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Brief Communication

Combined drug medium with isoniazid and rifampicin for identification of multi-drug resistant *Mycobacterium tuberculosis*

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Abstract

A low-cost method of detecting multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) with the possibility of quick adoption in a resource limited setting is urgently required. We conducted a study combining isoniazid and rifampicin in a single LJ medium, to detect MDR-TB strains. Combined and individual drug media showed 100% concordance for the detection of MDR-TB and susceptible strains by proportion method. Considering the results, combined isoniazid and rifampicin containing medium could be considered for use in settings where the sole detection of MDR-TB strains is justified.

Key Words: *Mycobacterium tuberculosis, MDR-TB, Isoniazid, Rifampicin, PST*

Introduction

Strains of *Mycobacterium tuberculosis* have acquired resistance to various drugs very soon after the first effective anti-tuberculosis (TB) chemotherapy began in 1952. The rising prevalence of multi-drug resistant (MDR) strains, defined here as *M. tuberculosis* resistant to isoniazid (H) and rifampicin (R), with or without resistance to the other drugs, has resulted in challenges both to the management of individual patients and to TB control programmes. According to the 4th World Health Organization report on anti-TB drug resistance,[1] 2.9% and 15.3% of new and previously treated TB cases respectively are MDR-TB. The enhancement of timely detection and treatment, and infection control measures in health care facilities to prevent the transmission of MDR-TB strains are crucial components in MDR-TB management programmes.

In this study, we had combined H and R, the two most effective first line anti-TB drugs, in a single LJ medium to assess the potency and action of the drugs in combination with the other and ability / efficiency to detect previously confirmed MDR-TB strain. In settings where resources are limited, the sole detection of MDR-TB cases could be justified from patients identified as "MDR-TB suspects" under DOTS-Plus activities and in drug resistance surveys. Theoretically it is expected that isolates exhibiting resistance to H and R would grow on the combined medium.

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Materials and Methods

A total of 107 *M. tuberculosis* strains isolated from patients enrolled in various clinical trials conducted at the centre were included in the study. The strains included both susceptible and resistant strains (mono-, poly- and MDR resistant) with respect to first line drugs. All the strains were randomized and used for further experiments. Individual drug medium were prepared as per standard methods[2] and the combined drug medium included the same concentrations of the 2 drugs in a single LJ slope. Drug susceptibility, testing was performed at critical concentrations of 0.2µg/ml for H and 40 µg/ml for R by proportionate method (PST) according to Canetti *et al*.[3] $H_{37}Rv$ was included at each occasion as control.

Results

Of the 107 isolates tested, 54 and 53 isolates were identified as non-MDR and MDR-TB [Table 1]. 100% (53/53) of the MDR-TB isolates showed similar resistance patterns in individual as well as combined drug medium. Hence the combined medium was able to identify all the MDR-TB strains included in the study without any discrepancy as compared to the results of the individual medium.36 of the 54 non-

Table 1: Comparative analysis of resistance pattern of combined media with standard individual drug media

Individual media (INH and RIF)	Combined media		Total
	Resistant	Susceptible	
RR	53	0	53
RS	0	09	09
SR	0	09	09
SS	0	36	36
Total	53	54	107

R – Resistant, S – Susceptible

MDR isolates were susceptible to H and R by individual drug medium and in the combined medium.

Discussion

Recent reports suggest that India and China carry approximately 50% of the global burden of MDR-TB.[1] It is necessary to identify these MDR-TB cases early in order to prevent further transmission in the community. Laboratory diagnosis of *M. tuberculosis* is mainly performed on conventional egg based LJ medium, which is considered as the "Gold Standard". Early detection can be achieved using liquid culture systems and/or molecular methods, but these are expensive and require sophisticated infrastructure and highly skilled personnel.

Here, we report on a modification in the LJ medium of combining the two most vital first line drugs, namely H and R. The combined medium is expected to identify only "true MDR" strains i.e. resistant to H and R, and not the mono-resistant strains that are inhibited by the action of the other drug in the medium.

Overall results indicate that primarily there was no inhibitory effect on the growth of the bacilli or with respect to action of the drugs in the combined medium as proven by the growth of the MDR-TB strains. Antagonistic effect between the drugs was not observed, as there was no inhibition on the growth of MDR strains.

The ability of the combined medium to accurately detect MDR-TB strains was seen in the 100% concordance with the results of the individual medium as it identified all the MDR-TB strains and susceptible strains without any discrepancy. Hence the combined medium could be considered for use as a routine laboratory method for the identification of MDR-TB strains.

As expected, the combined medium could not detect all of the 18 mono-resistant isolates for H or R. This reinforces the theory that strains exhibiting mono-resistance would be effectively killed by the action of the other drug in the combined medium, and is further proof of the bactericidal action of the drugs in the combined medium.

Prevalence of R mono-resistance strains was low (<2%) as previously reported from the clinical trials at TRC and by the recent state representative DRS survey conducted in the state of Gujarat in western India.[4-6] Rifampicin containing drug medium at the concentration of 40 µg/ml could however, be included with the combined medium for the detection of R mono-resistant strains if required. If so, then the interpretation of results would be as follows: any *M. tuberculosis* isolate showing growth in combined and R medium is a "true MDR-TB" strain; isolates with growth only on R medium without growth in the combined medium can be considered as R mono-resistant; and if no growth on both medium, the strain can either be susceptible to both H and R or be a H mono-resistant strain.

Combination of the two drugs in a single LJ slope simplifies the workload during preparation of the drug slopes, and minimizes the usage of glassware and the number of drug slopes used during the DST procedure. As drug medium are used within a month of preparation, there are no issues regarding the deterioration of the drug during storage.

In conclusion, the combination of H and R in a single LJ medium showed complete concordance with the results of individual drug medium in relation to MDR--TB strains and could be considered for the detection of MDR-TB isolates. Currently, the combined drug medium is being evaluated for the detection of MDR-TB strains directly from sputum specimens. The combined medium can be included in disease control programmes and in DRS surveys where the detection of MDR-TB alone is justified.

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