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Commentary

Dilemmas with ethionamide susceptibility testing of *Mycobacterium tuberculosis*: A microbiologist & physician's nightmare

Multidrug resistant tuberculosis (MDR-TB) has been a topic of growing importance in the last decade. Emergence of MDR-TB has complicated infections caused by human immunodeficiency virus (HIV) and the re-emergence of tuberculosis in the West. MDR-TB is a major therapeutic challenge as the organism is resistant to two key anti-tuberculosis drugs, isoniazid and rifampicin. Programmatic management of drug resistant tuberculosis using second line therapy has been standardized through the development of international guidelines[1]. In India, the Revised National Tuberculosis Control Programme (RNTCP) advocates second line regimen comprising of six drugs - kanamycin, levofloxacin, ethionamide, pyrazinamide, ethambutol and cycloserine for 6-9 months of intensive phase therapy and levofloxacin, ethionamide, ethambutol and cycloserine for the next 18 months of the continuation phase[2]. Second line therapy is more complicated than the first line therapy as it has reduced potency, severe side effects and reduced efficacy. Hence, RNTCP advocates that poor response to treatment should prompt an examination of the programmatic, clinical or microbiological causes[2].

The lack of rapid, accurate and reliable drug susceptibility testing of the second line anti-tuberculosis drugs makes it difficult to examine the microbiological causes of poor response. *In vitro* results of drug susceptibility testing to the second line drugs also show poor clinical correlation leading the physicians to rely more on their clinical acumen than factual data. Empirical treatment is advocated, not taking into account the risk of medication to which the *Mycobacterium tuberculosis* isolate may be resistant. Pharmacokinetic variability whereby poor circulating drug concentrations may impair killing of isolates with borderline susceptibility is also not considered[3]. This may further amplify resistance. For a drug like ethionamide, where drug susceptibility pattern is difficult to determine and the therapy is empirical, development of resistance may be a serious cause of concern. Lakshmi *et al*[4] in this issue have thus taken up a crucial investigation in the wake of emergence of MDR-TB and extensively drug resistant tuberculosis (XDR-TB).

Testing for drug susceptibility to ethionamide, a crucial part of second line anti-tuberculosis therapy, is problematic as the difference characterizing drug resistance or susceptibility is small. Moreover, the minimum inhibitory concentration (MIC) is very near to the therapeutic index as commented by Lakshmi *et al*, in their article in this issue[4]. In addition, the drug is thermolabile, which makes drug susceptibility testing more difficult[5]. Due to these issues, there is poor clinical and laboratory correlation[5].

Inspite of the importance of ethionamide in the treatment of MDR-TB, there are only a few reports on the drug susceptibility testing of this agent. The gold standard of drug susceptibility testing against anti-tuberculosis agents is the proportion method that uses a single critical concentration of drugs to determine resistance or susceptibility. Criteria for measuring resistance such as critical concentrations and critical proportions of drugs affect the treatment predictive value of the drug susceptibility assay. The World Health Organization (WHO) has defined critical concentrations for various second line drugs taking into account the *in vitro* criteria for resistance using representative clinical samples and recommended a concentration of 40 µg/ml for proportion method of drug susceptibility testing of ethionamide[6,7]. Lakshmi *et al*[4] also used the critical concentration of 40 µg/ml to perform the drug susceptibility assay by proportion method. However, the thermolabile nature of ethionamide may play a role in reducing the potency of the drug during inspissation

of the drug containing medium. Deterioration of the drug during prolonged incubation period required for *M. tuberculosis* can also be crucial. Technical errors, as mentioned by Lakshmi *et al*[4] like inoculum preparation, incubation and interpretation can add to erroneous results and should be minimized.

Various investigators have standardized the critical concentration of ethionamide to be used in assays other than the proportion method, such as MIC and MGIT960. Kim *et al*[8] used a critical concentration of 5 µg/ml for MGIT 960. Mpagama *et al*[3] used 5 µg/ml of ethionamide as the critical concentration to segregate borderline susceptible from resistant through MIC on MycoTB sensititer plates (Trek Diagnostics, Cleveland, Ohio, USA). Lakshmi *et al*[4] recommended a much higher concentration of 80 µg/ml for MIC on Lowenstein-Jensen (L-J) medium. The sensitivity of the MIC improved using 80 µg/ml as the cut-off. Though the MIC method has been observed to be more efficient than the proportion method[5], it has not yet been recommended to drive therapeutic decisions. The need of the hour is to generate more data on ethionamide susceptibility profile of a specific geographical region, since it has been reported that geographical variation in strains also leads to a shift in the MIC value[9]. This would entail re-calibration of MIC values for each geographical region.

In future, molecular tests may help in overcoming the problems encountered during testing for ethionamide susceptibility. Structural analogues, ethionamide and isoniazid share the same molecular target, the NADH-dependent enoyl-acyl carrier protein reductase InhA, which is involved in the synthesis of mycolic acids. Hence, cross-resistance to isoniazid and ethionamide may be observed in clinical isolates. Ethionamide acts by inhibiting mycobacterial cell wall mycolic acid synthesis and requires activation by a prodrug activator, NADPH-specific flavin adenosine dinucleotide containing mono-oxygenase encoded by *eth*A[10]. The expression of EthA is negatively regulated by EthR, a regulator that interacts directly with the *ethA* promoter region. Ethionamide resistance has been shown to be associated with mutations at *eth*A and *eth*R[10,11]. It has been reported that the presence of a mutation in the *inhA* regulatory region together with a mutation in the *inhA* coding region is associated with high-level resistance to ethionamide among the MDR-TB isolates[12]. In a study at Mumbai, Vadwai *et al*[13] studied 69 phenotypically determined ethionamide resistant isolates and found that *inh*A promoter mutation was associated with ethionamide resistance in 21 isolates. They concluded that *inh*A mutation could be considered as a marker for ethionamide resistance in India. However, since 69.5 per cent of the ethionamide resistant isolates did not have *inh*A promoter mutations, alternative mechanisms could not be ruled out[13]. Identifying the molecular basis of ethionamide resistance could be an important focus for future studies. Further studies are needed to address the basis for mechanisms of ethionamide susceptibility.

The future holds a lot of promises with newer molecular assays. Considering the problems faced due to phenotypic drug susceptibility profile, future assays for drug susceptibility may include a combination of molecular and conventional assays for second line drug susceptibility testing. What is also needed is better microbiological-clinical correlation for drug susceptibility profiles since there is a vast difference between *in vitro* and *in vivo* results. Bigger cohorts correlating the drug susceptibility profile with patient's clinical outcome are also needed to retard the emergence of extensively drug resistant tuberculosis.

Mandira Varma-Basil[1,*] & Rajendra Prasad[2]
Departments of [1]Microbiology &
[2]Respiratory Medicine,
Vallabhbhai Patel Chest Institute
University of Delhi, Delhi 110 007, India
[*]*For correspondence*:
mandirav@rediffmail.com

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