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GenoType MTBDR*plus* as a Complementary Tool for the Typing of *Mycobacterium tuberculosis*

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Abstract

The aim of this study was to investigate the usefulness of combining profiles obtained by using a line probe assay (LPA) originally intended to characterize the resistance of two major anti-tuberculosis drugs to the association of spoligotyping and MIRU-VNTR, in order to improve its discriminatory power. For this purpose, 74 strains of *Mycobacterium tuberculosis* belonging to the same cluster after spoligotyping were further typed by using the 24 loci MIRU/VNTR. These strains were then tested by the GenoType MTBDR*plus*, and profiles obtained were analyzed within previously obtained clusters. The combination of spoligotying and MIRU-VNTR led to the consolidation of 56 of them (75.7%) in 9 clusters. Most of the strains (54, 96.4%) were multidrug resistant (MDR). From the 9 initial clusters, the addition of GenoType MTBDR*plus* helped to define 26 profiles including 11 unique profiles, and 3 original clusters remained undifferentiated. Results obtained express the relevance of combining this method which improved quite significantly the discriminatory power in typing *Mycobacterium tuberculosis*.

Keywords

***Mycobacterium tuberculosis*; MIRU-VNTR; Spoligotyping; GenoType MTBDR*plus*; Côte d'Ivoire**

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1. Introduction

Molecular methods used to type isolates belonging to *Mycobacterium tuberculosis* complex (MTBc) are relevant in both phylogenetic and epidemiological studies [1]. Because of the limited discriminatory power of classical genotyping methods such as Spoligotyping, MIRU-VNTR and IS6110-RFLP, analyses of SNPs and whole genome sequencing (WGS) have recently begun to be used in epidemiological investigations for instance to identify outbreaks [2] [3]. WGS has the best discriminatory power at DNA level but its implementation is still costly and not possible in all settings, especially in developing countries. In addition to conventional typing methods, there are drug resistance genotyping tests based on line probe assays (LPAs) which were endorsed by the World Health Organization [4] and can be widely used. By detecting a large number of mutations involved in resistance to anti-tuberculosis drugs, they can generate a broad range of patterns. Indeed, even if all pansusceptible strains by these methods display the same pattern, the putative number of possible combinations for resistant and in particular multidrug resistant (MDR) strains can be relatively substantial.

The aim of this study was to evaluate the relevance of combining a LPA to spoligotyping and MIRU-VNTR in order to type MTBc strains.

2. Material and Methods

During a previous study, 74 MTBc strains belonging to the same cluster by spoligotyping (SIT53) were typed by 24 loci MIRU-VNTR and led to the consolidation of 56 of them (75.7%) into nine clusters [5]. GenoType MTBDR*plus* (Hain Lifescience, Nehren, Germany) was implemented for drug resistance detection and profiles which were obtained were analyzed. Mutations detected and LPA patterns were described and discriminatory power was assessed by calculating the Hunter-Gaston Discriminatory (HGDI) rate [6].

3. Results

Fifty-four strains (96.4%) were MDR and 2 (3.6%) resistant to isoniazid. No susceptible strains were observed. While 9 clusters were previously defined after the combination of spoligotyping and MIRU-VNTR, 27 profiles were described after adding GenoType MTBDR*plus*. Those included 13 unique patterns and 14 clusters containing 43 strains, resulting in the increase of HGDI rate from 74.2% to 96.6% (**Table 1**). Only 3 clusters remained undifferentiated while the 6 others defined 11 new clusters including 5 in the largest one. Mutations detected in *katG* gene and *mabA-inhA* promoter were all already known. Regarding *rpoB* however, resistance patterns were not related to known mutations for 12 strains. All of the strains harbored S315T mutation in *katG* but some clusters displayed specificities. Thus, only 2 clusters included mutations in *mabA-inhA* and for 2 other, wild type 7 was always absent in *rpoB*.

4. Discussion

In this study, patterns obtained with GenoType MTBDR*plus* are used for the first time to help in differentiate mycobacterial strains. If drug resistance profiles established by phenotypic methods seem not reliable enough [7], identification of the genetic support of resistance is assumed to be more consistent. Indeed, LPAs are, intrinsically and above all, genotyping methods as they also characterize the genome. Mycobacterial strains studied here were clustered after performing spoligotyping and 24 loci MIRU-VNTR which discriminatory power is at least equal to that of RFLP IS6110 [8]. Both methods were associated in many studies and achieved a high level of discrimination, minimizing the rate of clustering [9] and then allowing expecting the same patterns with GenoType MTBDR*plus*. Anticipated results have therefore been mainly observed as interestingly, profiles previously defined showed similar characteristics with the LPA. Thus, most of the strains were still clustered, and the patterns observed were fully similar in some clusters and nearly identical in others. However, two significant facts emerge from the use of GenoType MTBDR*plus* and indicate a marked improvement in the discrimination power. On one hand, there is a subdivision of the previously obtained clusters which result in the increase of HGDI index and, on the other hand, 13 unique profiles are obtained from the 9 previous clusters. Eventually, results that were obtained express the relevance of this method which is relatively easy to perform within a single working day and perfectly reproducible. Thus, it could be associated to classical genotyping methods in order, at least, to support arguments in favor of an outbreak or not. That would be done before resorting to techniques

Table 1. Characteristics of *Mycobacterium tuberculosis* complex isolates tested.

Cluster (**n**)	MIRU-VNTR profile[1]	Gene						Result of LPA	Number of strains
		rpoB		*katG*		*mabA inhA*			
		wt[2]	mutation	wt	mutation	wt	mutation		
Cluster 1 (**6**)	264333233531436253213422	7	H526D	-[3]	S315T	+[4]	-	MDR	4
		7	Unknown	-	S315T	+	-	MDR	2
		8	S531L	-	S315T	+	-	MDR	5
		7	H526Y	-	S315T	+	-	MDR	5
		7	H526Y	-	S315T	2	T8C	MDR	4
		7	H526D	-	S315T	2	T8C	MDR	3
		3/4	D516V	-	S315T	2	T8C	MDR	3
Cluster 2 (**27**)	264333233431436253213422	3/4/7	Unknown	-	S315T	2	T8C	MDR	1
		7	H526D	-	S315T	+	-	MDR	1
		7	Unknown	-	S315T	2	T8C	MDR	1
		7	Unknown	-	S315T	2	T8A	MDR	1
		8	S531L	-	S315T	2	T8A	MDR	1
		+	-	-	S315T	+	-	INHR	1
		+	-	-	S315T	2	T8C	INHR	1
Cluster 3 (**3**)	254333233431436253213422	7	Unknown	-	S315T	+	-	MDR	2
		3/4	D516V	-	S315T	+	-	MDR	1
Cluster 4 (**3**)	244333233431436253213422	7	Unknown	-	S315T	+	-	MDR	2
		1/7	Unknown	-	S315T	+	-	MDR	1
		8	S531L	-	S315T	+	-	MDR	3
Cluster 5 (**6**)	274333233431436253213422	7	Unknown	-	S315T	+	-	MDR	1
		3/4	Unknown	-	S315T	+	-	MDR	1
		7	H526Y	-	S315T	+	-	MDR	1
Cluster 6 (**2**)	264333233431426253213422	7	H526Y	-	S315T	2	T8C	MDR	2
Cluster 7 (**3**)	263333233431436253213422	3/4	D516V	-	S315T	+	-	MDR	2
		8	S531L	-	S315T	+	-	MDR	1
Cluster 8 (**2**)	274333133431436253213422	3/4	D516V	-	S315T	+	-	MDR	2
Cluster 9 (**4**)	264333133431436253213422	3/4	D516V	-	S315T	+	-	MDR	4

1) MIRU-VNTR loci are displayed in the following order: 580, 802, 2996, 960, 1644, 3192, 424, 577, 2165, 2401, 3690, 4156, 1955, 2163b, 4052, 154, 2531, 4348, 2059, 2687, 3007, 2347, 2461, and 3171; 2) Missing wildtype(s); 3) No wildtype present; 4) All wildtypes are present.

known to provide better discrimination such as WGS, potentially the ultimate tool for diagnosis and typing but which is not routinely used so far.

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