Molecular epidemiologic tools for diagnosis of tuberculosis: A Review of literature and its’ applicability in Nigeria

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Accepted 10 September, 2012

There is scarcity of data on molecular epidemiology of tuberculosis (TB) in many of the high burden countries of sub-Saharan Africa including Nigeria. This may be due to inadequate diagnostic and research capacities. There is a need to build capacity on molecular epidemiologic techniques in order to enhance TB diagnosis, treatment and prevention strategies. Some of the more popular molecular typing methods being used globally include IS6110-based restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR)-based methods such as spacer oligonucleotide typing or spoligotyping, mycobacterial interspersed repetitive units-multilocus variable number tandem repeats (MIRU-VNTR), single-nucleotide polymorphisms and large-sequence polymorphism analysis. Among other things, molecular technique(s) to be adopted for use in a particular setting depend on the state of the infrastructure including laboratory facilities, presence of skilled personnel and cost. In Nigeria however, availability of PCR-based diagnostic assay –MTBDR plus assay (HAIN- Lifesciences, Germany) in some TB reference laboratories may serve as a springboard for introduction of advanced TB molecular epidemiologic tools in the country.

Key words: Molecular epidemiologic tools, tuberculosis, Nigeria.

INTRODUCTION

Nigeria is one of the most populous countries in sub-Saharan Africa with a population of about 160 million. It is one of the countries with the highest burden of tuberculosis (TB) in the world with an incidence of 460 smear-positive TB cases per 100,000 inhabitants (WHO, 2010). The Directly Observed Treatment Short Course (DOTS) strategy was adopted by the National TB Control Program (NTBCP) in 2003. Nigeria’s treatment success rate of 78% and new smear positive case detection rate of 24% ranks among the lowest of the high-burden countries (WHO, 2010). The resurgence of TB in the world but more importantly in developing countries has renewed interest in understanding the epidemiology and pathogenesis of the disease (Rastogi and Sola, 2007). The advancement of molecular techniques that allow identification and tracking of strains within Mycobacterium Tuberculosis Complex (MTBC) has revolutionalised TB research. This method differentiates between recent active infection from endogenous reactivation (Garcia de Viedma et al., 2011). The contribution of molecular epidemiological methods in the prevention and control of TB is made possible through their ability to differentiate between infecting strains, assessment of the overall diversity of circulating MTBC strains, pinpointing differences by regions and subpopulations, and measurement of prevalence of endemic strains (Van Soolingen et al., 1995; Van Soolingen, 2001). Molecular epidemiologic tool is essential to provide the estimation of TB infection among epidemiologically linked patients, the forecast of next epidemic outbreak, and the analysis of risk factors associated with TB infection.

Molecular typing of M. tuberculosis is a powerful adjunct to TB control. It is useful to monitor the disease transmission, to detect or confirm outbreaks and laboratory error/cross contamination, to distinguish endogenous reactivation from exogenous re-infection, and to identify the clonal spread of successful clones, including multi-drug resistant ones (Mathema et al.,
2006). However, the application of these methods in high TB-burden countries has been hampered by constrained resources, technical limitations of standard IS6110 fingerprinting (Barnes and Cave, 2003), and in general, by the dominance of geographically specific, genetically homogenous strain lineages which renders molecular discrimination of unrelated clones difficult (Cardoso et al., 2011).

Some of the more popular MTBC typing methods being used globally include IS6110-based restriction fragment length polymorphism (RFLP) (Van Embden et al., 1993), PCR based methods such as spoligotyping (Kamerbeek et al., 1997), mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) (Frothingham et al., 1998), single –nucleotide polymorphisms (Gutacker et al., 2006) and large – sequence polymorphism analysis (De Jong et al., 2009; Mulenga et al., 2010).

This review is intended to stimulate TB research in Nigeria by highlighting molecular tools that can be adopted for use in a country with high burden of the disease.

**IS6110-BASED RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)**

Insertion element (IS)6110 RFLP typing has been considered as the gold standard for molecular epidemiology of TB (Affolabi et al., 2009), but this approach is technically demanding and requires weeks to culture a certain amount of isolates in order to obtain the necessary quantities of DNA (Bolotin et al., 2009). In addition, it provides insufficient discrimination power especially for isolates with less than 6 copy numbers of IS6110 (Cowan et al., 2002). This method provides excellent differentiation but requires specialized software for analysis of the data as well as relatively long turnaround times for reporting of the results. Genotyping methods that could employ amplification of nucleic acids have been assessed in efforts to address shortcomings of RFLP analysis.

**SPACER OLIGONUCLEOTIDE TYPING OR SPOLOGOTYPPING**

Spacer oligonucleotide typing or spoligotyping was the first widely adopted PCR-based method for genotyping spoligotyping based on the direct repeat (DR) region of *M. tuberculosis*, has advantages over IS6110: (i) Small amount of DNA sample is needed for clinical examination and strain testing from liquid culture (ii) Only digital number is used to express the results (iii) It can be used for genotyping of isolates with less than 6 copies of IS6110 (Guo et al., 2011).

Spoligotyping data which is represented in absolute terms (digitally) can be readily shared among laboratories thereby enabling the creation of large international database (SpolDB). This development allows investigators to survey strain diversity and uncover global strain families such as the Beijing and the Latin American Mediterranean (LAM) families. The method has been very successful in providing a tool for the rapid acquisition of MTB genotyping information and for the establishment of a global picture of MTB diversity (Brudey et al., 2006). It is highly reproducible and has been developed into a high –throughput assay for large molecular epidemiology surveys (Driscoll, 2009). The strengths of this method include its low cost, its digital data results, and good correlation of its results with other genetic markers, its fair level of overall differentiation of strains, its high-throughput capacity, and its ability to provide species information. Its weaknesses however, include its inability to differentiate well within large strain families such as Beijing family, the potential for convergent evolution of patterns, the limited success in improving the assay through expansion, and the difficulty in obtaining the specialized membranes and instrumentation. Important usefulness of spoligotyping method includes the following:

**For species identification within the MTBC group**

MTBC is made of closely related species – *M. tuberculosis*, *M. africanum*, *M. bovis* and less common ones such as *M. canetti* and *M. microti*. The presence or absence of certain spacer sequences acts as a signature for presumptive species identification (Streicher et al., 2007). For example, *M. bovis* do not hybridize to spacers 39 to 43 but do generally hybridize to spacers 33 to 36 (Kamerkeek et al., 1997) while *M. africanum* isolates do not hybridize to spacers 8, 9 and 39 but do generally hybridize to 33 to 36 (Driscoll, 2009).

**For integration into public health program**

Spoligotyping is a very useful tool for public health program. Universal genotyping that is genotyping of every isolate has many beneficial effects such as: earlier identification of false-positive MTB cultures (due to laboratory cross contamination), discovery of unsuspected cases of MTB transmission (linking patients who had not previously been identified as contacts through conventional methods), confirmation of species identification within MTBC group, and capability to generate a database to examine strain diversity in a particular region for monitoring program success in TB control (Clark et al., 2006). Moreover, universal genotyping enables shorter turnaround times in as much as a method like spoligotyping can be performed as a routine activity in the laboratory workup of a patients’
MTB strain (Gori et al., 2005). Selective genotyping (genotyping only certain MTB strains) on the other hand, entails requests for analysis of isolates weeks or months after the laboratory has received the specimen and also in situation where retrieval from archival storage may be difficulty.

For establishing commonality of spoligotypes

Knowledge of the MTB strain diversity in an area is important in establishing the significance of genotyping matches for all fingerprinting methods especially for spoligotyping. For example, a finding that two patients match by one of spoligotype does not in itself prove that the two strains are identical. Additional genotyping data is required through mycobacterial interspersed repetitive unit (MIRU) or RFLP analysis to establish the significance of the typing match. However, in a well–characterized population, the appearance of two strains with a matching unique spoligotype pattern is likely to be significant, especially if other certain factors are present. These factors include:

a) Strains showing a “common” spoligotype for example, Beijing pattern 
b) Patients share one or more epidemiologic links 
c) Strains match using MIRU and or RFLP 
d) Strains share a “rare” spoligotype 
e) Patients are contacts of one another

For epidemiological uses

Incorporating spoligotyping data into the TB control programs is useful to detect direct contact investigations and to identify cases of false –positive cultures (for example, laboratory cross-contamination) (Ellis et al., 2002). Genotyping program in an area with low TB incidence may reveal few matches among patient isolates, suggesting a low occurrence of recent transmission whereas, in high incident area, a greater number of MTB strains with the more frequently observed spoligotypes may be encountered. This may obscure the picture of recent transmission versus distant transmission, thereby necessitating the use of additional genotyping assays such as RFLP and MIRU (Sintchencho and Gilbert, 2007).

For identification of false-positive TB cultures

Laboratory cross-contamination of patient samples continues to be a problem that results in false TB diagnosis. The commonest example has been cross-contamination with common laboratory strains H37Ra and H37Rv. These two isolates share the same spoligotype and have similar but un-identical RFLP patterns (Bifani et al., 2000). To date, there have been no reports of a clinical isolate sharing the H37 spoligotype (Driscoll, 2009). Therefore when a clinical isolate is found to have a spoligotype matching H37, a laboratory cross-contamination event is likely to have occurred (National TB controllers association/CDC, 2004). Cross-contamination of a patient sample with another patient specimen or mislabeling either at point of collection or in the laboratory usually requires further investigation such as performing additional genotyping assays and review of the patient clinical data.
Applicability in Nigeria

There is scarcity of indigenous data on molecular epidemiology of TB in many high burden countries of sub-Saharan Africa. In Nigeria however, the few available data were generated Oversea in collaboration with local researchers (Cadmus et al., 2006; Ani et al., 2010; Thumano et al., 2011). The paucity of information on molecular epidemiology of TB in Nigeria may be ascribed to inadequate infrastructure including laboratory facilities, low level of TB research and inadequate funding. The need to strengthen laboratory facilities for TB diagnosis in Nigeria has been previously highlighted (Kehinde et al., 2005).

Diagnosis of TB in Nigeria relies majorly on sputum microscopy while isolation of the causative organisms in pure culture is only available in reference laboratories. Of recent however, some research centers in the country including the University College Hospital, Ibadan and the Nigerian Institute for Medical Research, Lagos have acquired PCR –based Geno Type MTBDR plus 96 assay (Hains Lifesciences, 2010) for molecular diagnosis of TB including MDR-TB.

In conclusion, molecular epidemiologit tools such as spoligotyping and MIRU-VNTR can be introduced in some centers using existing PCR-based facilities in order to fast-track TB molecular diagnostic research in Nigeria.

REFERENCES


