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Direct drug susceptibility testing of *Mycobacterium tuberculosis* using the proportional method: A multicenter study

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ABSTRACT

Introduction: Conventional indirect drug susceptibility testing (DST) of *Mycobacterium tuberculosis* with solid media is inexpensive and reliable, but time-consuming. This study aimed to evaluate direct DST for testing sputum samples without culture to significantly reduce the time required to detect multidrug-resistant tuberculosis (MDR-TB).
Methods: Direct and indirect DST of isoniazid (INH), rifampicin (RIF) and ethambutol (EMB) were performed on 334 sputum smear-positive specimens.
Results: There was full agreement between the results obtained from direct testing and after isolation of the bacteria by culture. Thus, the sensitivity and specificity were observed to be 100% for all three tested drugs when compared with indirect DST. In comparison with indirect DST, none of the samples with the direct method took >25 days to report the DST (between 15–25 days with a mean detection time of 20 days).
Conclusions: Direct DST on solid media was shown to give reliable results at a much earlier stage than conventional phenotypic DST. The direct method was found to be more rapid, more accurate and simpler. In addition, it reduced the handling of pathogenic bacteria and thus reduced the bio hazards related to conventional DST.

1. Introduction

Tuberculosis (TB) is a global public-health problem. Its drug-resistant forms are of increasing concern in many low-income and middle-income countries. Although the global mortality and incidence rates have decreased during the last decade, the increasing prevalence of multidrug-resistant TB (MDR-TB) – defined as resistance to at least isoniazid (INH) and rifampicin (RIF) – has led to new challenges [1]. As an intensifying threat, it is reported that MDR-TB accounts for 4.1% of new TB cases and 19% of previously treated TB cases in the world [1]. MDR-TB is typically associated with worse treatment outcomes than drug-susceptible TB, and demands more expensive drugs for prolonged periods [2–6]. For better management of MDR-TB, early detection of resistance and starting effective treatments are important to reduce the transmission of MDR-TB and ensure successful treatment of the individual patient [7–10]. Without any doubt, rapid identification of drug resistance plays an important role in the detection and control of MDR-TB [11–15]. Whenever possible, molecular tools such as the World Health Organization-recommended line probe assays and the GeneXpert MTB/RIF should be used. However, these

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are still rather costly and not available on a level to meet the global demand. An alternative fairly rapid way of detecting MDR-TB is by using liquid medium-based automated culture systems such as the BACTEC MGIT 960 system; the above-mentioned limitations also apply to this technique.

Thus, many countries still perform conventional indirect drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (*M. tuberculosis*). In this method, DST is performed after a culture is isolated from a clinical specimen, which normally takes around 4 weeks [15]. Although conventional indirect DST is well established and offers reliable results, the results are typically unavailable within 2 months after sampling. This is such a long time that it can hardly be seen as acceptable, at least not in places where MDR-TB is a problem. Direct DST on solid medium has the potential to significantly reduce the time to detect drug resistance when directly used on sputum smear-positive specimens [16,17].

This multicentre study aimed to assess the accuracy and time savings of direct DST against indirect DST.

2. Methods

2.1. Setting and samples

This study was carried out at five different regional TB laboratories in Iran (Tehran, Mashhad, Ahvaz, Shiraz and Isfahan) from July 2015 to January 2017. These regional reference laboratories are well-equipped biosafety level III laboratory facilities, and standard biosafety precautions were followed for specimen processing, inoculation and DST. The Swedish Institute for Infectious Disease Control monitored and supervised the laboratories' quality. Sputum specimens from suspected TB cases and cases under TB treatment, which were found to be smear positive for acid-fast bacteria (AFB), were included in the study. The Ethics Committee of Tehran University of Medical Sciences approved the study.

2.2. Microscopy examination and identification of mycobacteria

All the smears were stained with the fluorochrome and confirmed with Ziehl-Neelsen methods [18]. For identification of mycobacteria, the slope cultures on Lowenstein-Jensen (LJ) medium were incubated at 37 °C and examined for growth once weekly up to 8 weeks. Bacterial isolates were identified as *M. tuberculosis* using standard biochemical tests, including production of niacin, nitrate reduction and catalase.

2.3. Molecular identification of M. tuberculosis

For molecular identification of *M. tuberculosis*, *IS6110* based-polymerase chain reaction (PCR) assay and Xpert MTB/RIF were used. Genomic DNA, for *IS6110* based-PCR assay, was extracted using QIAamp DNA Mini Kit (QIAGEN, USA) according to kit instructions. A 123 bp fragment of insertion element *IS6110* of the *M. tuberculosis* complex was used as a target and amplified using previously described PCR primers [19]. Genomic DNA of *M. tuberculosis* H37Rv (ATCC27294) and *Mycobacterium fortuitum* (ATCC 49404) were used as positive and negative controls, respectively. Xpert MTB/RIF was performed on clinical specimens as previously described [20].

2.4. Indirect drug susceptibility testing

The indirect DST of confirmed *M. tuberculosis* isolates to INH, RIF and ethambutol (EMB) was determined using the proportion method on LJ medium. Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drugs: 0.2 μg/mL for INH, 40 μg/mL for RIF, and 2 μg/mL for EMB. Interpretation was made according to the usual criteria for resistance (i.e., 1% for all drugs). *M. tuberculosis* H37Rv strain (ATCC 27294) was used for quality control testing in DST [21].

2.5. Direct drug susceptibility testing

The direct DST on LJ medium was performed with smear-positive isolates and the inoculation was adjusted according to the treatment history and numbers of AFB found in specimens. The same recommended critical concentrations of the INH, RIF and EMB drugs were also used for the indirect testing. For specimens obtained from patients, the suspension was not diluted if the AFB was 1+. If the AFB was 2+, DST was performed, without dilution and with 1:2 dilutions. For AFB with 3+ grade, DST was performed with 1:2 and 1:3 dilutions. An aliquot of 100 μL of the dilution was inoculated in two tubes of LJ medium with and without antibiotics. All tubes were incubated at 37 °C. Preliminary results could be reported earlier for resistant strains, sometimes as early as 15 days. In case of contamination, LJ medium was checked in the first 24–48 h. Contaminated media were discarded and then performed again with 5% oxalic acid for decontamination.

2.6. Statistical analysis

Data were analysed with MedCalc 14 statistical software. Sensitivity and specificity of the direct DST were calculated according to standard indirect DST as the reference standard in this study.

3. Results

A total of 334 clinical isolates of *M. tuberculosis*, from the same number of patients, were included in this study and tested for their susceptibility to INH, RIF and EMB by indirect and direct methods (Table 1).

Table 1
Comparison of direct DST and indirect DST in 334 isolates of *Mycobacterium tuberculosis*.

Site 1 (Total number of tests)	Direct DST		Indirect DST	
	Resistance	Sensitive	Resistance	Sensitive
Site 1 (220)				
INH	11	323	11	323
RIF	4	330	4	330
EMB	2	332	2	332
MDR	11	323	11	323
Site 2 (56)				
INH	4	330	4	330
RIF	5	329	5	329
EMB	8	326	8	326
MDR	4	330	4	330
Site 3 (20)				
INH	1	333	1	333
RIF	1	333	1	333
EMB	1	333	1	333
MDR	1	333	1	333
Site 4 (19)				
INH	3	331	3	331
RIF	1	333	1	333
EMB	0	334	0	334
MDR	1	333	1	333
Site 5 (19)				
INH	2	332	2	332
RIF	1	333	1	333
EMB	1	333	1	333
MDR	1	333	1	333

Site 1: Tehran; Site 2: Mashhad; Site 3: Ahvaz; Site 4: Shiraz; and Site 5: Isfahan. Abbreviations: DST, drug susceptibility testing; INH, isoniazid; RIF, rifampicin; EMB, ethambutol; MDR, multi-drug resistant.

3.1. Comparison of direct with indirect drug susceptibility testing

When the 334 *M. tuberculosis* isolates clinical samples were tested for their susceptibility to INH, RIF and EMB, an agreement of 100% with indirect DST was seen. The sensitivity (i.e. the ability to detect true drug resistance) was observed to be 100% for INH, RIF and EMB. Specificity (i.e. the ability to detect true drug susceptibility) was also 100% for these drugs.

3.2. Turnaround time

Most of the results were available within 20 days of test reading. None of the samples took more than 25 days to report the DST by a direct method. Generally, direct results were rolled out at the same time as culture.

4. Discussion

The prevalence of MDR-TB has been globally increasing, and rapid DST methods play an important role in the detection and control of MDR-TB. Direct DST in the conventional solid medium has a time-saving advantage which can be set up in low- and middle-income settings.

The most important aspect of the current findings is the accuracy of results obtained by the direct DST method. This was similar to previous studies. According to Siddiqi et al., the results of direct DST using the BACTEC MGIT were compared with those of indirect DST; there was 95.1% concordance with INH and 96.1% with RIF [15]. Likewise, subsequent investigations have reported that direct DST is highly sensitive and reliable when compared within direct DST [16].

Another important finding of this study was the reduced turnaround time of results obtained by the direct DST. The time to report direct DST from AFB smear varied from 15–25 days, which is consistent with previous reports [15]. The total time for report of indirect DST was calculated as the time to isolate a culture, the time required to set up DST, and the time to get results of the susceptibility test from a positive culture. In another words, the time to report direct DST was the time to achieve culture results. The results indicate that direct DST significantly reduces the time to report DST results.

Although direct DST offers several advantages in detecting MDR-TB, it also has some limitations. It cannot compete with molecular assays or liquid based methods for early detection of drug resistant TB. Therefore, these techniques should be used if possible. In places where this is not the case, direct DST can be the only alternative to significantly reduce time to detect MDR-TB. Direct DST can only be used in smear-positive cases, where it provides reliable results for specimens with 1+, 2+ and 3+ in direct smear microscopy. Drug susceptibility testing for specimens with scanty smears should be conducted with an indirect conventional method due to poor growth of mycobacteria in the direct method. Another limitation of this method is that inoculation should be induced in the first 24 h to have optimum output, whereas inoculation in the indirect method is from mycobacterium colony.

In conclusion, direct DST is a rapid, low-cost, accurate and simple method of determining drug resistance from sputum in comparison with indirect DST. It is easily implemented in laboratories experienced in TB DST.

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Competing interests

None.

Ethical approval

Not required.

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