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Short Communication

Use of rapid molecular test for multidrug-resistant tuberculosis detection among relapse cases in Cote d'Ivoire

K. N'guessan [a,*], J.M. Ahui Brou [b], J.S. Assi [a], G.D. Adagra [c], O. Adade [c], J.M. Assande [a], A. Guei [a], J. Kouakou [c], M. Dosso [a]

[a] Institut Pasteur de Côte d'Ivoire, Laboratoire National de Référence de la Tuberculose, 01BP 490 Abidjan 01, Cote d'Ivoire
[b] Service de Pneumologie CHU de Cocody, Cote d'Ivoire
[c] Programme National de Lutte contre la Tuberculose, Cote d'Ivoire

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ABSTRACT

Tuberculosis is explicitly recognized as a major global public health problem. In Côte d'Ivoire, relapse cases represent 66.5% of patients eligible for retreatment according to the National Tuberculosis Control Program. This study objective was to detect multidrug-resistance tuberculosis among relapse cases. Patients were recruited in tuberculosis centers in routine. A standardized questioning was administrated. Two sputum samples were collected and transported at Institut Pasteur. Sputum samples were decontaminated by NALC method. The DNA extraction was realized with 500 μl of decontaminated sputum sample with smear-positive. MTBDRplus assay version 2.0 was performed according to the manufacturer's instruction. An internal quality control program with positive and negative controls was implemented for interpretation of results. In total 146 relapse cases with smear positive were studied. Out of selected patients, 130 had received the 2RHZE/4RH regimen and 16, the 2RHZES/1RHZE/5HRE. In group of relapse cases previously treated with 2RHZE/4RH regimen, 40 (31.3%, IC95%: [0.23; 0.39]) had punctual mutations at codon 526 in *rpoB* gene. Although, in patients under treated with 2RHZES/1RHZE/5HRE, a mutation in *rpoB* gene was identified in 12 of 16 sputum samples. Thirteen mutations conferring a resistance to Isoniazid were observed of which 9 in *katG* gene and 4 in *katG* and promoter region of inhA gene. The comparison (Chi-square with Yates correction) of resistance rates to Rifampin estimated showed a statistically significant difference.

Conclusion: Use of a rapid method to detect drug-resistance in recurrent TB cases has permitted to identify patients eligible for first-line drugs or not.

Introduction

Tuberculosis (TB) is a disease that affects the whole society and its drug-resistant form adds another obstacle to its control. Rapid diagnosis of TB and multidrug-resistant (resistance to at least Rifampin and Isoniazid) TB (MDR-TB) is an important challenge to ensure a quick and adequate course of TB therapy is initiated to limit the dissemination of

* Corresponding author. Tel.: +225 07894275.
E-mail address: kouassinguessan@pasteur.ci (K. N'guessan).
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multi-resistant strains [1]. Rifampin and Isoniazid are the cornerstones of first-line drugs used for the treatment of TB.

With DNA fingerprinting, the cases of recurrent TB can be categorized as relapse cases due to the original infecting strain or reinfection with a new strain of *Mycobacterium tuberculosis* [2]. Considering that recurrent TB cases are also the previously treated patients for TB, they are consequently a group with high risk for MDR-TB [3].

At the operational level, the recurrent TB cases are often identified as relapse cases. In fact, a relapse case is a patient previously declared cured or treatment completed and subsequently diagnosed with a new episode of TB bacteriologically confirmed (sputum smear or culture) [4]. The emergence of MDR-TB associated with relapse may be considered as an additional challenge in TB control [5,6], notably in developing countries. The prevalence of MDR-TB has been estimated to be low in sub-Saharan Africa, where surveillance of drug resistance is limited [1]. In Côte d'Ivoire, the prevalence of MDR-TB cases among new cases with a positive smear decreased from 5.3% to 2.5%, respectively from 1995 to 2006 [6].

These last 10 years, relapse cases represented 66.5% of retreatment cases notified by the National Tuberculosis Control Program. Relapse cases may be to a certain extent assimilated with new TB cases. Also, there is limited data available for the principal two first-line drugs in Côte d'Ivoire concerning relapse cases.

New diagnostic tools for TB were developed and validated, particularly molecular methods [7,8]. Molecular methods are highly efficient for Rifampin resistance detection and are well adapted to search for the most relevant Isoniazid resistance. Some methods require minimal infrastructure and equipment [9].

The study objective was to detect resistance to Rifampin and Isoniazid among relapse cases diagnosed in TB centers in Côte d'Ivoire.

Patients and methods

Study sites

Patients were recruited from the Pneumology service of CHU de Cocody and also referrals from regional centers for TB in Côte d'Ivoire. In a sanitary pyramid, the pneumology service is at the central level, and the regional centers are at the intermediate level. A standard questionnaire was administered by trained medical staff. Symptomatic patients were included consecutively based on their history [4]. Data collected included age, gender, previous anti-TB treatment, episodes of TB and results of their smears for AFB detection after Ziehl-Neelsen staining.

Samples collection and transport

For each patient enrolled, two sputum samples (spot, early morning) were collected and put in individual bags. Samples collected were transported at 4 °C in the icebox from the study site to the National Reference Laboratory for Tuberculosis. This laboratory is integrated in the Mycobacteria Unit of Institut Pasteur de Côte d'Ivoire.

Resistance detection

DNA extraction

Sputum samples were processed using the US Centers for Disease Control and Prevention (US CDC) recommended method of N-acetyl-L-Cysteine 4% NaOH-2.9% Citrate. Five milliliters of sputum were transferred in 50 ml of plastic centrifuge tube. Five milliliters of NALC solution were added to the sputum. The preparation was mixed by vortexing for 1 min and incubated at room temperature for 15 min. In the centrifuge tube, 35 ml of sterile phosphate buffer pH 6.8 were added and centrifuged at 3.000*g* for 20 min. Supernatant was carefully eliminated. Pellet was re-suspended with 2 ml of sterile phosphate buffer; 200 μl of re-suspended pellet were used to perform a smear which was stained using the Ziehl-Neelsen method.

The "GenoLyse®" kit was used for bacterial DNA extraction; 500 microliters of sediment were transferred in an eppendorf tube of 1500 μl. The suspension was centrifuged at 10.000*g* in an aerosol-tight rotor for 15 min. The supernatant was discarded; 100 μl of lysis buffer were added to the sediments. The bacterial preparation was homogenized by vortexing. The bacterial suspension was inactivated at 95 °C for 5 min; 100 μl 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 to 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 MTBDR 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× 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: 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).

Prior to hybridization, a migration of 5 μl of each PCR product was performed during 30 min at 100 V in 1% agarose gel stained with ethidium bromide. DNA amplified was revealed with a UV-transilluminator lamp (wave's length 365 nm).

Hybridization

Hybridization and detection were performed with a TwinCubator 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 with 20 μl of amplified sample. The 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