Correspondence

Tracing transmission of tuberculosis by random amplified polymorphic DNA (RAPD) analysis within same family & neighbourhood

Sir,

Molecular epidemiology that integrates molecular approaches into the convention epidemiologic investigations has proved quite useful in tracing the transmission of drug resistant *Mycobacterium tuberculosis* in USA. Molecular epidemiological tools for tuberculosis include both restriction fragment length polymorphism (RFLP) as well as PCR methods. The most useful methods are the IS 6110 RFLP analysis and the two PCR-based methods—spoligotyping and mycobacterial interspersed repetitive unit (MIRU) analysis. In our previous report, the typing of Indian isolates of *Mycobacterium tuberculosis* by random amplified polymorphic DNA (RAPD) analysis using an arbitrary primer 986 FP, was found to be reproducible and promising to establish similarity in profiles, and clustering was found among isolates from Ranchi, New Delhi and Chandigarh. In this report we have focused on the application of this system on analysis of *M. tuberculosis* isolates from a small group of patients living in two closely located slum areas of Agra city in north India to find out its utility in rapid discrimination of isolates.

A total of 16 coded isolates of *M. tuberculosis* from 16 patients (2 from the same family and 14 from neighbourhood) stored in the Mycobacterial Repository Centre of National JALMA Institute for Leprosy & other mycobacterial Diseases, Agra, obtained from TB sputum samples of Meena Charitable Hospital Lohamandi, Agra, were included. Isolates obtained from the sputa received from TB patients living in two closely located slum areas of Agra city viz., Jagdeeshpura and Lohamandi during April 2004 to September 2004, were studied. The isolates used in the study were obtained as a part of surveillance study on drug resistance of Government of India which was duly approved by the Ethical Committee of the Institute. DNAs from these isolates were isolated and were analyzed RAPD technique as described earlier taking the usual precautions.

There was a clear cluster of isolates from patients from same family (DAU-166, DAU-168) and neighbourhood (DAU-171, DAU-180, DAU-165, DAU-162, DAU-156, DAU-40, DAU-16) (Figs. 1 and 2). These observations indicate utility of RAPD analysis in rapid discrimination of isolates from cases within a family and also from those who were living in these slum areas. Similar findings of showing evidence of transmission have been reported from Taiwan city using this system. RAPD reproducibility is known to be affected by several factors such as the thermal cycler used, the enzyme involved in

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Fig.1. RAPD profiles from different isolates of *M. tuberculosis* from a slum area of Agra city.
the amplification\(^1\) and the age of the strain studied\(^2\). If the effect of these factors is not properly controlled, the RAPD analysis may not be suitable to be implemented as a molecular epidemiological tool. According to some earlier studies\(^3,13\), the RAPD analysis, although using different primer compared to the present study, showed a low sensitivity, reproducibility and discriminatory power in comparison with most prominent genotyping methods like IS 6110, MIRU-VNTR and spoligotyping.

Unlike these studies, our RAPD analysis using primers described earlier did not produce different pattern for duplicate DNA sample\(^8\). Reproducibility was also reported to be good in other studies\(^7,9\).

Though RAPD analysis has the advantage of being a simple, easy, fast and relatively low cost molecular typing method, important aspects like reproducibility, comparability and complimentary role with other discriminatory method like IS 6110\(^4\), MIRU-VNTR\(^6\), spoligotyping\(^5\) and amplified fragment length polymorphism (AFLP)\(^14\) need to studied and are to be taken into consideration. There is an intense need to conduct well planned studies specially in densely populated areas such as slum areas of major cities of India and to find complementary role of RAPD with other discriminatory methods for studying dynamics of transmission, especially after introduction of DOTS. Our study indicates that RAPD analysis seems to have potential in tracing transmission of tuberculosis within same family and neighbourhood.

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