purification protocols have to be rigorous to avoid contaminating endonucleases. In contrast, *Ofo*I purification is relatively simple compared to *Ava*I purification[10], and the purified restriction enzyme *Ofo*I is free from non-specific nucleases and any other restriction enzyme.

More than 200 cyanobacterial restriction endonucleases have been reported[11]. Amongst these, *Ava*I is the well-studied restriction enzyme from *A. variabilis*. Till date, thirteen isoschizomers and one neoschizomer (*Nli3877I*) of *Ava*I have been found in various cyanobacterial genera like *Agmenellum*[12], *Anabaena, Anabaenopsis*, *Nostoc*[13], and *Phormidium*. Widespread occurrence of these isoschizomers in various cyanobacterial species[14] belonging to different genera and families, strongly suggests that the genes for the enzyme are likely to be of common origin, and also indicates efficient horizontal gene transfer within cyanobacteria. Near identical behaviour in reaction characteristics of *Ava*I and *Ofo*I provides further credence to this hypothesis.

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Comparison of Etest with MIC method on Lowenstein–Jensen medium for susceptibility testing of *Mycobacterium tuberculosis*

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The emergence of multiple drug resistance in tuberculosis (TB) has led to increased interest in determining the rifampicin and isoniazid susceptibility rapidly for management of individual cases as well as for epidemiological purposes. In TB, a major problem appears to be different cut-off levels recommended in different media/systems. To evaluate the comparative performance of Etest, it was compared to standard Lowenstein–Jensen (LJ) MIC method using 55 *Mycobacterium tuberculosis* clinical isolates, among which 25 (45.45%) were resistant to rifampicin. The concordance of Etest (1 μg/ml) with standard LJ MIC method (64 μg/ml) was 100%. In the case of INH there was 85% concordance between the two methods when 1 μg/ml on the Middlebrook agar by Etest was used as cut-off point, whereas 1 μg/ml was the cut-off MIC on LJ medium. However there was 100% concordance when the cut-off point was changed to 0.75 μg/ml. The study supports the utility of Etest for timely detection of drug resistance in *M. tuberculosis*.

TUBERCULOSIS (TB) is a growing health problem both in terms of disease burden and increasing resistance to conventional chemotherapy[1]. The regions where TB is more prevalent lack the resources to implement appropriate measures to control the disease[2]. The standard treatment of TB as recommended by the World Health Organization (WHO) is a multi-drug regimen that includes two important drugs, rifampicin and isoniazid[3,4], and the resistance to these two drugs together[5] has been defined as multi-drug resistance (MDR). The early recognition and appropriate treatment have been proven to be one of the most effective strategies to control MDR TB[6], even in human immunodeficiency virus (HIV)-infected population[7].

Currently recommended methods for susceptibility testing include the proportion method, resistance ratio method and MIC method on Lowenstein–Jensen medium[8] and on defined media by using Middlebrook agar method, including the BACTEC[9]. Although relatively expensive, the Etest method will be valuable for developing countries, as it is relatively easy to perform, provides

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susceptibility results in about a week, and does not require expensive instruments and media. To further evaluate the Etest method for susceptibility testing of *Mycobacterium tuberculosis*, we have compared the Etest with LJ MIC method by a double-blind coded procedure using two most important anti-tuberculous agents active against *M. tuberculosis*.

M. tuberculosis isolates identified according to standard criteria[10] and reported to be resistant/sensitive to rifampicin and isoniazid from various regions of India and deposited in the Mycobacterial Repository Centre of our Institute were included in the study. A total of 55 strains along with control strain *M. tuberculosis* $H37R_V$ were tested.

Rifampicin and isoniazid were obtained from Novartis India Ltd. These were incorporated in LJ medium at 64 µg/ml and 1 µg/ml concentration, respectively. A standard bacterial suspension (4 mg/ml) prepared according to the procedure of Canetti *et al.*[8] was used as inoculum for inoculation onto LJ medium slants with a loop of 3 mm internal diameter[3,8,11]. All the culture bottles were incubated at 37°C. Readings were taken at the end of four weeks of inoculation. MIC was determined using standard criteria of counting the colony-forming units and comparing with culture controls[8,11,12]. An isolate was considered resistant if it yielded a growth of 20 colonies or more at a particular concentration of drug. Culture control was read to assess the quality of inoculum as growth of ++ using 150–200 colonies[11,13] on control was considered to be adequate for reading the result.

Growths of all the wild isolates were scraped from freshly-growing (3 to 4 weeks) LJ slants into 1 ml of Middlebrook 7H9 broth (Difco, USA). The tubes were vortexed vigorously to homogenize the suspension. The large particles were allowed to settle and the supernatant was adjusted to turbidity equivalent to a Mc Farland 3.0. The inoculum was swabbed onto the plate of freshly-prepared Middlebrook 7H11 agar with OADC supplement (depth $G \pm 0.5$ mm) by streaking the entire surface in three directions[14], and excess liquid was removed. The plates were pre-incubated at 37°C in BOD incubator for 24 h after which the Etest strip containing gradients of rifampicin (0.002 to 32 µg/ml) and isoniazid (0.016 to 256 µg/ml) was placed on the agar surface. The plates were then incubated under the same conditions until an inhibition ellipse zone was visible (5–7 days). The MIC was interpreted as the point at which the ellipse intersected the Etest strip, as described in the *Etest Technical Guide*[14]. When hazes and isolated mutant colonies were seen in the ellipse, the MICs were read where these were completely inhibited.

In this study, 45.45% (25 out of 55) of the isolates were resistant to rifampicin at 64 µg/ml and 36.36% (20 out of 55) of the isolates were resistant to isoniazid at 1 µg/ml by standard LJ drug incorporation method using MIC approach. Using Etest method, 45.45% (25 out of 55) of isolates were resistant to rifampicin at 1 µg/ml and 30.78% (17 out of 55) of the isolates were resistant to isoniazid at 1 µg/ml. When the cut-off point was changed to 0.75 µg/ml, 36.36% (20 out of 55) of the isolates were resistant. By Etest (INH at 0.75 µg/ml), MDR was 27.27% (15 out of 55). There was 100% concordance between LJ MIC method and Etest method. Both the techniques showed similar trends overall (100% concordance) when using the standard cut-off points for rifampicin (64 µg/ml on LJ and 1 µg/ml on Middlebrook agar by Etest). In case of isoniazid, there was 85% concordance between the two methods when 1 µg/ml on Middlebrook agar by Etest was used as a cut-off point. However, there was 100% concordance when the cut-off point was changed to 0.75 µg/ml (Figure 1).

Rifampicin is one of the important constituents of the anti-tuberculosis drug regimen. However, drug resistance to this drug has been increasing and global prevalence has been estimated to be around 3% (refs 15, 16). On the other hand, rifampicin resistance in treatment failure cases has been reported to be as high as 20 to 30% or even higher[11,16]. Such trends are reflected in India also[11,15] in the strains deposited at the Mycobacterial Repository Centre at our institute. For rifampicin in LJ medium, the recommended cut-off points have varied from 30 to 40 µg/ml (refs 8, 17) and 50 µg/ml (ref. 18) by the proportion method and 64 µg/ml (refs 11, 12) by MIC, whereas in Middlebrook agar broth by different methods, a cut-off point of 1.0 µg/ml has been considered significant[9].

For the Etest method, 1.0 µg/ml has been considered as the cut-off point[19,20]. For INH, sensitivity screening of *M. tuberculosis* by drug incorporation in LJ and Middlebrook showed MICs of 0.5 to 1.5 µg/ml (refs 8, 10), 0.5 µg/ml

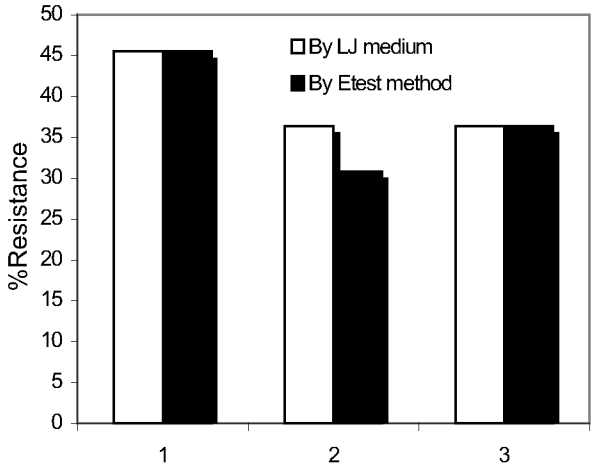

Figure 1. Proportion of rifampicin and INH resistance levels in *M. tuberculosis* isolates by LJ MIC and Etest method. 1, RFM levels of 64 µg/ml by LJ and 1 µg/ml by Etest; 2, INH level of 1 µg/ml by LJ and Etest; 3, INH levels of 1 µg/ml by LJ and 0.75 µg/ml by Etest.

(ref. 21) and 1 to 2 μg/ml (ref. 22). In our study, there has been good concordance between LJ MIC method and Etest. There is apparent need to analyse the relationship between cut-off points. There was 85% concordance between the two methods when 1 μg/ml on Middlebrook agar by Etest was used as cut-off. However, there was 100% concordance when the cut-off was changed to 0.75 μg/ml. This needs to be confirmed in a large number of isolates.

This study shows that Etest compares well with LJ medium MIC method. Though it needs to be compared in a large number of isolates, minor changes in cut-off point for INH to 0.75 μg/ml are possibly required. With minor changes among the criteria, Etest should be applicable for susceptibility testing of *M. tuberculosis* globally, even in countries like India where LJ medium is still used due to economic and logistic reasons.

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Psilotum complanatum Sw., a rare epiphytic fern ally of Great Nicobar Island: Exploration and habitat monitoring

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The Great Nicobar Island is considered as a unique zone of biodiversity housing many rare, endangered and endemic plant species. The pteridophytic species of the region possess a narrow range of adaptability and narrow ecological amplitude. *Psilotum complanatum* Sw., a rare epiphytic fern ally of the Great Nicobar Island is found in specific localities, particularly associated with tropical evergreen formations. Detailed assessment of biodiversity and its distribution pattern at species and community levels is necessary for bioprospecting. In this respect, the present study aims to establish species–habitat relationship based on field observations and also highlights interspecific relation of *P. complanatum* Sw. with another important fern species, *Sphaeropteris albosetacea* (Bedd.) Tyron (*Cyathea albosetaeca* Bedd; the tree fern).

THE Great Nicobar Island (Figure 1) is blessed with unique flora, interesting fauna and a network of five rivers (Alexandra, Jubilee, Galathea, Amrit Kaur and Dogmar). The tall and well-stratified trees provide a suitable habitat for growth of epiphytic ferns. Kurz, in 1876, reported a few ferns from here; later, in 1987, Ellis reported 120 species of pteridophytes, among which 60 are from the Great Nicobar Island alone[1]. Members under the genera *Psilotum* are referred to as a 'living rhyniophyte' by many palaeobotanists and plant morphologists. Further critical analysis of its morphology and shoot organization reveals that despite its simplicity, it exhibits the same level of organization as seed plants[2].

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