ORIGINAL ARTICLE

IN-VITRO EVALUATION OF SUSCEPTIBILITY OF M. TUBERCULOSIS TO SECOND LINE OF DRUGS

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ABSTRACT: Drug resistant tuberculosis is an emerging as a major challenge to tuberculosis control programme. With increased access to second line of drugs to treat these cases, in-vitro evaluation of susceptibility for some second line of drugs was undertaken. Study included 150 strains of Mycobacterium tuberculosis from newly diagnosed cases and 110 strains from patients with history of previous anti- tuberculosis treatment isolated from pulmonary tuberculosis cases. Drug susceptibility testing was done using Lowenstein –Jensen media by absolute concentration method. 170 strains were susceptible to all drugs. 80 strains showed resistance to any one or more primary drugs. 3.3% showed any resistance to Isoniazid (INH) and 4% to Rifampicin (RMP) among new cases where as it was 8.2% and 6.3% among the strains from previously treated cases. Multi - drug resistance was more commonly observed among previously treated cases (7.2%) as against newly diagnosed cases (1.3%). Any resistance to Ofloxacin among both groups was higher than that of primary drugs. i.e., 5% and 14.5%. Similarly resistance to Moxifloxacin was 6.6% and 8.2%. Any resistance observed to Amikacin and Cycloserine among two groups was (4%, 5.4%) and (5.3%, 4.5%) respectively. Analysis of resistant pattern to second line of drugs among 10 Multi drug resistant (MDR) strains and 170 strains, which were susceptible to all primary drugs showed that resistance to Ofloxacin and Moxifloxacin was higher (30%, and 20%) among MDR strains than resistance among susceptible strains. Resistance to Amikacin and Cycloserine among MDR and susceptible strains was not significantly different.
KEYWORDS: M. tuberculosis, –in vitro susceptibility, second line of drugs.

INTRODUCTION: Tuberculosis remains one of the major causes of death from single infectious agent worldwide. A total of 99% of the new cases occur in middle and low income countries. India ranks first in terms of absolute number of cases.(1) Management of this disease is through chemotherapy How, describe. But today there is great concern in the control of this disease due to emergence of drug resistance and HIV infection. Resistance of M. tuberculosis to anti-tuberculosis drugs is man-made amplification of natural phenomenon of spontaneous genetic event. Currently Multidrug resistant (MDR) tuberculosis accounts for 5.3% of all TB cases in the world, with the prevalence of initial and acquired resistance of 2.9% and 15.3% respectively.(2) In India, MDR among all new TB cases is 2.8% and among previously treated cases it is 17%.(3) Stop TB strategy launched by World Health Organization (WHO) in 2006 describes interventions that should be implemented to achieve the 2015 targets. Guided by Stop TB partnership working group on MDR-TB and Green Light committee recommends easy accessibility to second line of drugs (SLDs) at low cost to improve cure rate, sputum conversion, shorten the course and prevent the death. Various second line drugs include Fluoroquinolones, Aminoglycosides,

Macrolides, Beta lactams, Cycloserine etc.[4] But these drugs are not primarily anti tuberculosis drugs and they are extensively used for treating other infections like full form infections, soft tissue infections, blood stream infections etc.

With increased access to second line of drugs, their utility to treat MDR cases is increasing throughout the world. In India there is easy availability of antibiotics and their use is uncontrolled. Kobaidze K et al[5] has discussed the hazards of over the counter availability of anti-tuberculosis drugs in the state with high burden of tuberculosis. Most of the data available (complete the sentence and give references) on drug susceptibility to second line of drugs is from tertiary or reference centers, where mainly treatment failure cases are referred. This data does not reflect the true burden of the drug resistance. So we decided to evaluate some second line drugs in the laboratory, to analyze the susceptibility pattern of M. tuberculosis isolated from newly diagnosed patients and patients with previous history of anti -tuberculosis treatment in semi –urban area mainly catering to surrounding rural population. We have used Isoniazid (INH), Rifampicin (RMP), Ethambutol (EMB), among primary line of drugs. Reframe the sentence.

MATERIAL & METHODS: Give the address of the department and institution where work was done. Mention the study period. The present work was permitted by the institutional Ethical Committee. There were no human risks involved.

A total of 260 strains identified as M. tuberculosis, of which 150 were from newly diagnosed cases and 110 from patients with history of previous anti- tuberculosis treatment, isolated from cases of pulmonary tuberculosis were included. This sample size was calculated according to the formula $N = \{z^2\ 1\text{-}\ \alpha\ /2p\ (1\text{-}p)\} / d^2$[6]

Primary isolation of strains from sputum samples was done using Lowenstein – Jensen(LJ) medium. All these strains were identified as M. tuberculosis. Indirect susceptibility testing was done using absolute drug concentration method. Medium used was Lowenstein –Jensen with following pre- inspissation drug concentrations incorporated in it.

Drugs used	Concentrations of drugs Incorporated in LJ (µg/ml)					Critical concentration (µg/ml)
INH	0.1	0.5	1.0	2.0	4.0	1.0
RMP	16	32	64	128	256	64
EMB	02	04	08	16	32	4.0
STM	02	04	08	16	32	8.0
OFLOX	0.5	1.0	2.0	4.0	8.0	2.0
MOXI	0.125	0.25	0.5	1.0	2.0	0.5
AMIKACIN	0.5	1.0	2.0	4.0	8.0	1.0
CYCLOSERINE	10	20	30	40	50	30

Each batch of media was subjected to sterility testing before use. Pure form of drug was procured from Hi-Media, India.

Inoculum preparation: Fresh culture not more than 15 days old after isolating the strain was used. With 22 SWG (wire diameter - 0.7 mm) nichrome wire loop of 3 mm diameter was used to sweep representative growth, which weighed 2 mg in dry weight. Then it was discharged into 0.4 ml of sterile distilled water with six 2mm (is it 2mm or 3mm diameter?) diameter glass beads contained in 07 ml sterile Mac Cartney bottle. Suspension was prepared by shaking the bottle for one minute. Turbidity of suspension was compared with 0.5 Mac Farland tube.

Inoculation: Then using a 27 SWG (wire diameter- 0.4 mm) of 3mm diameter nichrome loop was used to take a loopful of suspension for inoculating the drug containing and drug free Lowenstein - Jensen media as standardized inoculum.

All the test strains were inoculated as above on various concentrations of drugs containing LJ and drug free LJ. In each batch one set of drug containing media was inoculated with standard $H_{37}R_v$ (ATCC 27294) strain. Inoculated media were incubated at 37° C. Slopes were observed daily for first week and twice weekly later on. Minimum Inhibitory Concentration (MIC) was recorded as the lowest drug concentration which inhibited 99% of the bacterial population growth as compared to drug free LJ. Preliminary readings were recorded after two weeks and final after four weeks. Strains showing no growth or few colonies were incubated up to six weeks as resistant strains are reported to grow slowly.[4, 7]

RESULTS: Among total 260 strains of M. tuberculosis, 170(65.38%) strains were susceptible to all drugs. 10 (3.8%) were MDR strains 80 (30.76%) showed resistance to one or more drugs.

In our study of resistance profile of 150 strains of M. tuberculosis isolated from newly diagnosed cases, 3.3% showed (any)? what do you mean by any resistance to INH and 4% to RMP, where as it was 8.2% and 6.3% among the strains from previously treated cases. This difference was statistically insignificant.

We did not observe any resistance to Ethambutol in newly diagnosed cases and only one strain (0.9%) showed resistance among previously treated cases.

Any resistance to Ofloxacin among both groups was higher than that of primary drugs. i.e., 5% and 14.5%. Even though any resistance to new Fluoroquinolone Moxifloxacin was lower (6.6% and 8.2%), cross resistance with Ofloxacin was observed. Any resistance to Amikacin and Cycloserine among both study groups was similar.

We observed higher MDR strains among previously treated cases (7.2%) than newly diagnosed cases (1.3%), which was statistically significant.

Analysis of resistant pattern to second line of drugs among 10 MDR strains and 170 strains**,** which were susceptible to all primary drugs showed that resistance to Oflaxacin and Moxiflxacin was higher (30%**,** and 20%) among MDR strains than resistance among susceptible strains**,** it was statistically insignificant. Similarly no statistical significant difference was observed in resistance to Amikacin and Cycloserine among MDR strains and susceptible strains.

DISCUSSION: Increasing threat of multidrug resistant tuberculosis (MDR-tb) has been documented in two worldwide surveys conducted by WHO and (full form) IUTLD. Resistance to first line of drugs is wide spread with MDR hotspots in many parts of the world including India. Spread of MDR-TB can be prevented only if patients with MDR-tb are identified and treated with effective drugs. Reframe the sentence. In India there is a high burden of tuberculosis with easy accessibility of all SLDs,[8] so we can know the resistant profile of M. tuberculosis in each geographical area.

Our observation of any resistance to INH and RMP is less than that reported by Jeon CY et al,[9] who has reported 18%, 72% for INH and 10% and 67% for RMP in newly diagnosed and previously treated cases respectively. Population based study from Georgia has reported initial MDR-Tb of 7% and acquired MDR-Tb- 27%.[10] Ramchandran R. et al also has reported any resistance of INH to be 11% and 37% among new and patients with previous treatment.[11]

WHO data on India shows MDR to be 2.8% and 17% among new and previously treated cases. Migliori GB et al in their data from Europe have reported initial and acquired MDR of 7.9% and 49.3%.[12] Similarly even from South Korea, MDR of 11% is reported.[13] Ramchandran R et.al[11] also has reported MDR of 2.4% and 17.4% among new and previously treated cases respectively, But our observation of MDR in two study groups is lower may be because most of these studies show the data of reference laboratories, where as ours is from a district place catering to patients referred from surrounding rural areas.

In 1990s many studies reported usefulness of Fluoroquinolones to treat tuberculosis. But recently study from John Hopkins university School of Medicine that even newly diagnosed TB patients might have Fluoroquinlone resistance as the result of their wide use for bacterial infections [14]. But Jeon et al[8] has reported higher resistance of 42% and 66% among newly diagnosed and previously treated cases to Ofloxacin. Any resistance observed to Cycloserine in the study from Canada shows 2% in new cases, which is lower than ours.[15]

Resistance profile of MDR strains to Ofloxacin by Sharma SK et al[16] is 9%, which is lower than ours and that of Ramchandran R et al[10] is 24%, which is similar to our observation. Fluoroquinolone resistance data from Canada shows any resistance to Ofloxacin ranging from 9% in 2005, 10% in 2002 and 42% in 2004, which consistent with our is observation.[15]

Resistance of MDR strains to aminoglycoside drug Amikacin varies from different reports. Migliori GB et al[17] has reported that 15% of MDR strains are resistant to Amikacin, whereas Moadab SR et al[18] has reported that only 5% of MDR strains to be resistant to Amikacin. Data from Canada shows that MDR strains show resistance of 6% in 2002, 16% in 2006.[15] Thus there is wide variation in resistant pattern of our observation and other reports.

In our study none of the strains showed resistance to any Fluoroquinolone and also Amikacin to label as XDR (full form) strain.

Guided by Stop-TB working group on MDR-TB and Green Light committee concurrent efforts by various private and public organizations focus on confronting challenges of drug resistant TB, and sharing information and strategies is an unprecedented collaborative way. Most of the SLD resistant data is available from developed countries, and from Reference Laboratories. In Country like India, where SLDs are available over the counter we need to study SLD resistant profile at the grass root level before wide spread use of these drugs.

CONCLUSION:

- MDR and XDR M. tuberculosis are emerging having negative impact on Tuberculosis control programme.
- Second line of drugs recommended by Green Light committee is extensively used in India to treat infections other than tuberculosis.
- To analyse the utility of second line of drugs burden of MDR in the community must be studied, which was 3.8% in our study.
- In-vitro evaluation of second line drugs against M. tuberculosis strains isolated is useful guide to select appropriate combination of drugs.
- In our study any resistance observed to quinolones is very high (for Ofloxacin initial and acquired resistance was 9.3% and 14.5% respectively.
- Cross resistance was observed between Ofloxacin and Moxifloxacin.
- Initial and acquired resistance to Amikacin, cCycloserine was also high.

REFERENCES:

1. WHO report 2008. Global tuberculosis control – surveillance, planning, financing. WHO>Programmes and projects>Tuberculosis(TB)>WHO publications on tuberculosis> Global tuberculosis control-epidemiology, strategy, financing > Global tuberculosis control – surveillance, planning, financing.
2. Anti-tuberculosis drug resistance in the world. Fourth global report. The WHO /IUATLD Global project on Anti-tuberculosis drug resistance surveillance; WHO, Geneva 2008. WHO/HTM/TB/2008. 394: 10-12.
3. Country profile, India. Global tuberculosis control. WHO report. 2008: 109.
4. Policy guidance on drug susceptibility testing (DST) of second –line antituberculosis drugs. WHO, Geneva 2008. WHO/HTM/2008.392: 8.
5. Kobaidze K, Salakaia A, Blumberg HM. Over the counter availability of Antituberculosis drugs in Tbilisi, Georgia in the setting of a high prevalence of MDR-TB. Interdisciplinary Perspectives on Infectious Disease. 2009. Doi; 10.1155/2009/513609.
6. Lwanga SK, Lemeshow S. Sample size determination in health studies. A practical manual. WHO, Geneva, 25, 1991.
7. Canetti G, Wallace F, Khomenko A, Mahler HT, Menon NK, Mitchison DA, Rist N, Smelev NA. Advances in Techniques of testing Mycobacterial Drug sensitivity and the use of sensitivity tests in Tuberculosis Control Programmes. Bull Org. mond. Sante. Bull WHO 1969; (41): 21-43.
8. Rajeshwari R, Balsubramanian R, Bose MSC, Sekar L, Rahman F. Private pharmacies in tuberculosis control - a neglected link. Int J Tuberc Lung Dis 2002; (6): 171-173.
9. Jeon CY, Hwang SH, Min JH, Prevots DR, Goldfeder LC, Lee H, et al Extensively Drug resistant Tuberculosis in S. Korea: Risk factors and treatment outcomes among patients at a tertiary referral hospital. Clin Infec Dis 2008(46): 42-49.
10. Lomtadze N, Aspindzelashvili R, Janjgava M. et. Al. Prevalence and risk factors for multidrug – resistant tuberculosis in the Republic of Georgia: a population based study. Int J Tuberc Lung Dis 2009; (13): 68-73.

11. Ramchandran R, Nalini S, Chandrasekar V, Dave PV, Sanghavi AS, Paramsivan CN, Narayan PR, et al Surveillance of drug resistant tuberculosis in the state of Gujrat, India. Int J Tuberc Lung Dis. (2009); 13(9)1154-1160.
12. Migliori GB, Besozzi G, Girardi E, Kilman K, Lange C, Toungoussova OS et al. Clinical and operational value of the extensively drug - resistant tuberculosis definition. Eur Respir J, 2007; 30(4) 623-626.
13. Emergence of M. tuberculosis with extensive resistance to second – Line Drugs- Worldwide, 2000-2004. Moebidity & Mortality Weekly Report.2006; 55 (11) 301-305.
14. Barclay L. Widespread use of Fluoroquinolones may be creating resistant TB strains. Am J Respir Crit Care Med 2009; (180): 287-289.
15. Tuberculosis: Drug resistance in Canada -2007.
http: //www.phac-aspc.gc.ca/tbpc-latb/index-eng..php.
16. Sharma SK, George N, Kadhiravan T, Saha PK, Mishra HK, Hanif M. Prevalence of extensively drug resistant tuberculosis among patients with multidrug – resistant tuberculosis: a retrospective hospital –based study. Ind J Med Res 2009; 130(4),: 392-395.
17. Migliori GB, Lange C, Centis R, Sotgiu G, Mutterlein R, Hoffmann H, Kliiman K, Laco GDe, Richardson MD, Spanevello A, cirillo DM, Resistance to second – line injectables and treatment outcomes in multidrug resistant and extensively drug resistant tuberculosis cases. Eur Respir J 2008; 31(6): 1155-1159.
18. Moadab SR, Rafi AAN. Susceptibility of Mycobacterium tuberculosis and Nontuberculosis Mycobacteria to Kanamycin, Amikacin. Pharm Scie Wint 2006(4): 47-54.

Drug	Any resistance among isolates from newly diagnosed cases. (150)	Any resistance among isolates from cases of previous treatment. (110)	Statistical significance*
INH	05 (3.3%)	09 (8.2%)	p>0.05
RMP	06 (4%)	07 (6.3%)	P> 0.05
EMB	00	01 (0.9%)	--
STM	03 (2%)	05 (4.5 %)	p>0.05
OFLOX	14 (9.3%)	16 (14.5 %)	p>0.05
MOXIFLOX	10 (6.6%)	09 (8.2 %)	p>0.05
AMIKACIN	06 (4%)	06 (5.4%)	p>0.05
CYCLOSERINE	08 (5.3 %)	05 (4.5 %)	p>0.05
MDR	02 (1.3%)	08 (7.2%)	P<0.05.

Table 1: Showing resistance among M. tuberculosis to individual drug

(* Statistical significance was calculated using Fischer exact test)
For any level resistance, give numerical range

	Any resistance			
	OFLOX	MOXIFLOX	AMIKACIN	CYCLOSERINE
MDR (10)	03 (30%)	02 (20%)	01 (10%)	01 (10%)
Susceptible strains (170)	27 (15.9%)	17 (10%)	11 (6.5%)	12 (7.1%)
Statistical significance*	P >0.05	P >0.05	P >0.05	P >0.05

Table 2: showing resistance to second line drugs among MDR and susceptible strains

(* Statistical significance is calculated using Fischer exact test.)

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