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EVALUATION OF FAST-PLAQUE TB-RIF FOR RAPID DETECTION OF RIFAMPIN SUSCEPTIBLITY IN CLINICAL ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS*

MYCOBACTERIUM TUBERCULOSIS KÖKENLERİNDE HIZLI RİFAMPİN DİRENCİ SAPTANMASINDA FAST-PLAQUE TB-RIF'İN DEĞERLENDİRİLMESİ

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Anahtar Sözcükler: *Mycobacterium tuberculosis*, mikobakteriyofaj deneyi, rifampin direnci

SUMMARY

A new rapid mycobacteriophage assay, Fast-Plaque TB-RIF (FPTB-RIF) (Biotec Laboratories Ltd.), for the detection of rifampin (RIF) resistance in Mycobacterium tuberculosis has recently been described, which gives results in a short period of time. This study aimed at comparing this test with the agar proportion method. Totally 62 clinical isolates of M. tuberculosis were studied with both methods. The mycobacteriophage assay was performed according to the manufacturer's recommendations. The phage assay showed concordance with agar proportion method for 10 of 11 (91%) resistant and 50 of 51 susceptibile (98%) isolates. The results with FPTB-RIF were comparable with those of the standard proportion method. Fast Plaque TB-RIF is easy to perform and presents a low cost, reliable means of screening for rifampin resistance in clinical isolates of M. tuberculosis.

ÖZET

Fast Plaque TB-RIF (FPTB-RIF) (Biotec Laboratories Ltd.) Mycobacterium tuberculosis'de hızlı rifampin direnci saptayan, mikofabteriyofaj temeline dayanan yeni tanımlanmış bir testtir. Bu çalışmanın amacı, bu yöntem ile agar proporsiyon yöntemini karşılaştırmaktı. Klinik örneklerden soyutlanan 62 M. tuberculosis izolatında FPTB-RIF, agar proporsiyon yöntemi ile karşılaştırıldı. Test üretici firmanın tanımladığı şekilde yapıldı. Agar proporsiyon testiyle dirençli bulunan 11 izolatın 10'u (% 91), duyarlı bulunan 51 izolatın 50'si (% 98) faj testiyle uyumlu olarak bulundu. Fast Plaque TB-RIF ile elde edilen bulgular standart proporsiyon yöntemi ile uyumluydu. Uygulanması kolay ve ucuz olması nedeniyle, Fast Plaque TB-RIF M. tuberculosis izolatlarında rifampin direncini taramak için güvenle kullanılabilir.

INTRODUCTION

Tuberculosis (TB) is the leading cause of death due to an infectious agent. It affects one-third of the world's population, and 95 % of the diease burden is born by devoloping countries (1). This situation is likely to deteriorate in the future, with annual disease rates expected to rise from 8.8 million in 1995 to 11.9 milllion per year in 2005 (2). As TB incidence has increased, there has been a corresponding rise in the proportion of drug-resistant cases, acquired largely as a result of

incomplete treatment regimens but also as a result of spread from index cases of resistant TB (3). The most worrisome trend is the increase in multi-drug resistant TB, i.e., resistance to at least isoniazid and RIF (4). One of the major factors influencing the clinical outcome and the control of the transmission of multidrug-resistant TB from patients is the time taken to obtain drug susceptibility data (5). The Centers for Disease Control and Prevention recommend that all isolates of *M. tuberculosis* be tested for their susceptibility to antibiotics, using the most rapid methods possible, and that susceptibility data for first-line drugs be available within 30 days of the arrival of the specimen (6). Conventional culture-based techniques for susceptibility testing take several weeks to complete, and although both radiometric anad nonradiometric liquid culture systems have significantly reduced turnaround times, results are still not avaible for 5 to 12 days after isolation of a strain (7). Molecular techniques have limited utility for detecting resistance to antibiotics other than RIF, for which mutations conferring resistance occur at a number of genetic loci. Attention is now being focused on rapid phenotypic methods, which use markers of viability other than increase in biomass. (8). The Fast-Plaque TB-RIF (FPTB-RIF) (Biotec Laboratories Ltd.) uses specific mycobacteriophage in order to determine the presence of viable tuberculous bacilli. The test is a rapid manual test to detect rifampin resistance within 48 hours in *M. tuberculosis* cultures (9).

In this study, the efficacy of Fast-Plaque TB-RIF test for screening resistance to RIF in *M. tuberculosis* was evaluated comparing it with agar proportion method.

MATERIALS AND METHODS

Isolates: Clinical isolates of *M. tuberculosis* were obtained from the culture collection of the authors' department. They were identified by DNA probe (AccuProbe; Gen-Probe, Inc., San Diego, Calif.). Isolates were subcultured on Loewenstein-Jensen (LJ) egg medium at 37°C.

Agar proportion method of susceptibility testing: Antimicrobial susceptibility testing was carried out on all isolates with the agar proportion method described by Kent and Kubica (10).

Fast-Plaque RIF-TB: The mycobacterophage assay was performed as described by the manufacturer. Briefly, *M. tuberculosis* test suspension was prepared from LJ slants. Test suspension was added to two separate plastic tubes containing solution without RIF (RIF -) and with RIF (RIF +). Supensions were icubated at 37°C for 24 hours. Pre-incubated samples were treated with mycobacteriophage, and incubated 37°C for 90 minutes to allow lytic phage enter the viable cells. To ensure that only intracelluler phage was carried over into the *M. smegmatis* cultures, extracellular viruses were destroyed with the phagicidal agent by incubating at room temperature for 5 minutes. As the last step, test suspensions were mixed with a suspension of sensor cells *(M. smegmatis)* in solid medium and incubated overnight at 37°C. Positive and negative controls were used to ensure that each run had been correctly performed. A sample was scored as sensitive if the number of plaques on the plate with rifampin treated organisms was less than 10. When the number was greater than 50 plaques, the sample was scored as resistant. Isolates were scored only if >100 plaques were present on the control plates containing organisms not treated with rifampin. Results were deemed noninterpretable if fewer than 100 plaques were present.

RESULTS

Of the 67 isolates tested by FPTB-RIF, 59 yielded a result on initial testing whereas eight had to be retested due to insufficient plaque numbers in the "no-antibiotic" control. On repeat testing of the latter group, it was seen that five cultures were contaminated. Of the remaining 62 isolates, 10 of 11 (91 %) resistant and 50 of 51 (98 %) susceptible isolates, determined by the agar proportion, were correctly assigned. Of the 11 isolates resistant to RIF, three were also resistant to INH by agar proportion method. The comparison of FPTB-RIF assay with agar proportion method for testing susceptibility of *M. tuberculosis* isolates to rifampin are shown in Table 1, and plates from FPTB-RIF assay are shown in Figure 1.

DISCUSSION

The phage amplified biological assay has been described previously (11) and applied to rifampin and isoniazid susceptibility testing in clinical isolates of *M .tuberculosis*

Table 1. Concordance of Fast-Plaque TB-RIF assay with results obtained by agar proportion method f testing susceptibility of *M. tuberculosis* isolates to rifampin

	Proportion resistant	Proportion susceptible	Total	% Concordance
FPTB-RIF resistant	10		11	9
FPTB-RIF susceptible		50	51	
Total	11	51	62	

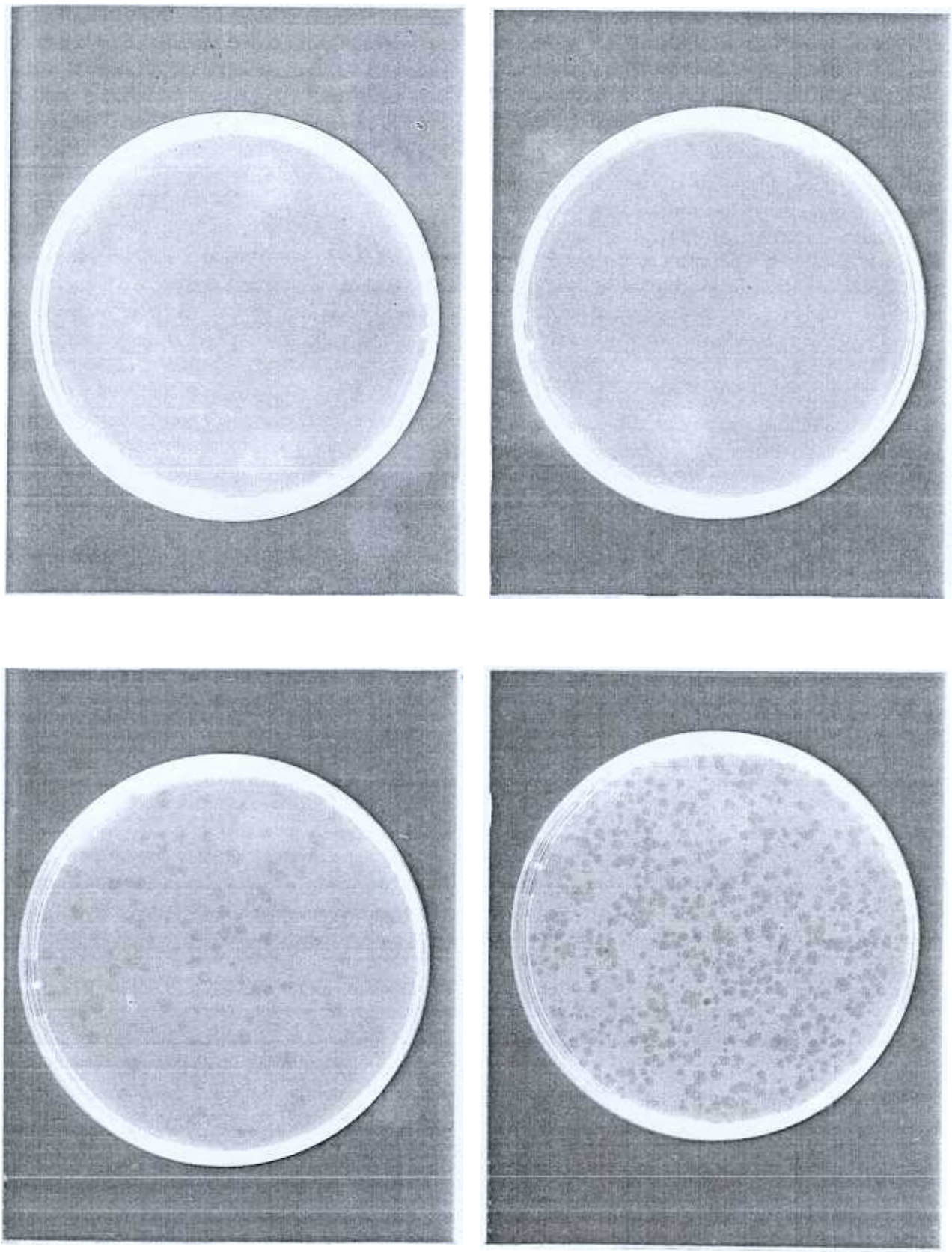

Figure 1. Plates from rifampin (RIF)-susceptible and -resistant *Mycobacterium tuberculosis* strains
Rifampin-susceptible (above): Left, RIF (-) plate; right, RIF (+) plate
Rifampin-resistant (below): Left, RIF (-) plate; right, RIF (+) plate

(12). The FPTB-RIF is developed for rifampin susceptibility testing of solid *M. tuberculosis* cultures as to give result within 48 hours (9). In the present study, the results of the rifampin susceptibility testing by the Fast Plaque TB-RIF was highly concordant (97 %) with the results of the agar proportion method. The sensitivity and specificity of the former assay were 91 %, and 98 %, respectively. Its turnaround time was 48 hours, being one week shorter than that of the agar proportion method. Results of preliminary studies with the FPTB-RIF procedure suggest performance comparable to the standard protocol (overall accuracy, 97-98 %) (9), in agreement with the results of the present study. In the United Kingdom over 90 % of rifampin resistant isolates are also resistant to isoniazid (8). However, in the present study, only three rifampin resistant isolates were resistant to isoniazid. One weakness of the FPTB-RIF assay is the primary failure rate when plaque counts on control plates are below 100. Thus three of 62 (5 %) of the viable cultures failed to produce adequate plaque counts on initial testing. The reason for this situation is unclear, but it could be the inadequancy of the inoculum in the initial test. The study demonstrated that this test can be used as a rapid screen in determining the rifampin resistance in *M. tuberculosis*.

It is concluded that the FPTB-RIF assay provides a reliable rapid means of susceptiblty testing with a total turnaround time of only 48 hours, also it presents advantages, particularly for developing countries, in terms of low technical demand and low cost. In addition, it may allow earlier reporting of rifampin resistant strains which may lead to appropriate therapeutic measures, thus decreasing the misuse of antimicrobial agents.

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