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Original Research Article

Molecular Characterization of the Resistance of *Mycobacterium tuberculosis* to Second Line Drugs in Côte d'Ivoire

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Abstract

Purpose: *To characterize the resistance of Mycobacterium tuberculosis to second line drugs using a line probe assay.*
Methods: *Multi-drug resistant strains of Mycobacterium tuberculosis isolated between December 2008 and December 2009 were tested for resistance to fluoroquinolones and second-line injectable drugs using GenoType® MTBDRsl.*
Results: *Thirty eight strains gave interpretable results. None of them had a mutation in the gyrA gene. Regarding second-line injectable drugs, 4 strains (11 %) were resistant to aminoglycosides/ capreomycin and all of them harbored A1401G mutation.*
Conclusion: *No extensive drug resistant strains were observed. A relatively high proportion of strains were resistant to at least one second-line injectable drug. Resistance mechanism seemed similar for all of them.*

Keywords: *Mycobacterium tuberculosis, Line probe assay, GenoType® MTBDRsl, Aminoglycosides Capreomycin, Mutation*

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INTRODUCTION

One of the priorities of the World Health Organization (WHO) for the control of tuberculosis infection was to intensify the detection and the treatment of cases [1]. This strategy, however, could be thwarted by the emergence of cases of multidrug-resistant (MDR) tuberculosis (TB) or even extremely-resistant (XDR) bacilli. The last survey conducted in Côte d'Ivoire on MDR-TB, that is, caused by TB bacilli resistant to at least the two major drugs simultaneously namely, isoniazid and rifampicin, was evaluated and a prevalence of 2.5 % was obtained in 2006 [2].

Among the MDR strains, it is possible to find XDR bacilli, in other words, resistant in addition to fluoroquinolones and to at least one of the second line injectable drugs (amikacin, kanamycin or capreomycin). The WHO has reported such cases in almost all Countries of each Continent [3], except for Africa, where a lower proportion of cases have been reported, possibly due to the limitation or lack of detection tools. Given the challenges presented by the implementation of conventional drug susceptibility testing (DST) for second-line drugs, genotypic methods appear to be, apart from their cost which is often high, an attractive alternative.

This study was undertaken to characterize the resistance of *Mycobacterium tuberculosis* strains isolated in Côte d'Ivoire to the second line drugs by using a Line Probe Assay (LPA).

EXPERIMENTAL

Bacterial strains

M. tuberculosis strains were isolated in TB patients undergoing retreatment regimen and whose sputum samples were received at the CeDReS, a Department of the Teaching Hospital of Treichville in Abidjan, Côte d'Ivoire. Samples of these patients were referred for conventional culture and DST which are part of routine activities of the CeDReS from December 2008 to December 2009. Among the isolates, those identified as MDR-TB were then tested for susceptibility to second-line drugs.

DNA extraction

DNA extraction was performed from liquid culture on the MP® which is a modified 7H9 medium (bioMérieux, Marne-la-Coquette, France). Bacterial isolates contained in 500 µl of liquid culture were heat- inactivated at 95 °C for 30 min and then sonicated for 12 min. Finally, the suspension was centrifugated at 13000 rpm for 5 min and the supernatant containing the DNA was stored at – 20 °C.

Line probe assay

Molecular characterization of resistance to second-line drugs was performed using the GenoType® MTBDR*sl* kit (Hain Lifescience, Nehren, Germany). Mutations targeted by the method included *rrs* gene (16S rRNA) leading to a resistance to amikacin, kanamycin and/or capreomycin and *gyrA* gene whose mutations cause resistance to fluoroquinolones. Mutations in *embB* gene resulting in resistance to ethambutol was also evaluated.

The technique was performed as described by the manufacturer. Briefly, the reaction was carried out in two stages. For amplification, 5 µl of DNA extract were added to 35µl of primers and nucleotides (supplied by the manufacturer), 5 µl of 10x amplification buffer (Qiagen), 1.2 µl of 25 mM MgCl, 1.25 U Hot Start Taq DNA polymerase (Qiagen, Hilden, Germany) and water for a final volume of 50 µl. The hybridization step took place in a hybridization buffer at 45 °C. Revelation of the hybridization was performed, after stringent washing, by colorimetric reaction.

After the amplification, the hybridization and the detection were performed in the GT-Blot 20® (Hain Lifescience GmbH, Nehren, Germany) which is an automated washing, stirring and heating system.

RESULTS

Five out of 43 DNA samples analyzed by the method did not give interpretable results. Figure 1 shows some representative DNA pattern obtained during the study.

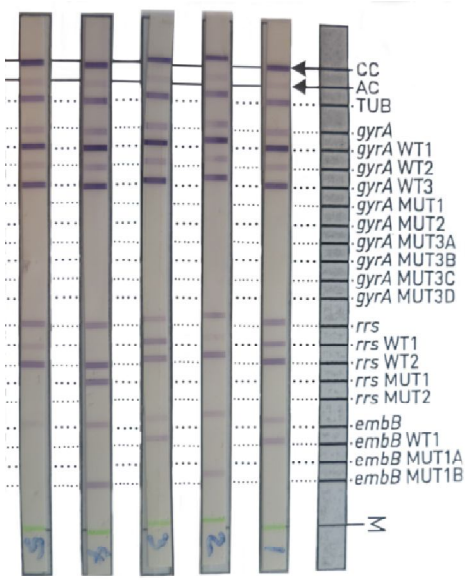

Figure 1: Representative DNA patterns obtained with Genotype® MTBDR*sl*
The positions of the oligonucleotides and control probes are given on the right. Targeted genes and specific probes lines are shown from top to bottom as follows: conjugate control (CC); amplification control (AC); M. tuberculosis complex-specific control (TUB); gyrA amplification control; gyrA wild-type probes WT1 to WT3 (85–90, 89–93 and 92–97); gyrA mutant probes MUT1, MUT2, MUT3A, MUT3B, MUT3C, and MUT3D for codons A90V, S91P, D94A, D94N, D94Y, D94G, and D94H, respectively; rrs amplification control; rrs wild-type probes WT1 (codons 1401 and 1402) and WT2 (codon 1484); rrs mutant probes MUT1 and MUT2, with A1401G and G1484T changes, respectively; embB amplification control; embB wild-type probe WT1, covering codon 306; and embB probes MUT1A and MUT1B for the mutations M306I and M306V, respectively. From the right to the left: Lane 1 : FLQs MISLs EMBs ; Lane 2 : FLQs MISLs EMBr ; Lane 3 : FLQs MISLs EMBs ; Lane 4 : FLQs MISLr EMBr ; Lane 5 : FLQs MISLs EMB invalid.

Analysis of the results for 38 strains with an interpretable profile (Table I) revealed for 19 strains the absence of any mutation for fluoroquinolones, aminoglycosides (kanamycin and amikacin), capreomycin and ethambutol.

Table I: Genotyping results obtained with the GenoType® MTBDR*sl*

Mutation observed (by gene)			
Number of strains	***gyrA***	***Rrs***	***embB***
19	wt	Wt	Wt
2	wt	A1401G	Wt
9	wt	Wt	M306I
2	wt	A1401G	M306V
6	wt	Wt	M306V

wt = wild type

Among the 19 other strains carrying a mutation in at least one of the three genes studied, 17 (45%) harbored a mutation in *embB*. Of these, 9 (53 %) had the M306I mutation and 8 (47 %), the M306V mutation. Concerning *rrs*, a mutation was observed in four strains (11 %), all carrying the A1401G mutation (100 %). No mutation was observed in *gyrA*. In 2 cases, the simultaneous presence of mutations in *rrs* and *embB* was observed.

DISCUSSION

While some molecular tests for the detection of resistance to first line drugs were endorsed by the WHO, this is still not the case for the Genotype® MTBDR*sl*. Initial assessments of the kit had however allowed to hypothesize a detection XDR strains within a period of 1 or 2 days, by combining it with another molecular method for the detection of resistance to rifampicin and isoniazid [4]. Eventually, several assessments have resulted in very different results. Thus, sensitivity was 57.1 to 100% for the detection resistance in fluoroquinolone and 42 to 100% for second-line injectable drugs (SILDs) [5]. The specificity varied meanwhile from 77.3 to 100 % for fluoroquinolones and from 47 to 100 % for SLIDs [5].

It should be noted that mutations observed for ethambutol were not analyzed because, in one hand, that it is not a second-line drug. In the other hand, the test was characterized, in addition to a very average specificity, by a low sensitivity for the detection of resistance to this drug [5].

No XDR strain was detected among the series studied. This finding can be explained by a fairly low negative predictive value of Genotype® MTBDR*sl* for the detection of resistance in fluoroquinolones [6]. To this must be added the fact that only *gyrA* is targeted by the test, although it is known that mutations in other genes such as *gyrB* are rarely observed [7].

In the absence of mutations in the fluoroquinolones, the analysis of mutations detected was confined to aminoglycosides and capreomycin. In the four strains exhibiting a mutation, the one that has been observed was only A1401G (100 %). This mutation is known to be the most frequently found with a prevalence of 62 to 98 % [5]. In addition, the A1401G mutation has significant consequences since it leads to a simultaneous resistance to amikacin, kanamycin and capreomycin [8].

Despite the absence of UR strains in the series, notably because of the absence of resistance to fluoroquinolones, the risk of selection of this kind of strain remains because of the relatively high consumption of these drugs in Côte d'Ivoire [9]. Moreover, the limited size of the sample should not foresee results that may be obtained within a larger sample. Indeed, several African countries have reported XDR-TB cases by end of 2011 and some of them are close to Cote d'Ivoire, namely Burkina Faso, Benin and Togo [10]. The importance of population movements between these countries belonging to the same sub-region could promote the exchange of strains, particularly since one of the cases detected in Burkina Faso was traced to a patient originally from Côte d'Ivoire [11].

CONCLUSION

No XDR strains were observed in the series. However, a relatively high proportion of strains harbored mutations for some second line drugs and should draw the attention of health authorities on the presence of strains which represent the stage before the appearance of XDR strains.

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