Original article

European Journal of Microbiology and Immunology 4 (2014) 3, pp. 166–173
DOI: 10.1556/EUJMI-D-14-00014

ASSESSMENT OF THE GENOTYPE MTBDRPLUS ASSAY FOR RIFAMPIN AND ISONIAZID RESISTANCE DETECTION ON SPUTUM SAMPLES IN COTE D'IVOIRE

K. N'guessan[1,*], J. S. Assi[1], T. Ouassa[2], J. M. Ahui-Brou[3], A. Tehe[4], M. Keita Sow[5], A. Guei[1], J. Kouakou[6] and M. Dosso[1]

[1] Institut Pasteur de Côte d'Ivoire, Laboratoire National de Référence de la Tuberculose, Cote d'Ivoire
[2] Centre de Diagnostic et de Recherche sur le SIDA, Cote d'Ivoire
[3] Service de Pneumologie CHU de Cocody, Cote d'Ivoire
[4] Projet Retro-CI/CDC, Cote d'Ivoire
[5] American Society for Microbiology, USA
[6] Programme National de Lutte contre la Tuberculose, France

Received: April 6, 2014; Accepted: May 10, 2014

We conducted an evaluation study on the GenoType MTBDRplus assay's ability to detect mutations conferring resistance to rifampin and isoniazid directly from sputum taken from 120 smear positive pulmonary patients from tuberculosis (TB) centers in Cote d'Ivoire.

The sputum was decontaminated by N-acetyl-l-cysteine (NALC) and comparatively analyzed with the MTBDRplus assay version 2.0 and the mycobacterial growth indicator tube (MGIT) 960 automated drug susceptibility testing (MGIT-DST). The GeneXpert *Mycobacterium tuberculosis* (MTB)/rifampicin (RIF) assay was performed for 21 sputa with absence of hybridization for at least one *rpoB* wild-type probes. Four and seven, respectively, discordant and concordant results were also analyzed.

The mutations in the *rpoB* gene were 21 (17.5%), 20 (16.7%), 7 (5.8%), and 10 (8.3%), respectively, for D516V, H526Y, H526D, and S531L. S315T mutation in *katG* gene associated or not with mutation in promoter of *inhA* was detected in 76 (63.3%) of the sputum. Compared to MGIT-DST, the sensitivity and specificity of the MTBDRplus for rifampin resistance detection were 100% (75–100%) and 73.2% (61.3–84%), respectively. For isoniazid resistance detection, the sensitivity and specificity were, respectively, 95% (90–99) and 95.1% (88.5–100%).

Interpretation of 16 sputa without hybridization of *rpoB* wild-type probe 8 compared to those obtained with MGIT-DST and GeneXpert MTB/RIF was discordant and concordant, respectively, for 11 and 5.

Keywords: molecular assay, rifampin, isoniazid, drug-resistance, assessment

Introduction

The emergence and spread of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) pose a major public health problem threat worldwide. Rapid identification of drug resistance, particularly for MDR-TB is of utmost importance to help mitigate disease spread [1]. New diagnostic tools for TB were developed and validated, particularly molecular methods [2]. Some of these methods are based on the knowledge that resistance to rifampin (RMP) and isoniazid (INH) in *Mycobacterium tuberculosis* is most often attributed to mutations in the *rpoB*, *katG*, and *inhA* genes.

Côte d'Ivoire has a population of about 20 million people and ranks among the world's high burden countries for human immunodeficiency virus (HIV) infection [3]. In Côte d'Ivoire, prevalence of MDR-TB cases among new cases with a positive smear decreased from 5.3% in 1996 to 2.5% in 2006 [4]. Among previously treated patients, prevalence of MDR-TB is estimated at 53.2% [5].

The U.S. President's Emergency Plan for AIDS Relief (PEPFAR) is one of the main partners of Côte d'Ivoire's National TB Control Program. The National TB Reference Laboratory (NTRL) of Cote d'Ivoire was renovated and equipped by PEPFAR. The NTRL developed MDR-TB diagnostic algorithms which include different assays such as the GenoType MTBDRplus.

The GenoType MTBDRplus assay has been validated in several countries [6–8] but not yet in Cote d'Ivoire. The study objective was to assess the assay's capacity to detect

* Corresponding author: K. N'guessan; 01 BP 490 Abidjan 01, Cote d'Ivoire; E-mail: kouassinguessan@pasteur.ci

ISSN 2062-509X / $ 20.00

resistance to RMP and INH among pulmonary TB cases diagnosed in Ivorian TB centers.

Patients and methods

Study site

Patients were recruited in the Pneumology service of Centre Hospitalier Universitaire (CHU) de Cocody, one of the three reference centers for TB in Côte d'Ivoire.

Samples collection and transport

For each patient enrolled, two sputum samples were collected and put in individual bags. Samples collected were transported at 4 °C in an icebox to the NTRL which is a component of the Mycobacteria Unit of Institut Pasteur de Côte d'Ivoire.

Pretreatment of sputum

Manipulations of infectious clinical specimens were performed in a Class II safety cabinet (The Baker Company, USA) in a BLS3 laboratory. Sputum samples were decontaminated with N-acetyl-$_L$-cystein (Alfa Aesar GmbH, Germany), 4% NaoH (Carlo Erba, France), 2.9% sodium citrate (Park Scientific Limited, Northampton, United Kingdom) (final concentration of NAOH 1%), followed by an incubation period at room temperature of 15 min. Sputum samples were centrifuged at 3000*g* for 20 min. Supernatant of concentrated sputum was carefully eliminated. Pellet was resuspended with 2 ml of sterile phosphate buffer.

Culture and drug susceptibility testing

One hundred microliters of resuspended pellet were used to perform a smear which was stained by the Ziehl–Neelsen method. Two hundred and five hundred microliters of suspension were used, respectively, to inoculate two Lowenstein–Jensen (Merck, Germany) tubes and one mycobacterial growth indicator tube (BD BACTEC™ MGIT™, USA) containing polymixin, amphotericin B, and naladixic acid (PANTA) (BD BACTEC™ MGIT™, USA) and oleic acid albumin, dextrose, and catalase (OADC) (BD BACTEC™ MGIT™, USA). Cultures in liquid medium were incubated in the automated MGIT 960 (BD, Biosciences, Sparks, Maryland, USA) for 6 weeks and Lowenstein–Jensen in an incubator at 37 °C for the same amount of time. All cultures flagged as positive were removed from the MGIT 960. MGIT positive tubes were examined for autofluorescence bronchoscopy (AFB) detection using Ziehl–Neelsen staining and put in an incubator at 37 °C. After 24 h, a protein excreted by the *M. tuberculosis* complex, the MPT64, was detected with 500 µl of broth in accordance with the manufacturer procedure (Standard Diagnostics, Seoul, South Korea). In parallel, the purity of the broth was evaluated by inoculating 1 ml on blood agar at 37 °C for 24 h. BACTEC™ MGIT™ 960 automated drug susceptibility testing (MGIT-DST) was performed on all the cultures of the clinical samples confirmed for containing a species of *M. tuberculosis* complex with negative test for purity. All strains were tested at 1.0, 0.1, 1.0, and 5 µg/ml, respectively, for streptomycin, isoniazid, rifampin, and ethambutol according to BACTEC™ MGIT™ 960 SIRE kit's procedure (BACTEC S.I.R.E., drug kit; BD Biosciences, USA). An internal quality control with H37Rv strain was performed for each batch of drug prepared.

Genotype resistance detection

DNA extraction

GenoLyse® kit (Hain Lifescience, Germany) for bacterial DNA extraction was used. Five hundred microliters of sediment were transferred in Eppendorf tube of 1500 µl. Suspension was centrifuged at 10,000*g* in aerosol-tight rotor during 15 min. Supernatant was discarded. One hundred microliters of lysis buffer was added to the sediments. The bacterial preparation was homogenized by vortexing. Bacterial suspension was inactivated at 95 °C for 5 min. One hundred microliters of neutralization buffer were added to the preparation. The inactivated suspension was centrifuged at 13,000*g* for 5 min. The DNA contained in the supernatant was transferred into a fresh tube. A negative control was included in each run of sputum sample decontaminated for DNA extraction.

Amplification of DNA extracted from sputum samples

With sputum containing AFB, Genotype MTBDRplus assay version 2.0 (Hain Lifescience, Nehren, Germany) was performed as recommended by the manufacturer. The amplification mixture contained 35 µl of primer-nucleotide Mix B, 10 µl of Mix A (5 µl 10´ polymerase chain reaction [PCR] buffer, 2 µl of MgCl2, 3 µl of molecular water, 1 unit of thermostable *Taq* DNA polymerase), and 5 µl of extracted chromosomal DNA solution.

Amplification parameters used were as follows: 15 min of denaturation at 95 °C, followed by 20 cycles of 30 s at 95 °C and 2 min at 65 °C, followed by 30 additional cycles of 25 s at 95 °C, 40 s at 53 °C, and 40 s at 70 °C, ending with a final extension step of 8 min at 70 °C (1 cycle).

Hybridization

Hybridization and detection were performed with a TwinCubator (Hain Lifesciences GmbH, Germany) semi-automated washing and shaking device according to the manufacturer's instructions and using the reagents provided with the kit. Twenty microliters of denaturation solution

was mixed to 20 µl of amplified sample. Mixed solution was incubated at room temperature for 5 min. One milliliter of pre-warmed hybridization buffer was added before the membrane strips were placed and shaken in the hybridization solution for 30 min at 45 °C. After two washing steps, a colorimetric detection of the hybridized amplicons was obtained by the addition of the streptavidin alkaline phosphatase conjugate.

An internal quality control process with positive and negative controls was implemented during the study. An interpretable MTBDRplus assay was defined as a test strip with all control markers positive, including results of the markers for positive control (H37Rv strain) and negative control for DNA extraction and for mix preparation.

Using GeneXpert MTB/RIF

For 21 sputum samples with absence of hybridization signal, with at least one of *rpoB* wild-type probes and 4 discordant results between drug susceptibility testing and MTBDRplus for RMP resistance detection, the GeneXpert *M. tuberculosis* (MTB)/rifampicin (RIF) assay (Cepheid, Inc., Sunnyvale, CA, USA) was performed with the sputum sample (pellet conserved). Drawing of lots of seven concordant results for RMP resistance was also analyzed. The GeneXpert MTB/RIF assay is a hemi-nested real-time PCR method that amplifies the 81-bp region of the rifampin resistance-determining region (RRDR) of the *rpoB* gene, positions 507–533. Five hundred microliters of supernatant were added to 1500 µl reagent buffer containing NaOH and isopropanol (3:1 ratio) ensuring a final volume of at least 2 ml. After 15 min of incubation with intermittent hand mixing, 2 ml of the liquefied inactivated sample was added to the cartridge that contained the wash buffer, reagents for lyophilized DNA extraction and PCR amplification, and fluorescent detection probes (five for the *rpoB* gene and one for an internal control, *Bacillus globigii* spores). After the cartridge was placed in the instrument module, the automated processes included the following: specimen filtering, sonication to lyse the bacilli and internal control spores, released DNA collection and combination with the PCR reagents, amplification, target detection by five-color fluorescence of overlapping molecular beacon probes, and one color fluorescence for the internal control. Results were automatically generated within 2 h and reported as *M. tuberculosis* complex negative or positive (with semi-quantification) and RMP sensitive or resistant. The GeneXpert MTB/RIF assay definition files version 4.4a was used in this study. Data analysis for RMP resistance detection was reported with cycle threshold differences superior to 4.5 as suggested by the manufacturer.

Data analysis

Data were entered in MS Excel 8.0 and analyzed using Epi-info 6.04 (CDC, Atlanta, USA). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the MTBDRplus assay were estimated using drug susceptibility testing results in liquid medium as gold standard.

Results

Sputum samples of 120 pulmonary TB cases with positive smears suspected of MDR-TB were collected, of whom 29 were female. The mean age of females and males was, respectively, 33.2 years and 34.5 years. Among recruited patients, 8, 30, and 57 were, respectively, new TB cases, failure after 2RHZE/4RH regimen, and relapse after 2RHZE/4RH regimen *(Table 1)*.

In the *rpoB* gene, with MTBDRplus assay, a specific mutation conferring RMP resistance was detected in 58 (73.4%) sputa for a total of 79 lacking the hybridization signal with the wild-type interpreted as phenotypical resistance. For 21 sputum samples, 16 lacked the hybridization signal with the *rpoB* wild-type probe 8 and 11 were interpreted susceptible after drug susceptibility testing. A D516V mutation in *rpoB* gene was detected in 21 (17.5%) sputum samples. At codon 526, the H526Y mutation and H526D were observed, respectively, for 20 and seven sputum samples. For ten sputa, S531L mutation was found at codon 531 *(Table 2)*.

Table 1. Patients recruited characteristics

Items	Female (n = 29)	Male (n = 91)	Statistical test/proportions
Mean ages ± SD	33.2 ± 12.3 Min: 15, max: 65	34.5 ± 9.4 Min: 15, max: 75	Student test: 0.61 SND
Patients' category			
New TB cases	1	7	8 (6.7%)
Failure after 2RHZE/4RH regimen	8	22	30 (25%)
Failure after retreatment	5	11	16 (13.3%)
Relapse after 2RHZE/4RH regimen	13	44	57 (45.5%)
Relapse after retreatment	2	7	9 (7.5%)

Min = minimum; max = maximum; SD: standard deviation; SND: statistically nonsignificant difference

Table 2. Patterns of GenoType MTBDRplus in comparison to drug susceptibility testing

Number of sputum samples (%)	DST		MTBDRplus assay			Results
	RMP	INH	RMP pattern *(rpoB)*	INH pattern		
				katG	*inhA*	
8 (6.7%)	R	R	ΔWT3,4, Mut1 (D516V)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3B (T8A)	MDR
8 (6.7%)	R	R	ΔWT3,4, Mut1 (D516V)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	MDR
5 (4.2%)	R	R	ΔWT3,4, Mut1 (D516V)	ΔWT, Mut1 (S315T)	WT	MDR
1 (0.8%)	R	R	ΔWT2,3,4	ΔWT, Mut1 (S315T)	WT	MDR
1 (0.8%)	R	R	ΔWT2	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	MDR
4 (3.3%)	R	R	ΔWT7, Mut2B (H526D)	ΔWT, Mut1 (S315T)	WT	MDR
1 (0.8%)	S	R	ΔWT7, Mut2B (H526D)	ΔWT, Mut1 (S315T)	WT	MDR
2 (1.7%)	R	R	ΔWT7, Mut2B (H526D)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	MDR
6 (5%)	R	R	ΔWT7, Mut2A (H526Y)	ΔWT, Mut1 (S315T)	WT	MDR
1 (0.8%)	S	S	ΔWT7, Mut2A (H526Y)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	MDR
3 (2.5%)	R	R	ΔWT7	ΔWT, Mut1 (S315T)	WT	MDR
11 (9.2%)	R	R	ΔWT7, Mut2A (H526Y)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	MDR
2 (1.7%)	R	R	ΔWT7, Mut2A (H526Y)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3B (T8A)	MDR
4 (3.3%)	R	R	ΔWT8, Mut3 (S53L)	ΔWT, Mut1 (S315T)	WT	MDR
2 (1.7%)	R	R	ΔWT8, Mut3 (S53L)	WT	WT	Rif monoR
1 (0.8%)	S	S	ΔWT8, Mut3 (S53L)	WT	WT	Rif monoR
1 (0.8%)	S	S	ΔWT8, Mut3 (S53L)	ΔWT, Mut1 (S315T)	WT	MDR
2 (1.7%)	R	R	ΔWT8, Mut3 (S53L)	ΔWT, Mut1 (S315T)	WT	MDR
11 (9.2%)	S	S	ΔWT8	WT	WT	Rif monoR
3 (2.5%)	R	R	ΔWT8	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3B (T8A)	MDR
1 (0.8%)	R	R	ΔWT8	ΔWT, Mut1 (S315T)	WT	MDR
1 (0.8%)	R	R	ΔWT8	WT	ΔWT 1, Mut1 (C15T)	MDR
27 (22.5%)	S	S	WT	WT	WT	Susceptible
9 (7.5%)	S	R	WT	ΔWT, Mut1 (S315T)	WT	Inh monoR
2 (1.7%)	S	R	WT	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3B (T8A)	Inh monoR
2 (1.7%)	S	R	WT	WT	WT	Susceptible
1 (0.8%)	S	R	WT	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	Inh monoR

Δ: Absence of hybridization signal with wild-type probes; WT: wild-type; Mut: mutation; DST: drug susceptibility testing; RMP: rifampin; INH: isoniazid; R: resistant; S: susceptible; MDR: multidrug-resistant; Rif monoR: rifampin monoresistant; Inh monoR: isoniazid monoresistant

For mutations conferring resistance to INH, the S315T mutation (*katG* gene) was detected in 76 (63.3%) sputum samples. S315T mutation was associated with nucleic acid substitution at position −8 in promoter region of *inhA* for 39 samples. For this purpose, 24 substitution cases of thymine by cytosine (T8C) and 15 with (T8A) adenine were described. One case of C15T mutation in promoter region was identified *(Table 2)*.

Drug susceptibility testing performed in liquid medium (MGIT 960) showed that 64 clinical isolates were resistant to RMP (1 μg/ml). Resistance to INH (0.1 μg/ml) was detected for 79 clinical strains *(Table 2)*.

The sensitivity, specificity, predictive positive value, and predictive negative value of MTBDRplus assay, for RMP resistance detection directly on sputum samples, were, respectively, 100% (75.6–100%), 73.2% (61.3–

84%), 81%, and 100%. For INH resistance detection directly with sputum, sensitivity and specificity were, respectively, 95% (90–99%) and 95.1% (88.5–100%) *(Table 3)*. The diagnostic performance of MTBDRplus compared to drug susceptibility testing in liquid medium (MGIT 960), for RMP and INH resistance detection from sputum samples, is summarized in *Table 2*.

Table 3. Performance of MTBDRplus compared to drug susceptibility testing

	Drug susceptibility testing		Performances
Rifampin	64	15	Sensitivity: 100% (75.6–100%)
			Specificity: 73.2% (61.3–84%)
			PPV: 81% (72–90%)
	0	41	NPV: 100% (69–100%)
Isoniazid	75	2	Sensitivity: 95% (90–99%)
			Specificity: 95.1% (88.5–100%)
	4	39	PPV: 97.4% (94–100%)
			NPV: 90.7% (82–99%)

PPV: positive predictive value; NPV: negative predictive value

The 21 sputum samples, for which there lacked a hybridization signal with at least one of the wild-type probes exploring the segment of *rpoB* gene, the GeneXpert MTB/RIF assay was performed. Results obtained showed that, for the 16 sputum samples with absence of hybridization of *rpoB* wild-type probes 8, interpreted RMP resistance with MTBDRplus assay, five were effectively resistant to RMP and 11 susceptible. For the five cases for which GeneXpert MTB/RIF detected resistance to RMP, results obtained were concordant with those obtained with the MTBDRplus assay excepted for the 11. For these last cases, a discordant result was observed between interpretations of MTBDRplus and GeneXpert MTB/RIF *(Table 4)*. We also observed that these 11 RMP monoresistant cases had no mutation in *katG* gene or in promoter region of *inhA* gene. These results obtained for these sputum samples were concordant with those obtained with the MGIT-DST *(Table 4)*.

For the five other sputum samples lacking a hybridization signal with wild-type probe 2, 3, and 4, interpretation of MTBRDplus results was concordant with those obtained with detection of RMP resistance with the MGIT 960 (1.0 µg/ml) and GeneXpert MTB/RIF assay.

For the seven sputum samples used as controls with the GeneXpert MTB/RIF assay, results obtained were concordant with those of the MTBDRplus assay and phenotypical test (MGIT DST) *(Table 4)*.

Discussion

In the recent years, a major emphasis has been given on rapid diagnosis of MDR-TB, which poses a great threat to the TB control programs worldwide [9]. According to the World Health Organization, 450,000 new cases of MDR-TB were notified worldwide in 2012 [3]. Taking care of MDR-TB patients is very expensive particularly in low income countries. Thus, a sensitive and specific diagnostic tool is required to initiate an appropriate therapy and reduce spread of multidrug-resistant *M. tuberculosis* strains.

Sequencing of targets (promoter of *inhA*, *katG*, *rpoB* genes, etc.) is the best gold standard for molecular assay evaluation such as the MTBDRplus assay, especially when the molecular method assay is compared to Bactec MGIT 960 automated drug susceptibility testing (MGIT-DST). In fact, with the MGIT-DST, false positive and negative results may occur.

The GenoType MTBDRplus assay validation in Côte d'Ivoire, directly on sputum, revealed that the sensitivity and specificity for RMP resistance detection were, respectively, 100% and 73.2%. Similar studies conducted in Brazil [10] and Nigeria [11] showed that sensitivity of MTBDRplus for RMP resistance detection directly with sputum was of 82% and 83%, respectively. Sensitivity of MTBDRplus for RMP resistance detection ranges from 92% to 99% globally [12–14].

This study demonstrated that the MTBDRplus assay allowed detecting 15 RMP resistance cases that were interpreted RMP susceptible with our gold standard (MGIT-DST). For four of these, a protein modifying activity of ARN polymerase was detected with the MTBDRplus assay. As we do not have any sequencer to confirm these results obtained, particularly for the 11 discordant results observed between interpretation of MTBDRplus assay and phenotypical test, we used the GeneXpert MTB/RIF assay.

The target of the two molecular assays is the 81-bp "core region" of the *rpoB* gene, where more than 95% of all RMP resistant strains can be detected [15]. For the 11 sputa, interpretation of MGIT-DST results for the RMP resistance detection and that of GeneXpert MTB/RIF were concordant except for the one obtained with MTBDRplus. Contrary to these 11 cases, 5 samples on which *rpoB* wild-type probe 8 was not detected, a resistance to RMP (1.0 µg/ml) was spotted with the MGIT-DST. The RMP resistance was also confirmed with the GeneXpert MTB/RIF assay.

Results described previously (16 sputa) could be due to a mutation occurring at the codon 533 of the *rpoB* gene. Indeed, *rpoB* wild-type probe 8 explores simultaneously codon 531 and 533 [12]. At codon 533, a mutation may induce a resistance to RMP or not [12]. Indeed, some authors showed that a mutation at codon 533 did not induce RMP resistance [12, 16, 17].

Other authors considered that mutation at codon 533 of *rpoB* gene is associated to low or high level of resistance to RMP [18, 19].

Note that, in most of cases, mutation in *rpoB* gene is associated with mutation in *katG* or promoter of *inhA*. Here, no mutation in *katG* gene or in promoter of *inhA* gene was observed. This is contrary to our usual experience, where

Table 4. Patterns of GenoType MTBDRplus in comparison to drug susceptibility testing and GeneXpert results

Number of sputum samples	DST		MTBDRplus assay			Results	GeneXpert	
	RMP	INH	RMP pattern *(rpoB)*	INH pattern			MTB	Rifampin
				katG	*inhA*			
1	R	R	ΔWT2,3,4,	ΔWT, Mut1 (S315T)	WT	MDR	Detected	Resistant
1	S	R	ΔWT7, Mut2B (H526D)	ΔWT, Mut1 (S315T)	WT	MDR	Detected	Resistant
1	S	S	ΔWT7, Mut2A (H526Y)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	MDR	Detected	Resistant
1	R	R	ΔWT2	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3A (T8C)	MDR	Detected	Resistant
3	R	R	ΔWT7	ΔWT, Mut1 (S315T)	WT	MDR	Detected	Resistant
1	S	S	ΔWT8, Mut3 (S531L)	WT	WT	Rif monoR	Detected	Resistant
1	S	S	ΔWT8, Mut3 (S531L)	ΔWT, Mut1 (S315T)	WT	MDR	Detected	Resistant
11	S	S	ΔWT8	WT	WT	Rif monoR	Detected	Susceptible
3	R	R	ΔWT8	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3B (T8A)	MDR	Detected	Resistant
1	R	R	ΔWT8	ΔWT, Mut1 (S315T)	WT	MDR	Detected	Resistant
1	R	R	ΔWT8	WT	ΔWT 1, Mut1 (C15T)	MDR	Detected	Resistant
3	S	S	WT	WT	WT	Susceptible	Detected	Susceptible
							Detected	Resistant
2	R	R	ΔWT3,4, Mut1 (D516V)	ΔWT, Mut1 (S315T)	ΔWT 2, Mut3B (T8A)	MDR	Detected	Resistant
2	R	R	ΔWT8, Mut3 (S531L)	ΔWT, Mut1 (S315T)	WT	MDR	Detected	Resistant

Δ: Absence of hybridization signal with wild-type probes; WT: wild-type; Mut: mutation; DST: drug susceptibility testing; RMP: rifampin; INH: isoniazid; R: resistant; S: susceptible; MDR: multidrug-resistant; Rif monoR: rifampin monoresistant; MTB: *M. tuberculosis* complex

the mutations in *rpoB* gene are often associated with mutation in *katG* or in *inhA* gene [5].

The results of seven sputa were concordant with the two first methods (MGIT-DST and MTBDRplus) and were also concordant with those obtained with the GeneXpert MTB/RIF.

Considering also that the GeneXpert MTB/RIF performs well as a rapid diagnostic for *M. tuberculosis* [20–22], observations described in *rpoB* gene at level of wild-type probe 8 could be due to mutation at the codon 533. Although DNA sequencing of *rpoB* gene is required for confirmation, the combination of results of MGIT-DST and GeneXpert (two methods with good performances for detection of RMP resistance) and hypothesis of mutation at codon 533 should be sustained. The difference observed with these two molecular assays is probably due to the principle of each method.

In total, in our setting, it appeared that interpretation of MTBDRplus assay directly on sputum based on absence of hybridization, particularly of *rpoB* wild-type probe 8, should be done carefully because RMP false resistance is not excluded.

Four sputa with a mutation in *rpoB* gene conferring RMP resistance were interpreted susceptible to RMP after MGIT-DST. These cases of false-susceptibility results were already evocated [23, 24] in particular with dysgonic *M. tuberculosis* strains. Probably, the proportion method in solid medium used as a gold standard would have given better results. In total, we note a variety of the sensitivity of the MTBDRplus. This may be due to the characteristics of the populations of *M. tuberculosis* according to geographic area considered or to the gold standard chosen [25].

Mutations that induce INH resistance are located in several genes and regions. In this study, sensitivity of the MTBDRplus assay for INH resistance detection was 95% (90–99%). In general, sensitivity of the MTBDRplus assay for INH resistance detection ranges from 73% to 92% [12–14]. Results of INH resistance detection were concor-

dant for 75 sputum samples. Most of the INH resistance observed was due to S315T mutation (*katG* gene) as previously described [15, 26]. For the six discordant results, the INH resistance could be due to mechanisms different from those studied here. Contamination could be also advocated as responsible of false resistance. In the two cases, sequencing of genes incriminated in INH resistance is required for confirmation.

Conclusion

For rifampin and isoniazid resistance detection directly with sputum, the MTBDRplus assay is an excellent tool for rapid diagnostic of MDR-TB. However, interpretation of absence of hybridization of *rpoB* wild-type 8 without specific protein detected needs to be confirmed.

Acknowledgements

We thank Natalia Shubladze and Gladys Anyo, consultants of the American Society for Microbiology, Dr. Daniela Cirillo of SRL of Milan, and Kekeletso Kao and Khye Seng, Expand TB consultants, PEPFAR's Team of Côte d'Ivoire for their contributions.

Conflict of interest

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this article.

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