HOSTED BY

ELSEVIER

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Drug susceptibility testing of *Mycobacterium tuberculosis* by a nitrate reductase assay applied directly on microscopy-positive sputum samples

Sana Kammoun [a], *Salma Smaoui* [a,*], *Chema Marouane* [a], *Leila Slim* [b], *Férièle Messadi-Akrout* [a]

[a] *Regional Laboratory of Hygiene, Sfax, Tunisia*
[b] *Microbiology Laboratory, Pneumology Hospital, Ariana, Tunisia*

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form
28 April 2015
Accepted 29 April 2015
Available online 19 May 2015

Keywords:
Drug resistance
M. tuberculosis
Drug susceptibility testing
Sputum

ABSTRACT

Aims and objectives: Current methods for drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB) are either costly or slow. As the prevalence of multidrug-resistant (MDR) strains increases, the need for fast, reliable, and inexpensive methods is obvious. This study evaluated a rapid colorimetric nitrate reductase assay (NRA) for direct DST of MTB directly from clinical sputum samples.

Methods: A total of 111 sputa with positive microscopy results for acid-fast bacilli (AFB) with more than 10 AFB per high-power field were used in the study. The samples were decontaminated using the modified Petroff method. The NRA results were compared with the reference indirect proportion method.

Results: The sensitivity and the specificity of the direct NRA were 90% and 97.3%, 92.6% and 98.2%, 52.9% and 100%, and 28.6% and 100% for rifampin, isoniazid, streptomycin, and ethambutol, respectively. The results were in most cases available in 28 days (84.3%).

Conclusions: The direct NRA could be used as a rapid, inexpensive, and accurate method to determine rifampin and isoniazid susceptibility directly from sputum. The technique might become a valid alternative to traditional methods, especially in low-income countries.

Introduction

Tuberculosis (TB) remains a major public health problem worldwide worsened by the emergence of multidrug-resistant tuberculosis (MDR-TB). In recent years, the incidence of TB has been rising, and the World Health Organization (WHO) has estimated the number of incident new cases at 9 million and 1.5 million people have died from the disease in 2013. The proportion of new cases with MDR-TB was 3.5% [1].

In order to fight this situation, a rapid and inexpensive drug susceptibility test (DST) is needed to allow a rapid initiation of a correct antibiotic (ATB) therapy. Standard methods for DST, such as the proportion method, are used globally, but depend on culture on solid media and are regrettably time-consuming [2]. The time lag is a significant threat to

* Corresponding author.
E-mail address: smaoui_salma@yahoo.fr (S. Smaoui).
Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2015.04.005

the patient, the community and healthcare workers. The current techniques, genetic as well as phenotypic, have been developed [3–6]. But those methods are globally either costly or slow and are consequently not feasible in most low-outcome countries. In view of these considerations, alternative rapid methods have been suggested, among them, the nitrate reductase assay (NRA) on Loëwenstein–Jensen (LJ) medium. It is simple to perform and has been successfully implemented in low-resources countries [7,8]. This test is based on the ability of *Mycobacterium tuberculosis* (MT) to reduce nitrate to nitrite, which is revealed as a color change in the culture medium, using the Griess method [9]. The indirect (using isolates) NRA yields results in less than 14 days, but requires an initial 3–4 weeks for the culture of the isolate. So far, only a few studies have evaluated the NRA applied directly on sputum samples.

The aim of the present study is to evaluate the performance of the NRA applied directly on microscopy-positive sputum samples from patients with pulmonary tuberculosis (PTB) for the detection of resistance to the first-line anti-tuberculosis drugs: rifampin (RIF), isoniazid (INH), streptomycin (STR) and ethambutol (EMB).

Material and methods

Setting

Currently, the laboratory receives samples from patients living in Sfax and suburbs and also from cities of the Tunisian South and Center. All strains are cultured on standard LJ medium, and the DST is performed with indirect proportion method (IPM). After processing, specimens are stored at −20 °C.

Specimen processing

From January 2009 to April 2014, a total of 111 sputa with positive microscopy results with AFB (acid-fast bacilli) having a positivity score of 1+ or more were processed using the Petroff decontamination method [10]. One milliliter of sterile distilled water was added to the sediment.

IPM

An LJ tube was inoculated with 0.2 ml of undiluted decontaminated suspension and incubated for up to 60 days. Isolates from this tube were used for IPM performed using LJ medium according to standard protocol. The following critical concentrations were used: 0.2 μg/ml for INH, 40 μg/ml for RIF, 4.0 μg/ml for STR, and 2.0 μg/ml for EMB.

Direct NRA DST

The NRA was performed as described previously by Ängeby et al. [11] on the difference in the use of sodium nitrate ($NaNO_3$) instead of potassium nitrate (KNO_3). Standard LJ medium was used with 1000 μg of $NaNO_3$/ml and with or without ATB. The same critical concentrations of ATB as those used in the IPM were applied.

Part of the decontaminated suspension was diluted 1:10 in sterile distilled water. For each specimen, 0.2 ml of the diluted preparation was inoculated into four drug-free LJ medium tubes containing only $NaNO_3$ (growth control tubes) and 0.2 ml of the undiluted suspension was inoculated into LJ medium containing $NaNO_3$ and each of the first-line ATB. The tubes were incubated at 37 °C.

After 7 days of incubation, 0.5 ml of freshly prepared Griess reagent (1 part 50% concentrated hydrochloric acid, 2 parts 0.2% sulfanilamide, and 2 parts 0.1% *n*-1-naphtylethylenediamine dihydrochloride) was added to one drug-free tube. If any color appeared, the tube with ATB was developed with the Griess reagent. If not, the other tubes were re-incubated, and the procedure was repeated at day 10 (D10), day 14, and finally at day 28. The medium color changes to weak or strong pink. An isolate was considered to be resistant if there was a color change in the ATB tube equal or greater than that in the diluted growth control. An isolate was considered to be susceptible if there was no color change or a color change less than that in the diluted growth control (Figs. 1–3). NRA was considered to be invalid if the nitrate reaction was negative in the drug-free medium at day 28 despite the presence of colonies.

Quality control

For each batch of medium, internal quality control was done using two known susceptibilities of MT strains: one fully susceptible and one MDR isolate.

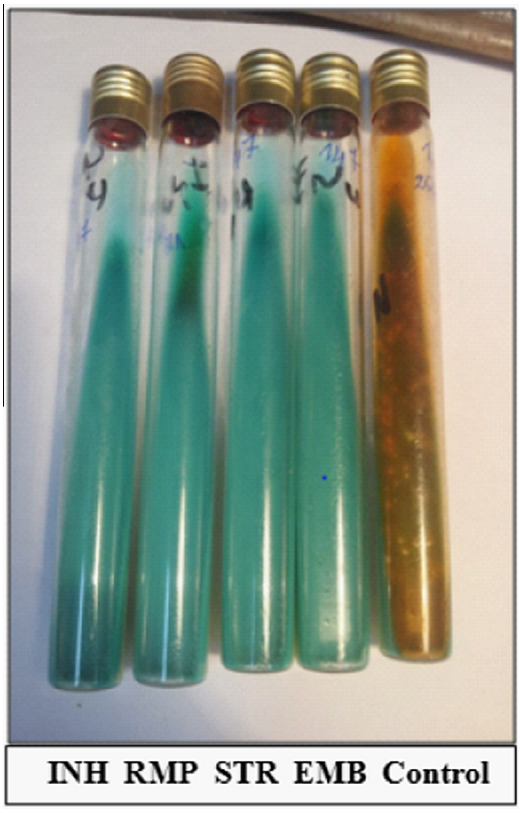

Fig. 1 – Sensitive strain to four antibiotics.