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ORIGINAL ARTICLE

DRUG RESISTANCE PATTERN AMONG PATIENTS WITH TUBERCULOSIS- A CLINICAL STUDY

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ABSTRACT:
Background: Tuberculosis (TB) is a contagious disease caused by the bacillus Mycobacterium (M.) tuberculosis and occasionally by Mycobacterium bovis, and Mycobacterium africanum. There are cases of drug-resistant TB throughout the world. Resistance of M. tuberculosis to drugs is a man-made amplification of spontaneous mutations in the genes of the tubercle bacilli. The present study was to conducted to evaluate the resistance of combinations of isoniazid (INH), rifampicin (RMP), streptomycin (SM), and ethambutol (EMB) and four resistant modes, i.e. mono, double, triple and quadruple resistance among cases of pulmonary tuberculosis patients. **Materials & Methods:** This study was conducted in Directly Observed Treatment Short-course (DOTS) centre in year 2014. It consisted of 200 newly diagnosed Sputum smear positive (SS+) for acid fast bacilli (AFB) pulmonary tuberculosis patients of both sexes and between the age group of 15 to 60 years. AFB culture and drug susceptibility test were done. Drug resistance was expressed in proportion method, where a strain was considered to be drug resistant if the number of colonies that grew on a drug containing medium was 1% or more of the colonies. Chest radiographs were taken of all the patients at the time of diagnosis of TB at the end of six-month treatment. Patients were evaluated by judging the site of lesions, zone of involvement, nature of the lesion (visible cavitary and non-cavitary area) in both lungs. Results were tabulated and subjected for correct inferences. P value < 0.05 was considered significant. **Results:** Out of 40 patients, 110 were males and 90 were females. Age group 15-30 years had males (50) and females (40). Age group 30-45 years had males (25) and females (35). Age group 45-60 years had males (35) and females (15). Sputum positivity grade was 1+ (60), 2+ (40), 3+ (45) or scanty (55). The difference was non - significant (P > 0.05). Socio economic status of patients was upper (2), upper middle (4), lower middle (4), upper lower (150) and lower middle (40). The difference was significant (P < 0.05). Lesions were cavitatory (36) and non cavitatory (164). The difference was significant (P <0.05). Culture on LJ medium was growth of bacteria in 185 cases, myobacteria other than tuberculosis in 3 cases, contamination in 10 cases and no growth of mycobacteria in 2 cases. Number of sensitive strains of rifampicin was 195, isoniazide (160), streptomycin (190), ethambutol (189) and resistant strains of rifampicin was 5, isoniazide (40), streptomycin (10), ethambutol (11). The difference of sensitive strains among all 4 drugs was non - significant (P > 0.05). Four most frequent drug resistance patterns of 21.3% strains of M. tuberculosis from mono drug, double drug, triple drug and quadruple drug resistance were 5.9%, 10.7%, 2.4% and 2.4% respectively. MDR was observed in 4.7% isolates. **Conclusion:** Drug resistant tuberculosis cases are now increasing day by day. It poses challenge to the treatment. Identification of resistant strains helps in proper management of cases of tuberculosis.
Key words: Drug resistant, Rifampicin, Streptomycin, Tuberculosis

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INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by the bacillus Mycobacterium tuberculosis and occasionally by Mycobacterium bovis, and Mycobacterium africanum. It is the most common infectious disease causing deaths in humans. TB is also more common among men than women, and affects mostly adults in the economically productive age groups; around two-thirds of cases are estimated to occur among people aged 15–59 years. TB is presently a global epidemic with over two billion people, equal to one third of the world's population currently estimated to be infected, with 8.8 million new TB cases identified worldwide and 1.4 million deaths annually.[1]

Only 5-10% of the people infected will be sick or infectious at any point of time, in the remaining the disease

being latent. TB is still a global public health problem since the World Health Organization (WHO) declared the disease a global emergency in 1993. Moreover, about 100,000 women are rejected by their families each year because of TB, strongly impacting their children and families. In India alone, 30,000 children leave school annually, on account of their parents' TB.[2]

In 2008, WHO declared that there were 9.2 million TB cases per annum with 41% sputum smear positive (SS+). India reported the largest number of incident cases (2.0-2.5 million) which alone accounted for an estimated one quarter (26%) of all TB cases worldwide.

There are cases of drug-resistant TB throughout the world. Resistance of M. tuberculosis to drugs is a man-made amplification of spontaneous mutations in the genes of the tubercle bacilli. Multiplication of drug-resistant strains develops due to irregular drug supply, inappropriate prescription, or poor adherence to treatment. Because drug resistance develops due to inadequate use of drugs, anti-tuberculosis drug resistance surveillance is, together with the monitoring of treatment outcome, an essential tool for evaluating the quality of tuberculosis control programmes, lack of laboratory resources and rapid accurate point-of-care tests. To avoid develop resistant against TB, an accurate and rapid diagnosis of TB is of paramount importance in establishing appropriate clinical management and infection control measures.[3]

The present study was to conducted to evaluate the resistance of combinations of isoniazid (INH), rifampicin (RMP), streptomycin (SM), and ethambutol (EMB) and four resistant modes, i.e. mono, double, triple and quadruple resistance among cases of pulmonary tuberculosis patients.

MATERIALS & METHODS

This study was conducted in Directly Observed Treatment Short-course (DOTS) centre in year 2014. It consisted of 200 newly diagnosed Sputum smear positive (SS+) for acid fast bacilli (AFB) pulmonary tuberculosis patients of both sexes and between the age group of 15 to 60 years. Patients were informed regarding the study and written consent was taken.

Patients with previous history of Anti-Tuberculosis Treatment (ATT); pregnant and lactating women; subjects known to be HIV positive/ or suffering from any immuno-deficient state; and use of corticosteroids or supplements containing Vitamin A, zinc, iron, etc. during the previous month were excluded from the study.

Patient information such as name, age, gender etc. was recorded. Clinical symptoms such as fever, cough, expectoration, chest pain breathlessness, wheezing, haemoptysis, dyspnea, night sweat, loss or improve of appetite and weight loss or gain were recorded.

AFB culture and drug susceptibility test were done.

Three sputum specimens on two consecutive days from each patient were collected in properly labelled screw capped, sterile disposable plastic bottles after oral gargling with normal water. Thus, there were three samples: spot, early morning and spot. Specimens contained mucoid or mucopurulent material with minimum amounts of oral or nasal material into the McCartney bottles and volume was of approximately 5ml. AFB smear examination was carried out by direct microscopy using the Ziehl Neelsen (ZN) method. Sputum smear result was examined and interpreted according to the AFB grading.

Culture examinations were done on all diagnostic specimens, regardless of AFB smear positivity. Sputum specimens from each patient were processed with sodium hydroxide (NaOH) method- Modified Petroff's procedure and cultured on Lowenstein-Jensen (LJ) slopes. All inoculated LJ drug and control media were incubated at 37ºC. All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Thereafter, cultures were examined weekly, up to eight weeks on a specified day of the week. Typical colonies of M. tuberculosis were rough, crumbly, waxy, non-pigmented (buffcoloured) and slow-growers, i.e., only appeared two to three weeks after inoculation. The colony was confirmed by ZN staining. Detection time for MOTT was 25 days. M. tuberculosis positive strains were culture negative when they grew on p-nitro benzoate (PNB) containing medium. Only a few colonies of non-tuberculous Mycobacteria (NTM – often pigmented, with smooth morphology or PNB positive) were grown as visible colonies on PNB containing medium. Drug resistance was expressed in proportion method, where a strain was considered to be drug resistant if the number of colonies that grew on a drug containing medium was 1% or more of the colonies that grew on a control drug free medium. The control (drug free) medium showed good growth at least 50 to 100 colonies.

Chest radiographs were taken of all the patients at the time of diagnosis of TB at the end of six-month treatment. Patients were evaluated by judging the site of lesions, zone of involvement, nature of the lesion in both lungs. Results were tabulated and subjected for correct inferences. P value < 0.05 was considered significant.

RESULTS

Table I shows that out of 40 patients, 110 were males and 90 were females. The difference was non- significant ($P > 0.05$). Table II shows that age group 15-30 years had males (50) and females (40). Age group 30-45 years had males (25) and females (35). Age group 45-60 years had males (35) and females (15). The difference was non- significant ($P > 0.05$). Graph I shows that sputum positivity grade was 1+ (60), 2+ (40), 3+ (45) or scanty (55). The difference was non - significant ($P > 0.05$). Graph II shows socio economic status of patients. It was upper (2), upper middle (4), lower middle (4), upper lower (150) and lower middle (40). The difference was significant ($P < 0.05$). Lesions

were cavitatory (36) and non cavitatory (164). The difference was significant (P <0.05) (Graph III).
Graph IV shows result of culture on LJ medium. It was growth of bacteria in 185 cases, myobacteria other than tuberculosis in 3 cases, contamination in 10 cases and no growth of mycobacteria in 2 cases. Graph V shows sensitivity pattern of M. tuberculosis. Number of sensitive strains of rifampicin was 195, isoniazide (160), streptomycin (190), ethambutol (189) and resistant strains of rifampicin was 5, isoniazide (40), streptomycin (10), ethambutol (11). The difference of sensitive strains among all 4 drugs was non - significant (P > 0.05).
Table III shows that among new cases, four most frequent drug resistance patterns of 21.3% strains of M. tuberculosis from mono drug, double drug, triple drug and quadruple drug resistance were 5.9%, 10.7%, 2.4% and 2.4% respectively. MDR was observed in 4.7% isolates.

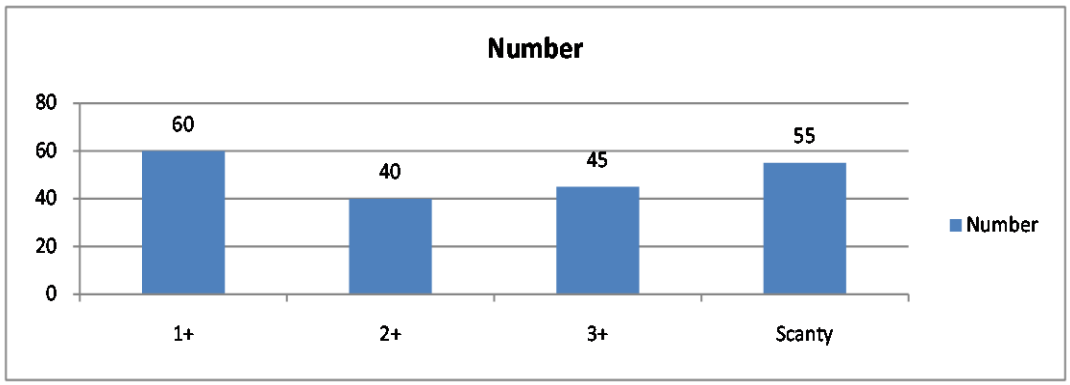
Table I Distribution of patients

Total - 40		
Male	**Female**	**P value**
110	90	0.2

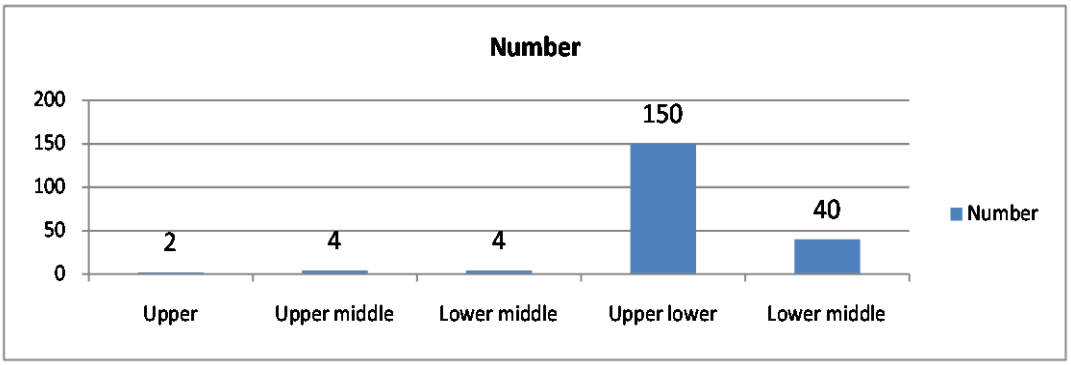
Table II Distribution of patients on the basis of age

Age group	Males	Females
15-30	50	40
30-45	25	35
45-60	35	15
Total	**110**	**90**

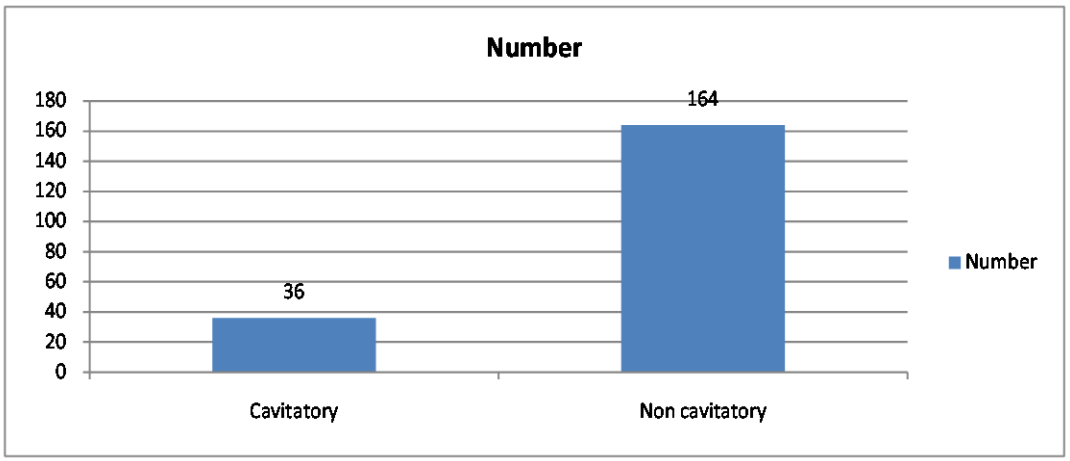
Graph I AFB positivity grade



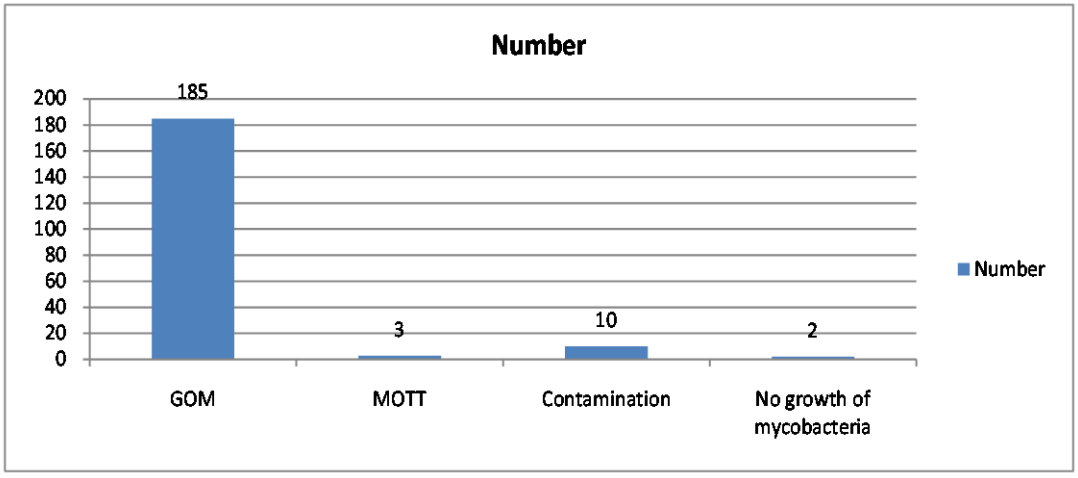
Graph II Socio economic status of patients



Graph III Radiographic lesion on chest radiograph



Graph IV Result of culture on LJ medium



Graph V Sensitivity pattern of M. tuberculosis to four anti-tuberculosis drugs in LJ medium by proportion method

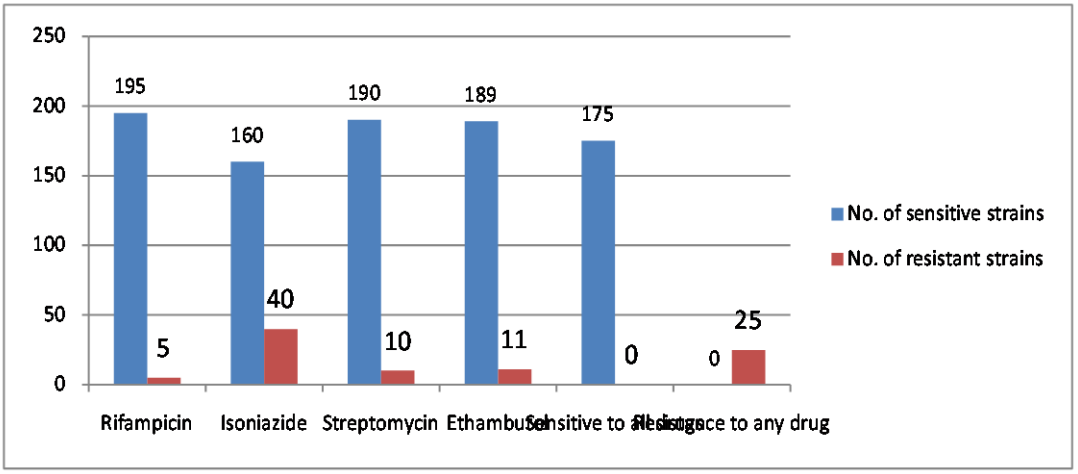


Table III Resistance pattern of 36 drug resistant strains of *M.* tuberculosis to four anti-tuberculosis drugs

Drug resistance pattern	Names of drugs	Number of resistant strains	Total
Mono drug resistance	Isoniazide (INH)	5	10
	Rifampicin (RMP)	2	
	Streptomycin (SM)	2	
	Ethambutol (EMB)	1	
Double drug resistance	INH+ RMP	2	19
	INH+SM	8	
	INH+EMB	7	
	EMB+SM	2	
Triple drug resistance	INH+RMP+SM	2	4
	INH+RMP+EMB	1	
	INH+RMP+SM+EMB	1	
Quadruple drug resistance	INH+RMP+SM+EMB	4	4
MDR			8

DISCUSSION

Surveillance and analysis of local rates of TB drug resistance are helpful in the detection and monitoring of the extent of MDR strains, indicating the quality of TB control in the country. Knowledge of the prevalence of drug resistance in new cases guided the selection of drugs used in initial treatment of tuberculosis. Accurate and rapid diagnosis of TB and drug-resistant TB is of paramount importance in establishing appropriate clinical management and infection control measures.[4] The present study was to conducted to evaluate the resistance of combinations of isoniazid (INH), rifampicin (RMP), streptomycin (SM), and ethambutol (EMB) and four resistant modes, i.e. mono, double, triple and quadruple resistance among cases of pulmonary tuberculosis patients.

Our study included 40 patients, males (110) and females (90). Age group 15-30 years had maximum number of males and females. Our results are in agreement with Michael Pereira et al.[5] We also found that sputum positivity grade in patients was 1+ (60), 2+ (40), 3+ (45) or scanty (55). Study conducted by Paramasivan CN[6] found that most of the M. tuberculosis strains were 2+ in her study in contrast to our study in which 60 strains were 1 positive.

We found that most of our cases were from upper lower economic status. Our results are in agreement with S.P Rai et al.[7] Lesions were cavitatory (36) and non cavitatory (164). This is in accordance to Sarala Menon et al.[8] We found that culture on LJ medium. It was growth of bacteria in 185 cases, myobacteria other than tuberculosis in 3 cases, contamination in 10 cases and no growth of mycobacteria in 2 cases. We evaluated the sensitivity pattern of M. tuberculosis. Number of sensitive strains of rifampicin was 195, isoniazide (160), streptomycin (190), ethambutol (189) and resistant strains of rifampicin was 5, isoniazide (40), streptomycin (10), ethambutol (11). Haji Khan et al[9] found similar results in their study.

In this study, among new cases, four most frequent drug resistance patterns of 21.3% strains of M. tuberculosis from mono drug, double drug, triple drug and quadruple drug resistance were 5.9%, 10.7%, 2.4% and 2.4% respectively. MDR was observed in 4.7% isolates. This is in accordance to Dursun Tartar et al.[10]

CONCLUSION

Drug resistant tuberculosis cases are now increasing day by day. It poses challenge to the treatment. Identification of resistant strains helps in proper management of cases of tuberculosis.

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