INTERNATIONAL JOURNAL OF MEDICAL AND APPLIED SCIENCES
Earthjournals Publisher
E-ISSN:2320-3137
www.earthjournals.org

RESEARCH ARTICLE

PREVALENCE OF DRUG RESISTANT TUBERCULOSIS IN RURAL GUJARAT

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ABSTRACT:
Background- Multi-drug resistant tuberculosis (MDR-TB) has emerged as a significant global health concern especially in developing countries like India. Increase in the drug resistance to commonly used drugs like rifampicin and isoniazid is a major cause of concern and is detrimental to the control of tuberculosis especially in a developing country like India. Limited data is available on the trends of prevalence of drug resistance in Gujarat state. The present study was undertaken to find prevalence of drug resistance in a tertiary care hospital catering to the rural community of Gujarat.
Materials & Method- In the present study, a total of 400 patients with pulmonary tuberculosis attending a tertiary care hospital catering to the rural population, were enrolled from January 2006 to December 2009. All the 90 Newly diagnosed patients and 310 Retreatment cases were subjected to sputum smear microscopy for acid fast bacilli (AFB) by Zeihl Neelsen method and inoculated onto the slants of Lowenstein-Jensen's (LJ) medium after decontamination. The positive cultures were evaluated for drug susceptibility pattern by Modified Proportion method on Lowenstein Jensen medium incorporated with individual first line ant tubercular drugs of recommended specified strength.
Results- The prevalence of MDR-TB in **new cases** was **3.3% (3/90)** and in **previously treated cases** was **47.2% (111/235)** in our study. Resistance to Rifampicin alone was low **4.4% (4/90)** in newly diagnosed cases whereas in previously treated cases, Rifampicin resistance was alarmingly high **52.7% (124/235).**
C**onclusion-** Our findings carry a significant importance as there has been a scarce data on the prevalence of MDR-TB in the rural population of India. This study also underlines the importance of continuous monitoring of the trends in drug resistance, which would provide useful inputs for shaping future policies to prevent the emergence and dissemination of MDR tuberculosis.

KEY WORDS: Tuberculosis, Multi-drug resistant (MDR), Prevalence. Previously treated cases. Rifampicin , RNTCP.

INTRODUCTION

Tuberculosis (TB) remains a major global health problem. It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). The majority of cases worldwide in 2012 were in the South-East Asia (29%), African (27%) and Western Pacific (19%) regions. India and China alone account for 26% and 12% of total cases, respectively. There were 8.6 million new TB cases and 1.3 million TB deaths in 2012. [1]

Multi-drug resistant tuberculosis (MDR-TB) has emerged as a significant global health concern. There are alarming reports of increasing drug resistance from various parts of the globe which is detrimental to the success of tuberculosis control program. [2] The level of drug resistance provides an

important indicator to assess the magnitude of the transmission of infection in the community.[3] With the widespread use of rifampicin, acquired resistance to rifampicin has been on the rise. [4] Due to high bactericidal and sterilizing action, Rifampicin along with Isoniazid forms the backbone of first line short course chemotherapy. [5,6] Increase in the drug resistance to commonly used drugs like Rifampicin and Isoniazid is a major cause of concern and is detrimental to the control of tuberculosis especially in a developing country like India. Limited data is available on the trends of prevalence of drug resistance in Gujarat. The present study was undertaken to find prevalence of drug resistance among the patients attending one of the tertiary care teaching hospitals catering mainly to the rural community of Gujarat.

MATERIALS AND METHODS

This study was conducted in a rural based tertiary care teaching hospital, serving the rural population of Gujarat state. The study protocol was approved by the Human Research Ethics Committee (HREC) of the Institute. A written informed consent was taken from each patient for inclusion in the study. Four hundred (n=400) clinically suspected patients with pulmonary tuberculosis attending the Shree Krishna Hospital were enrolled from January 2006 to December 2009. A detailed history of these patients including age, sex, occupation, address and previous history of anti-Koch's treatment were recorded. Based on the prior history of drug intake, cases were classified into untreated newly diagnosed cases and previously treated cases which included treatment failure cases, relapse cases and treatment after default (TAD) cases as per the RNTCP guidelines. Out of total 400 patients, 90 (22.5%) patients were newly diagnosed cases of pulmonary tuberculosis. The remaining 310 (77.5%) patients were previously treated cases further classified into Relapse, treatment failure and treatment after default (TAD) cases as per the RNTCP guidelines. Sputum smear microscopy for acid fast bacilli (AFB) was carried out by Zeihl Neelsen method. All sputum specimens were decontaminated by Modified Petroff's method using 4% NaOH. After the decontamination process, sediment was inoculated onto the slants of Lowenstein-Jensen's (LJ) medium and incubated for 8 weeks. *M. tuberculosis* isolates were identified according to standard criteria. [7] The positive cultures were evaluated for drug susceptibility pattern by Modified Proportion method. [8] on Lowenstein Jensen medium incorporated with individual first line ant tubercular drugs of recommended specified strength.(**Rifampicin-40 µg/ml, Isoniazid-1.0 µg/ml, Ethambutol-2.0µg/ml, Streptomycin-4.0 µg/ml).** Sensitivity testing to Pyrazinamide was not done due to a constraint of maintaining acidic pH of the culture media. The standard reference strain H_{37} RV was used as control strain.

RESULTS

Mycobacterial growth was isolated in all the 90 sputum samples from the newly diagnosed cases. Among the previously treated cases, **75.8% (235 sputum samples out of 310 cases)** showed mycobacterial growth. All the strains were identified as *M. tuberculosis.* Pattern of Primary drug resistance in newly diagnosed cases is shown in **Table 1**.

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E-ISSN:2320-3137
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TABLE- 1 Pattern of Drug Resistance in Newly Diagnosed Cases (n=90)

TOTAL SAMPLES TESTED	NO. of STRAINS RESISTANT TO			TOTAL RESISTANT STRAINS
90	1 DRUG	2 DRUGS	3 DRUGS	**18 (20 %)**
	S-3	***HR-3**	SHE-1	
	H-4	HE-1		
	E-2	SH-3		
	R-1			
SUB TOTAL	**10 (11.1%)**	**7 (7.7%)**	**1 (1.1%)**	

*** Multi- drug resistance in new cases -3.3% (3/90)**

S-Streptomycin, H-Isoniazid, E-Ethambutol, R-Rifampicin

Among the isolates from newly diagnosed cases, 18 strains out of the 90 cases (20%) were found to be resistant to one or more drugs. Resistance to Rifampicin and Isoniazid with or without resistance to other drugs was detected in 3 /90 (3.3%). **The prevalence of MDR-TB in newly diagnosed cases in our study was 3.3%** (Table-1). The prevalence of drug resistance to one drug either as a mono drug or together with other drugs was as follows- **Rifampicin in 4/90 (4.4%),** Isoniazid in 12/90 (13.3%), Streptomycin in 7/90 (7.7%) and Ethambutol in 4/90 (4.4%) **(Table-2)** .

TABLE-2 Drug Resistance to One Drug with or without Resistance to other Drugs in Newly Diagnosed Cases (n = 90)

TOTAL SAMPLES TESTED	NO. Of STRAINS RESISTANT TO EITHER SINGLE OR COMBINED WITH OTHERS.			
90	**S**	**H**	E	R
	7 (7.77%)	12 (13.33%)	4 (4.44%)	4 (4.44%)

S-Streptomycin, H-Isoniazid, E-Ethambutol, R-Rifampicin

Among 235 retreatment cases, 167 (71.06 %) strains were found to be resistant to one or more drugs. Resistance to Isoniazid and Rifampicin with or without resistance to other drugs was found in 111 (47.2 %) . **The prevalence of MDR-TB in previously treated cases was found to be significantly high, 47.2% (111/235) (Table-3).**

TABLE-3Pattern of Drug Resistance in Retreatment Cases (n = 235)

NO (Samples Tested)	**Number of Strains Resistant to**				**Resistant Strains (%)**
235	**1 DRUG**	**2 DRUGS**	**3 DRUGS**	**4DRUGS**	**167 (71.06%)**
	S-09	SH-06	SHE-02	***SHER-26**	
	H-13	SE-03	***SHR-32**		
	E-04	SR-01	SER-01		
	R-09	HE-06	***HER-17**		
		***HR-36**			
		ER-02			
TOTAL	**35 (14.89 %)**	**54 (22.97 %)**	**52 (22.12 %)**	**26 (11.06 %)**	

***Multi-drug resistance in previously treated cases- 47.23% (111/235)**

S-Streptomycin, H-Isoniazid, E-Ethambutol, R-Rifampicin

In the retreatment group (n=235), prevalence of drug resistance to one drug either as a mono drug or together with other drugs was as follows - Streptomycin 80/235 (30.04%), Isoniazid 138/235 (58.72%), Ethambutol in 6/235(25.95%) and **Rifampicin in 124/235 (52.76%). (Table-4)**

TABLE-4 Drug Resistance to One Drug with or without Resistance to other Drugs in Retreatment Cases (n = 235)

NO (Samples Tested)	**Number of Strains Resistant to Mono-Drug or more Drugs**			
235	**S**	**H**	**E**	**R**
	80 (34.04 %)	138 (58.72 %)	61 (25.95 %)	124 (52.76 %)

DISCUSSION

Rifampicin, first introduced in 1972 as an ant tubercular drug, is extremely effective against TB bacilli and has brought in a revolution in the management of Tuberculosis. Rifampicin targets mycobacteria by binding to the mycobacterial DNA dependent RNA polymerase and thereby kills the organism by interfering in the transcription process. Due to its high bactericidal and sterilizing action, Rifampicin along with Isoniazid forms the backbone of first line and short course chemotherapy.[5,6] Increase in the drug resistance to commonly used drugs like Rifampicin and Isoniazid is a major cause of concern and is detrimental to the control of tuberculosis especially in a developing country like India.

Though drug resistance in new cases is found to be low in developed countries, it is common in India and varies widely from area to area. Trivedi et al. [9] from Gujarat reported in 1986 that there was no drug resistance (0%) to Rifampicin in new cases while in the present study, drug resistance to Rifampicin in new cases was 4.4% **(Table-5).** In the present study, the drug resistance to Isoniazid in new cases was 13.3% which is lower than that reported in several other studies; Chandrasekharan et al (17.4% & 32.8%). [10,11], Paramsivan et al (15.4%). [12], Gupta et al (19.5%). [13] and Rajendra Prasad et al (15.6%)[17] **(Table-5)**. This shows that drug resistance to Rifampicin in new cases has been rising in Gujarat over a period of time. The prevalence of MDR TB in newly diagnosed cases in the present study was 3.3% which is lower than that reported by R.Prasad et al (5%)[17] and Malhotra et al (4.5%) [19] but higher than that reported by Paramsivan et al (2.5%) [18], Sofia et al (2.2%). [20] **(Table-5)**.

Table- 5 Drug Resistance in New Cases in India

Study		Total Prevalence	H %	S %	R %	H+R%
Trivedi et al (1988) [9]		20	13.9	7.4	0	0
Chandrasekharan et al (1990) [10]		21.2	17.4	5.7	3	1.3
Chandrasekharan et al (1992)[11]	Rural	34.9	32.8	5.1	4.4	3.4
	Urban	20.5	17.3	4.1	2.9	1.4
Parmasivan et al (1993)[12]	North Arcot (1985-89)	25	13	4	2	1.6
	Pondicherry (1985-91)	13.9	6	4	0.9	0.7
Gupta et al (1993)[13]		19.5	10.1	7.6	3	0.7
Jain et al (1993)[14]		-	18.5	-	0.6	0.4
Jena et al (1996) . [15]		7.9	2.9	4.9	1	0.4
Parmasivan et al (2000) . [16] (Tamil Nadu)		18.8	15.4	6.8	4.4	3.4
R.Prasad et al (2001) .[17]		27.4	15.6	11	3.9	5
Paramasivan et al (2002) . [18]	North Arcot (South) 1999	27.7	23.4	12	2.8	2.8
	Raichur (South) 1999-2000	21.9	18.7	7.2	2.5	2.5
Malhotra et al . [19]	Jaipur2002	-	13.6	-	6.8	4.5
Sofia et al(2004) . [20]	Bangalore city	27.7	13.7	22	2.6	2.2
Mahadeo et al. (2005)[21]	Mayurbhanj 2000-2002	5.3	2.5	3.9	0.7	0.7
	Hoogli 2000-2001	16.7	10.3	13	3	3
Zignol M, et al (2006) . [22]	-	-	-	-	-	2.4
Present Study	**2006-2009**	**20**	**13.33**	**7.77**	**4.44**	**3.3**

Although there is a very limited data available on the prevalence of drug resistance to Isoniazid and Rifampicin in previously treated cases, it is invariably higher than drug resistance in new cases. In the present study , the resistance to Isoniazid in previously treated cases was high (58.7%) followed by Rifampicin resistance (52.7%) which is comparable to the prevalence reported in different studies carried out in other parts of the country **(Table-6).**

Table-6 Drug Resistance in Previously treated cases in India

Study	**Total Prevalence**	**H %**	**S %**	**R %**	**H+R%**
Trivedi et al (1988) [9]					
1980	50.1	34.5	26	2.8	95% of Resistant were resistant to H or S or both
1986	65.3	55.8	-	37.3	-
Dutta M et al.1993 [23]	-	7	26	12	6
Jain et al (1992) [14] Dehli	50.07	50.07	-	33.3	33.3
Outside Delhi	78.8	78.8	-	61.5	61.5
Chowgule et al (1998) [24]	25.6	15	53.6	66.8	10.7
Shah AR et al (2002) [25]	58.6	97.5	57.26	63.9	56.2
Deivanayagam (2002) [26]	71	66.3	35.6	55.5	54.8
Paramasivan et al (2002)[18] North Arcot(South)	81	81	56.2	69	69
Raichur (South)	100	100	36.4	100	100
Malhotra et al 2002[19]	-	39.7	-	28.2	24.3
R.Prasad et al (2003)[27]	79.2	48.6	36.6	34.4	29.5
Hanif et al 2006[28]	52	52	14.2	47.1	47.1
Present Study	**71.06**	**58.7**	**34**	**52.7**	**47.23**

Trivedi et al[9] from Gujarat (1980-1986), reported resistance to Isoniazid as 55.8 %, and resistance to Rifampicin as 37.3 %. Shah et al[28] from Gujarat (2000- 2001) reported highest resistance to Isoniazid (97.5%) followed by Rifampicin (63.9%). This shows that resistance to first line anti-tuberculosis drugs in Gujarat is increasing.

The prevalence of MDR-TB in previously treated cases was found to be significantly high, 47.2%. However it was lower than that reported by Shah et al[25](53.6%) from Gujarat in 2002

(Table6). It could be attributed to the fact that our hospital is a tertiary care referral hospital and most of the patients are referred to the hospital due to unresponsiveness to the conventional Anti-Koch's treatment. Since such patients have already been exposed to antituberculosis agents, they are at high risk for harboring multi-drug resistant strains.

CONCLUSION

Our findings carry a significant importance as there has been a scarce data on the prevalence of MDR-TB in the rural population of India. This study also underlines the importance of continuous monitoring of the trends in drug resistance, which would provide useful inputs for shaping future policies to prevent the emergence and dissemination of MDR tuberculosis. Moreover, our study is a prospective study conducted over a period of four years in a tertiary care referral Centre. Although newer drugs for tuberculosis are there in the pipeline, but availability of those drugs is still a distant dream. Hence the key to success remain in adequate case finding, prompt and correct diagnosis and effective treatment.

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