Editorials

Typing of mycobacteria using spoligotyping

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The potential use of spoligotyping—a polymerase chain reaction based typing system for differentiation of strains of Mycobacterium tuberculosis—for studying the epidemiology of tuberculosis in developing countries which do not routinely perform mycobacterial culture is described in this issue of Thorax by Heyderman et al in a study from Zimbabwe. How does this new method compare with the now more established molecular typing methods for tuberculosis?

The most commonly used typing system for tuberculosis is IS6110 based restriction fragment length polymorphism (RFLP). Extensive experience with IS6110 based RFLP typing has shown that there is a high degree of heterogeneity in typing patterns. This means that the technique is highly discriminatory so that it is unlikely that two organisms will share indistinguishable typing patterns by chance. This property has allowed RFLP typing to be used in a variety of ways. For example, typing can be used to confirm outbreaks of tuberculosis or incidents of suspected laboratory cross contamination, and the technique has been used to investigate the relative importance of reactivation versus reinfection in patients with a second episode of tuberculosis. Studies which type all available isolates from a defined geographical area over a specified time period can help to estimate the importance of recent transmission and to identify risk factors for the spread of tuberculosis.

There are a number of difficulties with standard IS6110 typing—e.g., the technique is less discriminatory in isolates with low copy numbers of IS6110 (secondary typing using techniques based on different genetic markers such as direct repeat (DR) typing or polymorphic GC rich repetitive sequence typing (PGCRS) typing may be needed to differentiate such isolates); it requires large quantities of DNA so that prolonged culture may be required before typing causing considerable delay before results are available; it is also relatively labour intensive and thus costly; and the typing patterns produced have varying numbers of bands which may also vary in position so sophisticated software is needed to analyse large numbers of typing patterns and to compare results between laboratories.

Spoligotyping has some major advantages over standard IS6110 typing. It is a PCR based technique so minimal quantities of DNA are required. This means that it has the potential to be used directly on clinical specimens without the need for prior culture but, as the authors report, it may be necessary to take several samples before successful typing can be performed on sputum specimens. In countries which do not routinely culture specimens of Mycobacterium tuberculosis the ability to type isolates using small amounts of DNA may be valuable. However, such countries are also unlikely to have resources for performing spoligotyping so that isolates may still need to be transported to a reference laboratory with appropriate equipment and expertise.

In countries which do perform culture routinely the organism could be typed at an earlier stage without the need to wait for substantial growth. This raises the possibility of using PCR based typing results more in “real time” which may allow identification of outbreaks and incidents of laboratory cross contamination in time for effective early intervention. Spoligotyping can also be used to type non-viable archived isolates of M tuberculosis. It is less technically demanding than standard IS6110 typing and experienced laboratories can process large numbers of isolates quickly using the technique. The fact that spoligotyping produces a “barcode” typing pattern (where “bars” may either be present or absent but will not vary in position) allows the patterns to be compared using simple spreadsheet software which will sort characters. It is also much easier to compare typing results between centres.

All of these features provide considerable advantages over standard IS6110 typing, but in its current stage of development spoligotyping is substantially less discriminatory than standard IS6110 typing. In Austria 60% of isolates have unique IS6110 RFLP typing patterns compared with only 28% with unique spoligotyping patterns. A few spoligotyping patterns are particularly common—for example, in Austria 36% of isolates were allocated to only three spoligotyping patterns. In a study in London the three most common spoligotyping patterns accounted for 20% of isolates. The 125 isolates with these three patterns were subdivided into 93 subtypes using secondary typing (PHLS unpublished data).

Spoligotyping has been compared with other typing techniques including IS6110 RFLP, PGRS RFLP, multiplex linked PCR, random amplified polymorphism DNA (RAPD), and direct repeat element PCR and has been found to be less discriminatory than these techniques. It was found to be more reproducible than most of these techniques except for mixed linker PCR and standard IS6110 typing. Thus, although spoligotyping has some considerable advantages over standard IS6110 typing in terms of ease of use, speed of typing, the small quantities of DNA required, and the ease of computer comparison of banding patterns, the results produced need to be treated more cautiously. Isolates with different spoligotyping patterns can be confidently assumed to represent different genotypes (although one band differences may result from technical difficulties in typing). This may allow a suspected outbreak or laboratory cross contamination incident to be disproved. Interpretation of indistinguishable spoligotyping patterns is more difficult. Although clusters of isolates with indistinguishable spoligotyping patterns may represent chains of recent transmission, they may also represent broad genetic similarities between isolates which share a more distant common ancestor. With standard IS6110 typing (particularly in isolates with multiple copies of IS6110) one can be more confident in saying that indistinguishable isolates represent recent transmission than with spoligotyping. To make this claim for spoligotyping a secondary typing technique would be needed to allow further discrimination. The spoligotyping technique
used by Heyderman et al/ was based on 43 different spacer oligo-
nucleotides between direct repeat areas of the genome. Develop-
ment of the technique to use more spacer oligo-
nucleotides is likely to lead to more discriminatory spoligo-
typing methods being available in future. It will be important
to evaluate these against other typing techniques before ac-
cepting that they are sufficiently discriminatory to be of
practical use.

An understanding of the ease of use, discriminatory power,
and the reproducibility of typing techniques is essential for
the design and interpretation of typing studies. Equally
important is the selection of patients for the study and the
and the collection of valid epidemiological data to allow inter-
pretation of results. In studies which are using typing to
identify the extent of recent transmission and risk factors for
transmission a number of questions should be asked. Are the
samples drawn from a geographically defined area which
makes epidemiological sense? For example, if isolates from
only one hospital in a city are typed, transmission to or from
patients with indistinguishable isolates who are from the
same city but were treated in a different hospital will be
missed. Was the study conducted over a sufficiently long
time frame? It has been estimated that most people who
develop tuberculosis near to the time of infection will do so
within two to three years (mainly within the first year).19
Thus, studies that are only a few months long will miss epis-
odes of transmission with longer incubation periods. What
proportion of all patients with tuberculosis in the study area
and study time have had isolates typed? The lower this pro-
portion the lower will be the estimate of recent transmis-
sion.20 What epidemiological information has been collected?
There is little point in typing isolates without collecting epi-
demiological information to allow interpretation of results.

IS6110 based RFLP typing studies have substantially
increased our understanding of the epidemiology of tuber-
culosus. The development of easily used PCR based
techniques which do not rely on culture of the organism
should be welcomed. Until these techniques have been
shown to provide a high degree of discrimination and repeatability, the results should be treated with caution. The
adoption of IS6110 based RFLP typing using standardised
methodology in countries throughout the world has been a
major achievement. If spoligotyping can be made suffi-
ciently discriminatory and reproducible, the ease of use and
simple computer analysis of results would make it a good
candidate for a standard PCR based typing system.

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1 Heyderman RS, Goyal M, Roberts P, et al. Pulmonary tuberculosis in
2 van Embden JDA, Cave MD, Crawford JT, et al. Strain identification
of Mycobacterium tuberculosis by DNA fingerprinting: recommendations
3 van Soolingen D, Hermans PWM, de Haas PEW, et al. Occurrence and sta-
bility of of insertion sequences in Mycobacterium tuberculosis complex
strains: evaluation of an insertion sequence-dependent DNA polymor-
phism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 1991;
29:2578–86.
4 Alland D, Kalkut GE, Moss AR, et al. Transmission of tuberculosis in
5 Small PM, Hopewell PC, Singh SP, et al. The epidemiology of tuberculosis
6 Genewew T, Telenti A, Bernasconi C, et al. Molecular approach to identify-
ing route of transmission of tuberculosis in the community. Lancet
7 CDC. Nosocomial transmission of multidrug-resistant tuberculosis among
40:585–90.
8 Frieden TR, Sherman LF, Maw KL, et al. A multi-institutional outbreak of
9 Bauer J, Thomsen VO, Poulsen S, et al. False-positive results from cultures
of Mycobacterium tuberculosis due to laboratory cross-contamination
confirmed by restriction fragment length polymorphism. J Clin Microbiol
10 Small PM, McClellan NB, Singh SP, et al. Molecular strain typing ofMyco-
bacterium tuberculosis to confirm cross-contamination in the mycobacteriol-
ology laboratory and modification of procedures to minimise occurrence
11 Daley CD. Tuberculosis recurrence in Africa: true relapse or reinfection.
12 Small PM, Shaper RW, Hopewell PC, et al. Exogenous reinfection with
multidrug resistant Mycobacterium tuberculosis in patients with advanced
13 van Soolingen D, de Haas PEW, Hermans PWM, et al. Comparison of vari-
r eositive DNA elements as genetic markers for strain differentiation
and epidemiology of Mycobacterium tuberculosis. J Clin Microbiol 1993;31:
841–4.
14 Vauterin P. Sharing fingerprint databases among different laboratories: prob-
lems and solutions. Proceedings of Second Meeting of the European
Community Concerted Action on Genetic Markers and Tuberculosis Epidemi-
15 Kamerbeek J, Schouls L, Kolk A, et al. Differentiation of
transmission-linked cases of tuberculosis. Proceedings of theConcerted
Actions on Tuberculosis (Genetic Markers for the Epide-
miology of Tuberculosis/Generic Markers for the Epidemiology of Tuberculosis/Molecular Epidemiology
and Control of Tuberculosis), Cordoba, Spain, June 1997.
16 Small PM, Shaper RW, Hopewell PC, et al. Exogenous reinfection with
multidrug resistant Mycobacterium tuberculosis in patients with advanced
17 Kamerbeek J, Schouls L, Kolk A, et al. Differentiation of
transmission-linked cases of tuberculosis. Proceedings of theConcerted
Actions on Tuberculosis (Genetic Markers for the Epide-
miology of Tuberculosis/Molecular Epidemiology
and Control of Tuberculosis), Cordoba, Spain, June 1997.
18 Goyal M, Saunders NA, van Embden JD, et al. Strain identification of
Mycobacterium tuberculosis isolates by spoligotyping and IS6110 restriction fragment
multidrug resistant Mycobacterium tuberculosis in patients with advanced
20 Glynn JR, Vynnycky E, Fine PEM. The influence of incomplete ascertain-
ment on estimates of recent transmission of Mycobacterium tuberculosis
using DNA fingerprinting techniques. Proceedings of Combined Meeting
of the Concerted Actions on Tuberculosis (Genetic Markers for the Epide-
miology of Tuberculosis/Molecular Epidemiology and Control of Tubercu-
losis), Cordoba, Spain, June 1997.