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Mycobacterial Typing Systems: The Good, the Bad and the Unattainable

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Introduction

What is the perfect typing method? One that provides rapid, reliable, cost effective discrimination between closely related strains to allow for real time epidemiological investigations. Effective and reliable typing of mycobacteria, particularly those that are responsible for infectious disease, is of utmost importance when trying to implement efficient infection control protocols. This is particularly the case for Mycobacterium tuberculosis complex (MTC) isolates in the general population. It is also important in finding the environmental source of nontuberculosis mycobacteria (NTM) infections/outbreaks in immunocompromised patient cohorts, as there are limited data to demonstrate effective transmission between patients [1,2]. Typing platforms can loosely be divided into Phenotypic - those that rely on exploiting differences in physical properties of an organism, and Genotypic - those that rely on differentiating organisms based on genetic differences.

The Good…

There have been numerous "gold-standard" genotypic typing methods of mycobacteria described over the years. The genomes of species of the MTC typically comprise over 4 million base pairs [3-5], however, it is highly conserved; the genome of Mycobacterium *bovis* was shown to be greater than 99.5% identical to that of *M. tuberculosis* [4]. Therefore, genotypic typing methods have traditionally had to take advantages of small differences to allow for reliable differentiation. Van Embden and colleagues proposed a standardised technique of insertion sequence (IS) typing, namely IS6110, which proved to have sufficient discriminatory power to be used as a typing tool for *M. tuberculosis* isolates [6]. However, the technique is technically demanding and aspersions have been cast over the specificity of the method [7]. Where there is a low IS6110 copy number, other typing methods must be employed to facilitate sufficient discrimination. Typing methods that rely on single nucleotide polymorphisms (SNPs) and polymorphic GC-rich repetitive sequences (PGRS) have proven to be effective [8-10].

The most frequently used modern methods of mycobacterial typing are spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing. The term spoligotyping was coined by Kamerbeek and colleagues to describe a PCR based typing tool for spacer oligotyping of the chromosomal direct repeat region (DR) of the genome of *Mycobacterium tuberculosis* (and *M. bovis* also) [11]. Spoligotyping produces a 15 digit code based on the presence/absence of spacer sequences found in the DR region. The method has been shown to be cost-effective, quick and reproducible, which has facilitated the sharing of epidemiological data through databases such as the Institute Pasteur [12]. MIRU-VNTR has been widely adopted and studied as a typing tool for MTB isolates. The method distinguishes between types based on the number of copies of tandem repeats at specific loci [13]. The number of loci used for MIRU typing can be 12, 15 or 24 depending on the level of discrimination required. The presence and number of copies is represented as a code of 12/15/24 digits long depending on the number of loci used. The code returned for an isolate can facilitate the assignment of a lineage. Gagneux and colleagues conducted an in-depth study of MIRU types and their geographical distribution. They described 6 lineages; Indo-Oceanic, East Asian, East African Indian, Euro-American, West African-1 and West African-2 [14]. There are sublineages associated with each lineage; EAI for Indo-Oceanic, Beijing for East Asian, Delhi-CAS for East African Indian, Haarlem, LAM, H37Rv, Cameroon, Ghana, S, TUR, X, Uganda I, Uganda II, New-1, URAL for European American Lineage and West African 1 and 2 for West African-1 and 2, respectively. The method is quite robust, commercial kits are available and the results are very reproducible, facilitating sharing of MIRU types on platforms such as miru-vntrplus.org. The miru-vntrplus.com database also allows for classifying the lineage if a closely related lineage/sublineage has been uploaded previously [15].

Most of the above focusses on the typing of *Mycobacterium tuberculosis* isolates, with little or no applicability to NTM isolates. The rates of NTM isolates are rising [16], particularly among immunocompromised populations, therefore reliable typing methods must be employed in epidemiological studies to find the environmental source of suspected NTM infection outbreaks. VNTR typing methods have been described with some success for Mycobacterium *abscessus* isolates [17] and Mycobacterium *avium* isolates [18]. There were caveats with the *M. abscessus* study; it showed good discriminatory power but the sample size was small. However, the use of VNTR for typing *M. avium* described shows potential as a discriminatory typing tool. The use of IS units for a myriad of NTM isolates has also been described [19-21]; however, it appears that none of the traditional typing systems are robust enough to facilitate typing of both MTC and NTM isolates.

The Bad…

Phenotypic typing methods, such as antimicrobial susceptibility testing (AST), bacteriophage typing, and methods based on surface protein typing and enzymatic tests, have traditionally proven to be less than satisfactory as typing tools for mycobacteria. There are many of reasons for this, including the highly conserved nature of the mycobacterial cell wall, the relatively uniform AST profiles of mycobacteria (with the exception of outbreaks of multidrug resistant isolates, particularly in low prevalence settings) and the fact that the slow rate of growth of mycobacteria doesn't easily facilitate reliable enzymatic typing methods [22,23]. Early bacteriophage typing of

mycobacteria had poor discriminatory power [24]. The exploitation of differences in surface proteins expressed by mycobacteria using mass spectrometry and Raman spectroscopy has showed some promise [25,26]. There needs to be further work to assess the utility of mass spectrometry as a typing tool and whether reported issues with reproducibility and lack of standardization can be overcome [27]. The limitations mentioned have stymied phenotypic typing. It should be noted, however, that mass spectrometry based methods show the most potential and may yet prove to be an effective typing method. They have the potential to satisfy the criteria of rapid and cost effective strain determination and could facilitate a real-time epidemiological study in the case of an outbreak, however, further work in this field is required.

The Unattainable?

Whole genome sequencing (WGS), particularly through next-generation sequencing (NGS) platforms, has, as the name suggests, the ability to provide the highest discrimination of all methods. WGS can detect small changes such as SNPs that occur between generations and can facilitate more powerful epidemiological studies than traditional genotypic typing methods [28]. The major downsides of WGS systems are cost and that they require huge computational power and highly skilled bioinformaticians to interpret the results [29]. So, can the current, good "gold standard" methods of typing truly be replaced with the seemingly perfect typing system of WGS? It all depends on whether the limitations can be overcome at regional levels worldwide. With the recent cost reductions in NGS platforms, increased training in the field of bioinformatics and improved cloud storage facilities, the seemingly unattainable may be attainable sooner rather than later.

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