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Commentary

Molecular epidemiology of tuberculosis: Opportunities & challenges in disease control

A pathogen *Mycobacterium tuberculosis* that produces nearly 10.4 million new infections and 1.4 million deaths in a year and is one of the top 10 causes of death worldwide[1], remains one of the most successful human pathogens today with enormous health and economic problems in both the developing and high-income countries. The success of propagation of tuberculosis (TB) is directly linked to the social and hygiene conditions of human populations. The *M. tuberculosis* complex (MTBC) emerged about 70,000 yrs ago as a genetic bottleneck and spread globally by clonal expansion. Increase in population during the Neolithic period and the accompanying migration of humans are believed to be some of the factors that led to the spread of this pathogen[2]. The organism has undergone several changes over the centuries; thus, though a genetically homogenous group, the genetic diversity of MTBC may be greater than previously envisaged[3]. This diversity may in turn impact the biological properties of the organism[4], which may further impact TB control programmes. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB has increased the challenges faced by TB control programmes manifold. Moreover, several studies have shown that diverse molecular types of the organism may have different abilities to acquire drug resistance. Hence, the increase in drug resistance has added to our need to understand new clones that are developing and about the clones that have become extinct.

The global impact of TB can, thus, be reduced only with a concerted effort by not only clinicians and laboratory specialists but also epidemiologists and public health officials. This implies that we need coordinated efforts to promptly diagnose TB, adequately treat the disease and detect outbreaks accurately[2]. The latter requires the use of a combination of conventional and molecular epidemiological tools.

Molecular epidemiology has gained importance in recent times as a resource to understand crucial issues in spread of TB, particularly MDR and XDR-TB, and has emerged as a combination of molecular typing techniques and classical epidemiological approaches. Proper control of TB requires knowledge of the strains circulating in a region, being able to differentiate between relapse and reinfection, identifying incidence of recent transmission, the risk factors involved, the ability to track geographic distribution and clonal expansion of specific strains. There is increasing evidence that genetic differences in MTBC strains are linked to the outcome of the disease and thus patient management[5]. Hence, information about the strain can help in disease control, especially during outbreaks.

Although, initially, the identification and discrimination of mycobacteria were dependent on individual strain phenotype, susceptibility to antimicrobial agents, biochemical differences and serological reactivity, the introduction of molecular techniques in the field of TB has improved our understanding of the dissemination dynamics and evolutionary genetics of the pathogen. The first molecular typing methods used for *M. tuberculosis* were based on restriction fragment length polymorphism (RFLP) analysis of bacterial DNA. Later, insertion sequences such as IS*6110* were used[6]. IS*6110* RFLP-based fingerprinting has been used extensively to study the mycobacterial population structure in several parts of the world, including India[7-11]. However, IS*6110* fingerprinting is of limited use since a significant proportion (40-44%) of isolates of *M. tuberculosis* in certain regions of the world including several parts of India have been reported with low copy numbers or lack of IS*6110*[7,8,12,13]. Furthermore, IS*6110* typing is labour intensive and requires several weeks for culturing the *M. tuberculosis* isolates. Molecular typing methods targeting spacer sequences in the

direct repeat region, including spoligotyping, have also been used. However, these methods if used alone may underestimate the clonal diversity of *M. tuberculosis*[14], though spoligotyping has been found to be useful in identifying strains belonging to different clades or lineages[2]. Methods based on variable-number tandem repeats (VNTRs) of genetic elements such as mycobacterial interspersed repetitive unit (MIRU) typing can also be used to differentiate between *M. tuberculosis* isolates with low copy number IS*6110* elements[15]. MIRU-VNTR has a discriminating power greater than that of spoligotyping. In fact, when used together, MIRU-VNTR and spoligotyping offer a powerful molecular epidemiological tool. In addition, these techniques are less cumbersome than IS*6110* and the results are available faster. Although these techniques target sequences that are genetically variable, these interrogate less than one per cent of the genome of *M. tuberculosis*.

The availability of genomic sequences of *M. tuberculosis* led to a new phase in the molecular epidemiological investigations of TB. Earlier studies used Sanger sequencing[16]. However, next generation sequencing (NGS) or high throughput platforms metamorphosed a mere research tool into a large-scale diagnostic and molecular typing platform since millions of DNA fragments could be sequenced at the same time[16].

With these new technological advances, the clinical implications of molecular epidemiological studies have increased[16]. Molecular typing of MTBC cannot only inform investigators about the strains circulating in a particular country or region but can also be used to monitor the spread of specific genotypes in a community or between patients. Moreover, molecular typing can be used to identify cross contamination in laboratories and thus avoid false detection of pseudo-outbreaks[16]. More importantly, genotyping data have provided important information on the risk factors involved in TB transmission. Risk factors that have been identified for recent transmission of TB are pulmonary TB, smear-positive disease, HIV, alcohol abuse, intravenous drug use and residence in urban settings[17,18]. Molecular typing can thus be used by health authorities to plan or modify TB control programmes.

In this issue, Pasechnik *et al*[19] have used spoligotyping and MIRU typing to identify *M. tuberculosis* strains circulating in Omsk, Siberia. They reported that a large number of their isolates belonged to the Beijing family which is known to have a high rate of multidrug resistance. It has been hypothesized that drug resistance leads to reduced virulence and transmissibility of *M. tuberculosis*. However, as in Omsk, large regions of the world have been seen to harbour drug-resistant isolates, the most notable being, India, China and Russia. It is believed that epistasis may play a role in the compensation of the fitness cost that is believed to be associated with drug resistance[14]. The spread of MDR strains differs in various regions. In Europe, though the predominant MDR strains vary between countries, the T, LAM and Haarlem families have been seen to harbour the maximum numbers of MDR strains, while in East Asia, the Beijing isolates make up the most MDR isolates[16].

India is a vast land with enormous genetic and ethnic diversities and contributes to one-fourth of the global incident TB cases[20]. The distribution of *M. tuberculosis* lineages globally and in India emphasizes the spread of TB linked to human travel. Studies from India showed that the Central Asian strain (CAS), a modern lineage, was predominant in the western, central and northern parts of India[10,11,21-23]. The Manu lineage has been found in West Central India, while the East African Indian lineage has been predominantly found in south India[21]. Beijing is the third most predominant lineage, but the dominant lineage in North Eastern India[21].

It has also been found that the modern lineages have acquired mutations faster than ancient lineages. Beijing has been significantly associated with MDR as compared to other lineages. Many studies have linked the *M. tuberculosis* genotypes with the clinical manifestations of disease. For example, CAS strains have been found to be associated with extrapulmonary disease[24]. Euro American lineage was associated more with pulmonary TB than extrapulmonary TB[25].

Although India is a diverse land, the number of epidemiological studies available does not do justice to the repertoire of strains circulating in the country. In addition, the number of isolates included in most of the studies is only a few. Moreover, though the relapse rate of TB in India is 10 per cent, there are no data on whether the relapse is due to reactivation of a previous infection or due to reinfection[26]. The need of the hour is large multisite studies that include strains from large parts of India. Given the recent increase in travel related to work and leisure, a continuous vigil is required to be able to arrest the spread of TB and the

emergence of new clones. In addition, it is now widely clear that missed diagnostic opportunities, particularly that of drug-resistant *M. tuberculosis*, can lead to the evolution of more transmissible organisms that may become increasingly drug resistant. Molecular typing tools can help public health officials to identify transmission links with confidence. In the future, we may see powerful high throughput technologies such as NGS being used for complete strain characterization, detection of drug resistance, monitoring emergence of new drug resistance mutations and mechanisms and outbreak investigation through identification of clades and lineages that will transform disease management and target interventions and resources for TB control more appropriately.

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