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Fast track method for the identification of multi−drug resistant tuberculosis on direct clinical specimen using combined drug media

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ABSTRACT

Objective: To combine isoniazid (INH) and rifampicin (RIF) in a single media to detect and evaluate multi−drug resistant *Mycobacterium tuberculosis* (MDR−TB) strains using clinical specimens by direct and indirect drug susceptibility testing (DST). **Methods:** Drug susceptibility testing for INH and RIF using individual and combined drug media was performed by minimum inhibitory concentration (MIC) method on direct clinical specimens. **Results:** The combined drug media showed complete concordance with individual drug media in the detection of MDR−TB by direct DST method and 89% efficiency with indirect DST method. Susceptibility results were available by 3 weeks after the receipt of clinical specimen using direct DST on combined drug media. **Conclusions:** Combined drug media can be used as a fast track method in large scale studies warranting detection of MDR−TB.

1. Introduction

Tuberculosis (TB) has reemerged as one of the leading causes of death[1]. The severity of the disease has increased in the developing countries because of global HIV pandemic and social restructuring due to industrialization. Delayed recognition of drug resistance results in delayed initiation of effective therapy is a major factor contributing to multi−drug resistant tuberculosis (MDR−TB) outbreaks[2]. It is needless to emphasize that early diagnosis and treatment of drug resistant TB is of paramount importance not only from the patient's perspective, but also for the community at large. The high infection and death rate pose an urgent challenge to rapidly detect multi−drug resistance[3]. The fourth global report on drug resistance indicated the India and China contributes to approximately 50% of resistant cases[4]. The situation is still alarming with the sporadic reports of extensively−drug resistant TB (XDR−TB) in Indian setting[5]. With the introduction of DOTS−PLUS regimen for the treatment of MDR−TB in the country, there exists prime necessity for a method that detects MDR−TB early so that patients can be put on to specific treatment regimens at the earliest possible. The method should also be cost effective in resource−limited setting and easily adaptable with the existing facility[6]. It is necessary to detect drug resistance from direct clinical specimen, namely sputum, there by minimizing time to detection[7]. Hence, a study was attempted to combine the two most potent drugs, isoniazid (INH) and rifampicin (RIF) in a single Lowenstein−Jenson (LJ) medium for the detection of MDR−TB strains. Evaluation of combined drug media using primary cultures with known susceptibility profile yielded excellent results[8]. Hence, the current study was aimed at the detection of MDR−TB from direct clinical specimen.

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2. Materials and methods

A total of 128 consecutive sputum specimens received

in laboratory for smear and culture of acid fast bacilli were included. Initially, the direct smear for fluorescence microscopy was prepared and then processed for culture by modified Petroff's method following standard procedures[9]. One loopful (approximately 10 μL) of the specimen was inoculated onto 2 slopes of drug free LJ medium. Susceptibility testing from direct clinical specimens (Direct DST) by MIC method was performed as described elsewhere[10,11]. Briefly, 10 μL of sputum deposit was inoculated onto one slope of drug media containing INH and RIF at their break point concentrations namely 0.2 μg/mL and 64 μg/mL. Presence of *Mycobacterium tuberculosis (M. tuberculosis)* was confirmed by simultaneously inoculating one slope of para−nitro benzoic acid (PNB) at a concentration of 500 μg/mL. All the slopes were incubated at 37 ℃ and growth reading was noted[11]. First week reading was noted after the 10th day of inoculation and henceforth weekly reading once for up to a maximum of 4 weeks. The efficiency of the combined media for the detection of MDR−TB isolates was evaluated with individual drug media.

3. Results

Fluorescence smear microscopy identified 100 (78%) and 28 (22%) as smear positive and negative respectively. Of the 128 sputum specimens processed for culture by modified Petroff's method, 84 (66%) were positive while 44 (34%) were negative for *M. tuberculosis*. Seven of the 84 positive isolates were excluded from further analysis as they were either negative, contaminated in control or in drug slopes by direct DST method and 77 were finally considered for analysis. Growth observed on individual drug medium by direct DST method showed 37 (48%) isolates resistant to INH and 26 (20%) to RIF. Forty isolates (31%) were susceptible to both INH and RIF. Twenty six (20%) isolates were identified as MDR−TB strains both by individual drug media and combined drug media (Table 1). Mono resistance to RIF was not encountered and none of the 11 INH mono resistance strains were detected in combined drug media. There was complete concordance between individual as well combined drug media for the detection of MDR−TB isolates by direct DST method.

Table 1
Comparison of combined drug media with individual drug media for the detection of MDR−TB isolates by direct DST method.

Individual drug media*		Combined drug media#(INH+RIF)		Total
RIF	INH	Negative	Positive	
Negative	Negative	40	0	40
Negative	Positive	11	0	11
Positive	Positive	0	26	26
Total		51	26	77

Negative: no visible growth on the media; Positive: visible growth in the media.
* − Method employed was conventional MIC method.
# − Combined drug media included both INH and RIF in a single medium.

4. Discussion

Inadequate detection of drug resistance and infrastructure for resistance testing has been identified as reasons for a mounting global tuberculosis burden[1, 12]. Recent report from the state of Gujarat indicated a high level of resistance to anti tuberculosis drugs among new and treated cases[4, 13]. Rapid detection of drug resistance not only helps to optimize treatment and improve outcome for patients with drug−resistant TB but also important in the prevention of transmission of drug−resistant tuberculosis[14, 15].

Detection of drug susceptibility from direct clinical specimens represents the actual situation *in−vivo* and reduces the time to detection considerably[11]. The efficiency of MIC method using breakpoint concentration for first and second line drug showed significant concordance with the conventional method[17]. As an extrapolation of the similar methodology, it was attempted to combine the vital first line drugs, INH and RIF at their critical concentrations in a single LJ medium.

Comparison of combined drug media with individual drug media by direct DST method showed a complete concordance in the detection of MDR−TB. This not only establishes that combined drug media is efficient in detecting MDR−TB isolates, but also indicates that it is efficient in determining resistance even with unknown measure of organism. The advantage of such comparison is that both the combined as well as individual drug media was evaluated together under similar conditions. The combined drug media is expected to detect only true MDR−TB strains resistant to both INH and RIF and not mono resistant isolates. As expected none of the mono resistant isolates were detected by combined drug media. Therefore, growth on combined media indicates the presence of MDR−TB strains and no growth do not exclude the presence of mono resistance strains. In the current study, mono resistance was encountered only to INH and not with RIF indicating the efficiency of the drug as well as the National TB control programme. The current study reiterates the earlier report by Santha *et al*[18] on the low percentage of RIF mono resistance in the same study setting. Majority of the resistant isolates were detected within 3 weeks of incubation and correlates with the earlier report[19, 20]. Hence, MDR−TB isolates can be identified within 4 weeks after receipt of the clinical specimen. Inclusion of single combined drug media and a slope of PNB along with drug free slopes will help to isolate, identify and perform DST at a single time point.

The fast track method when used in direct clinical specimens will reduce the utilization of drug media considerably in comparison with the conventional DST method. Detection of MDR−TB by conventional MIC method utilizes 6 slopes for both INH and RIF, whereas, the combined media requires just a single slope incorporated with the break point concentration of INH and RIF. This will have a great impact both financially and technically.

Adaptation of the methodology in resource limited setting is easy as LJ and MIC methods are still being routinely being used.

Overall, the study has evaluated a fast track method for the detection of MDR–TB isolates using direct clinical specimen. The method has shown good concordance with individual drug media. The turn around time for the detection of MDR–TB isolates was reduced to 3 weeks. The method is a modification of conventional LJ medium based detection system and can be well adapted by any mycobacteria laboratory without much of prior standardization. The methodology can be recommended for routine usage in large scale DRS studies, high–through put laboratories and in resource limited setting for the early and economical identification of MDR–TB strains.

Conflict of interest statement

We declare that we have no conflict of interest.

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