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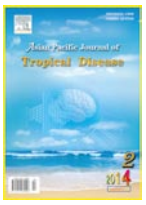

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# *In vitro* drug susceptibility pattern of *Mycobacterium tuberculosis* in CAT I and CAT II pulmonary tuberculosis patients in Aligarh, Uttar Pradesh, India

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ABSTRACT

**Objective:** To evaluate *in vitro* resistance pattern of the first line anti-tubercular drugs in new and previously treated cases of pulmonary tuberculosis patients in Aligarh region.

**Methods:** This study was carried out involving 975 suspected tuberculosis patients. All the specimens of patients were subjected to Ziehl-Neelsen staining, cultured on Lowenstein-Jensen medium and resistance pattern was evaluated by standard proportion method. All patients diagnosed with pulmonary tuberculosis were placed in CAT I and II under Revised National Tuberculosis Control Programme guidelines.

**Result:** Out of 220 patients, 129 (58.7%) were from CAT I and 91 (41.3%) were from CAT II. Totally 44.5% were resistant to one or more than two drugs and 18.6% patients showed resistance to both isoniazid and rifampicin. The individual resistance pattern of these first line drugs were as follows: 37.7% patients were resistant to isoniazid, 22.2% to rifampicin, 8.6% to streptomycin and 10% were resistant to ethambutol.

**Conclusions:** Our findings concluded a high prevalence of *in vitro* drug resistance of *Mycobacterium tuberculosis* isolates, especially multidrug resistant tuberculosis, in both the categories. So there is an urgent need to further study the risk factors for transmission and multidrug resistant tuberculosis in these settings.

## 1. Introduction

India, designated as a high burden country for tuberculosis, has also been identified as a hot spot region for multidrug resistant tuberculosis (MDR-TB) infection[1,2]. World Health Organization estimated 8.7 million incident cases and 12 million prevalent cases worldwide in 2011. India and China accounted for almost 40% of the world's tuberculosis cases[3]. The prevalence of MDR-TB is increasing throughout the world in both new tuberculosis cases *i.e.* CAT I as well as previously-treated tuberculosis cases *i.e.* CAT II[4]. Although previous treatment for tuberculosis is the strongest risk factor for development of MDR-TB, treatment-naive patients are also at risk due to either spontaneous mutations or transmission of resistant strains[5]. The risk of transmission of resistant strains from close contacts is increasing day-by-day because of overcrowding and growing burden of MDR-TB patients.

Globally, there were an estimated 630 000 cases of MDR-TB among the world's 12 million prevalent cases of tuberculosis in 2011. Worldwide, 3.7% of new cases and 20% of previously treated cases were estimated to have MDR-TB. India, China, the Russian Federation and South Africa have almost 60% of the world's cases of MDR-TB[3]. Extensively drug-resistant TB (XDR-TB) has been reported by 84 countries; the average proportion of MDR-TB cases with XDR-TB is 9.0%[6]. Several studies reveal that India has the second largest number of MDR-TB cases in the world. Hence, with the increasing incidence of MDR-TB cases in the country, there is a possibility that the emergence of its more resistant counterpart, XDR-TB, would also increase. The present study was undertaken with the objective to evaluate the

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*in vitro* drug resistance pattern of first line anti-tubercular drugs and to determine prevalence of MDR-TB in suspected cases of new and previously treated pulmonary tuberculosis patients in Aligarh region over a period of four years.

## 2. Materials and methods

The present study was carried out on 975 patients attending outpatient and inpatient departments of Jawaharlal Nehru Medical College, AMU, Aligarh from January 2009 to December 2012, who were clinically suspected to be suffering from pulmonary tuberculosis. The two consecutive sputum samples were collected, one on the spot and second next day morning, in a wide mouth sterile container with a leak proof cap. A detailed questionnaire was used to determine the symptoms like fever, cough, weakness, continuous weight loss, shortness of breathing, previous history of anti-tubercular treatment and patients were categorized as having initial or acquired resistance.

### 2.1. Specimen processing

The specimens were processed by standard conventional method; one portion of the sputum was subjected to routine direct microscopic examination by Ziehl-Neelsen (ZN) method using Revised National Tuberculosis Control Programme grading system. The rest of the sputum was digested and decontaminated by 4% NaOH with modified Petroff's method and concentrated by centrifugation at 6 000 r/min for 20 min; and then the pellet was washed with sterile distilled water. From the pellet a smear was prepared for ZN staining and two Lowenstein-Jensen media slant were inoculated which were then incubated at 37 °C for 6-8 weeks. The inoculated Lowenstein-Jensen media were examined every second day during the first week and then weekly for upto 8 weeks for presence of growth. If growth was present, it was identified by niacin test, nitrate reduction test and p-nitrobenzoic acid test[6,7].

### 2.2. Drug susceptibility test

Drug susceptibility test was carried out by standardized 1% proportion method using the critical concentration of first line anti-tubercular drugs as follows: rifampicin (40 μg/mL), isoniazid (0.2 μg/mL), ethambutol (2 μg/mL) and streptomycin (4 μg/mL) (Sigma Aldrich, India). The bacterial suspension was prepared by adding approximately 4 mg moist weight of a representative sample of the bacterial mass into 0.4 mL of sterile distilled water in a test tube containing four glass beads with diameter of 2-3 mm and vortexed for 30 seconds to produce a uniform suspension, and finally 3.6 mL of sterile distilled water was added to cover specimen to get a concentration of 1 mg/mL. From this suspension a 10 fold dilution was made by adding 0.2 mL to 1.8 mL sterile distilled water. And finally bacterial suspension was inoculated into the drug containing and drug free media and incubated at 37 °C and observed at Day 28 and again at Day 42. Fully susceptible H37Rv reference strain was used as control.

### 2.3. Ethical approval

The study protocol was approved by the Institutional Ethical Committee of Jawaharlal Nehru Medical College, AMU, Aligarh.

## 3. Results

The present study included 975 patients with provisional diagnosis of pulmonary tuberculosis attending outpatient and inpatient departments of Jawaharlal Nehru Medical College and Hospital during a period of four years. Majority of patients 680 (69.7%) were males and 295 (30.3%) were females.

### 3.1. Mycobacteriological examination of pulmonary tuberculosis patients

Out of 975 suspected tuberculosis patients, 180 (18.4%) patients were smears positive for acid-fast bacilli by ZN staining technique, while 220 (22.5%) specimens showed growth on Lowenstein-Jensen medium, they were slow growing non-chromogenous; not growing on p-nitrobenzoic acid were presumed to be *Mycobacterium tuberculosis* (*M. tuberculosis*) and were confirmed by niacin and nitrate reduction test. According to Revised National Tuberculosis Control Programme guideline, out of 220 *M. tuberculosis* positive patients, 129 (58.6%) were in CAT I and 91 (41.4%) were in CAT II (Table 1).

### 3.2. Anti-tubercular drug resistance

Out of total 220 *M. tuberculosis* isolates, 122 (55.4%) were fully susceptible to four first line anti-tubercular drugs, 98 (44.5%) were resistant to one or more than one anti-tubercular drug in which forty six (20.9%) were resistant to any one drug, thirty four (15.4%) resistant to any two drugs, thirteen (5.9%) resistant to three drugs, and five (2.2%) resistant to all four first line anti-tubercular drugs. Totally 83 (37.7%) *M. tuberculosis* isolates showed resistance to

isoniazid, 49 (22.2%) to rifampicin, 22 (10%) to ethambutol and 19 (8.6%) to streptomycin (Table 1).

**Table 1**
Drug resistance pattern of first line anti-tuberculosis drug resistance in new and previously treated patients ($n$=220).

| Drug resistant pattern | Number of cases $n$ (%) | | Total (%) |
|---|---|---|---|
| | New cases or CAT I (%) | Previously treated cases or CAT II (%) | |
| Total cases | 129 (58.6) | 91 (41.30) | 220 (100.0) |
| Fully susceptible | 89 (68.9) | 33 (36.20) | 122 (55.4) |
| Total resistant | 40 (31.1) | 58 (63.70) | 98 (44.5) |
| Single drug resistant | 29 (22.4) | 17 (18.60) | 46 (20.9) |
| Only resistance to H | 20 (15.5) | 13 (14.20) | 33 (15.0) |
| Only resistance to R | 6 (4.6) | 1 (1.09) | 7 (3.1) |
| Only resistance to E | 1 (0.7) | 0 (0.00) | 1 (0.4) |
| Only resistance to S | 2 (1.5) | 3 (3.20) | 5 (2.2) |
| Two drug resistant | 10 (7.7) | 24 (26.30) | 34 (15.4) |
| H+R | 7 (5.4) | 21 (23.00) | 28 (12.7) |
| S+E | 0 (0.0) | 1 (1.09) | 1 (0.4) |
| H+E | 2 (1.5) | 1 (1.09) | 3 (1.3) |
| R+E | 1 (0.7) | 0 (0.00) | 1 (0.4) |
| H+S | 0 (0.0) | 1 (1.09) | 1 (0.4) |
| Three drug resistant | 1 (0.7) | 12 (13.10) | 13 (5.9) |
| R+H+S | 0 (0.0) | 2 (2.10) | 2 (0.9) |
| R+H+E | 1 (0.7) | 5 (5.40) | 6 (2.7) |
| H+S+E | 0 (0.0) | 5 (5.40) | 5 (2.2) |
| Four drug resistant | 0 (0.0) | 5 (5.40) | 5 (2.2) |
| MDR pattern | 8 (6.2) | 33 (36.20) | 41 (18.6) |
| HR | 7 (5.4) | 21 (23.00) | 28 (12.7) |
| HR+S | 0 (0.0) | 2 (2.10) | 2 (0.9) |
| HR+E | 1 (0.7) | 5 (5.40) | 6 (2.7) |
| HR+S+E | 0 (0.0) | 5 (5.40) | 5 (2.2) |
| Any resistance to H | 30 (23.2) | 53 (58.20) | 83 (37.7) |
| Any resistance to R | 15 (11.6) | 34 (37.30) | 49 (22.2) |
| Any resistance to E | 5 (3.8) | 17 (18.60) | 22 (10.0) |
| Any resistance to S | 2 (1.5) | 17 (18.60) | 19 (8.6) |

R: Rifampicin; H: Isoniazid; S: Streptomycin; E-Ethambutol.

In CAT I, out of 129 pulmonary tuberculosis cases, 40 (31.1%) were resistant to one or more than one firstline anti-tubercular drugs and 30 (23.2%) were resistant to isoniazid, 15 (11.6%) to rifampicin, 5 (3.8%) to ethambutol and 2 (1.5%) to streptomycin; while in CAT II most of the patients were resistant to either of the first-line anti-tuberculosis drugs tested. Out of total 91 patients in CAT II, 53 (58.2%) were resistant to isoniazid, 34 (37.3%) to rifampicin and 17 (18.6%) to both streptomycin and ethambutol. Overall 41 (18.6%) *M. tuberculosis* isolates were resistant to both rifampicin and isoniazid or they were MDR-TB. Among the new and previously treated cases, MDR-TB was found to be associated with 8 (6.2%) and 33 (36.2%) cases, respectively.

## 4. Discussion

This study regarding *in vitro* drug resistance pattern among new and previously treated cases has generated valuable information in context of the drug resistant tuberculosis in this region. This is the pioneer study in which pattern of anti-tubercular drug resistance of this region has been evaluated. In our study 37.7% resistance were observed against isoniazid, 22.2% to rifampicin, 8.6% to streptomycin and 10% to ethambutol. Tripathi *et al.* in 2012 reported similar resistance pattern in North India[8]; they observed 37.8% resistance to isoniazid followed by rifampicin (42.9%), streptomycin (12.4%) and ethambutol (7.9%). Menon *et al.* in 2012 observed in Mumbai slightly different resistance pattern and they reported maximum resistance to rifampicin (74.4%) followed by streptomycin (70.0%), isoniazid (53.2%) and ethambutol (21.7%)[9]. They observed higher resistance against all the four anti-tubercular drugs. Resistance to drugs also varies from place to place and time to time. It is evident that prevalence of anti-tubercular drug resistance varied considerably throughout the world and particularly in India. The reasons of this variation are selection of resistant criteria, the misuse of drugs, the quality of questionnaire used for eliciting history of previous treatment, inadequate laboratory support and reporting systems.

In our study newly diagnosed 129 pulmonary tuberculosis cases, the anti-tubercular drug resistance to first-line drugs was comparing; 30 (23.2%) were resistant to isoniazid, 15 (11.6%) to rifampicin, 5 (3.8%) to ethambutol and 2 (1.5%) to streptomycin. Similarly Sethi *et al.* in 2013 reported 26.4% resistance towards isoniazid[6], 9.9% to rifampicin, 14.9% to ethambutol and 28.1% to streptomycin, which is in accordance with our study. In India there are fewer surveys of acquired drug resistance, and the rate of acquired resistance is usually higher than primary resistance. Our study has observed that in 91 previously treated cases, most of the patients were resistant to either of the first line anti-tuberculosis drugs. Out of total 91 patients, 53 (58.2%) were resistant to isoniazid, 34 (37.3%) to rifampicin and 17 (18.6%) to both streptomycin and ethambutol. In other studies, the range of acquired resistance to isoniazid ranged from 4% to 53.7%, to streptomycin from 0% to 19.4%, to rifampicin from 0% to 14.5% and to ethambutol from 0% to 13.7%[3,10].

Several factors have been identified in the causation of drug resistant tuberculosis, of which the three most important are previous treatment with anti-tubercular drugs which may be inappropriate and incomplete, high prevalence of drug resistant tuberculosis in the community and contact with a patient known to have drug resistant tuberculosis. In patients with previous treatment or disease, the odds of resistant tuberculosis were 4-7 times higher than that of person with no history of past treatment[11].

Drug resistance in tuberculosis has been reported since early days of anti-tubercular chemotherapy, but recently

MDR-TB has been an area of growing concern. The rate of MDR-TB was very low in most of the surveys ranging from 0%-10.8% in the case of primary resistance and from 0%-48% for acquired resistance[10]. MDR-TB was reported to range from 0.5%-14.3% in surveys where there was no distinction between primary and acquired resistance. In our study there was totally 41 (18.6%) MDR-TB cases and the prevalence of MDR-TB was 36.3% (33 out of 91) in previously treated patients and 6.2% (8 out of 129) in new pulmonary tuberculosis patients. We also observed different combination of drug resistant pattern in MDR-TB strains of previously treated cases: 23% to rifampicin and isoniazid, 2.1% to rifampicin, isoniazid and streptomycin, 5.4% to rifampicin, isoniazid and ethambutol, and 5.4% to rifampicin, isoniazid, streptomycin and ethambutol. While in new cases only 5.4% resistance to two drug combination rifampicin and isoniazid, and 0.7% resistance to rifampicin, isoniazid and ethambutol were observed. Another study in India also showed similar resistance to rifampicin and isoniazid, rifampicin, isoniazid and ethambutol, rifampicin, isoniazid and streptomycin, and rifampicin, isoniazid, streptomycin and ethambutol with 12.2%, 7.6%, 7.6% and 15.7%, respectively among previously treated cases, and overall MDR-TB prevalence was 43.3%[12]. Sangaré *et al.* reported different resistance patterns to rifampicin and isoniazid, rifampicin, isoniazid and ethambutol, rifampicin, isoniazid and streptomycin, and rifampicin, isoniazid, streptomycin and ethambutol with 8.6%, 5.4%, 1.1% and 35.5%, respectively among previously treated cases, and total overall resistance was 50.5%[13].

In conclusion, our high levels of drug resistance (44.5%) mainly result from that our pulmonary tuberculosis patients belong to previously treated cases, which were harboring more resistant isolates. We also reported high prevalence (36.2%) of MDR-TB in previously treated pulmonary tuberculosis cases in this region, which occurring primarily as a consequence of poor treatment services that could lead to emergence of XDR-TB if MDR-TB is not managed properly. Culture and drug susceptibility test for patients with clinically suspected MDR-TB must not be cover looked, so there is need for surveillance of emerging and increasing trend of MDR-TB and to optimise patient management accordingly. The use of rapid, sensitive and specific methods for detecting MDR-TB must be recommended to combat the problem of MDR-TB. This strongly highlights the need to make useful strategies for testing, surveillance and effective clinical management of MDR-TB cases in India.

## Conflict of interest statement

We declare that we have no conflict of interest.

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