implication of isoniazid-monoresistance particularly in pediatric population remains debated. Moreover, role of isoniazid-monoresistance as a precursor of MDR-Tb has been considered. (4) In its supplemental guideline, WHO recommends addition of levofloxacin to the regimen for 6 months without streptomycin for isoniazid-resistant, rifampicin-susceptible cases while NTEP advocates 3-6 months intensive phase regimen containing kanamycin and levofloxacin and 9 months levofloxacin in continuation phase. (2) In contrast, similar outcomes in terms of culture conversion rateand all-cause mortality has been observed in is oniazid-monoresistant tuberculosis compared to drug susceptible cases. (4) In presence of such conflicting guidelines and studies in the setting of underdiagnosis, it is imperative to accurately detect isoniazid-monoresistant Indian patients so that a large number of such patients can be randomized to WHO or RNTCP or drug sensitive regimens and actual treatment outcome can be determined. Moreover, need for different regimens for low level (MICs-0.5-2 μg/ml) and high level (MICs > 2 μg/ml) isoniazid-monoresistance should be reassessed. Our finding of 11.5% isoniazid-monoresistance, all being high-level, is only a tip of the iceberg and actual burden of isoniazid-resistance in tuberculosis high-burden India needs to be calculated by recruiting large number of incident cases.

Accurate diagnosis of drug resistance can be achieved by LPAs including commercial ones with low turn-around time of 24-48 hours in contrast to gold standard culture based methods which are time consuming. In fact, more than 90% concordance has been observed between sequencing and LPA in samples with discrepant results between LPA and MGIT-based susceptibility. (3) However, LPAs, including Geno Type MTBDR plus ver2.0have shown variable results in direct smear negative samples which underscores the importance of proper standardization and validation of these assays in smear negative cases. The assays which show high level of valid results can be considered to be included in the national programme for detection of isoniazid resistance.

HIGHLIGHTS

- National Tuberculosis Elimination Program, India advocates the usage of molecular test like Xpert MTB/RIF assay for detection of rifampicin resistance directly from samples for diagnosis of MDR-Tb but isoniazid-monoresistance remains underdiagnosed in the absence of properly validated assays and lack of guidelines.
- Isoniazid-monoresistance is hypothesized to be precursor of multidrug-resistance tuberculosis (MDR-Tb). There are significant differences in treatment guidelines of isoniazid-monoresistant tuberculosis recommended by

WHO and NTEP probably because of the lack of adequate studies.

- Proportion of isoniazid-monoresistance in newly diagnosed cases is significant even in pediatric population (>10%) in high-burden countries like India.
- Isoniazid-monoresistance should be routinely diagnosed in addition to rifampicin resistance by using assays with high levels of diagnostic accuracy. Well standardized and validated line probe assays can diagnose isoniazid-monoresistance directly from samples in paucibacillary disease as in pediatric patients with a low turn-around time.

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