Decreasing prevalence of multi-drugs resistant *Mycobacterium tuberculosis* in Nashik City, India

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ABSTRACT

Objective: In India, increasing prevalence of multi-drug resistant tuberculosis (MDR) has aggravated the control of tuberculosis problem. In many urban and semi-urban regions of India, no surveillance data of multidrug resistance in *Mycobacterium tuberculosis* is available.

Methods: A surveillance study on multidrug resistance was carried out in semi-urban and rural regions in and around Nashik City of Maharashtra, India. The surveillance study was conducted in this region found that the prevalence of combined resistance to first and second-line anti-tuberculosis drugs is remarkably high. The isolates of *M. tuberculosis* was identified and subjected to drug susceptibility testing. The patterns of drug susceptibility of isolates of *M. tuberculosis* during the periods 2000 and 2004 were compared with drug susceptibility patterns of the organisms during the period 2008 to 2011.

Results: The 260 isolates identified as *M. tuberculosis* show mean drug resistance prevalence of 45.6% for more than any two drugs and the MDR rate as 37% in the years 2000 to 2004 whereas 305 isolates of the organism show mean drug resistance prevalence of 30.2% and the MDR rate as 25% in the years 2008 to 2011.

Conclusion: The researcher found that, though the prevalence of multidrug resistance to the drugs tested is remarkably high, it has come down noticeably during the past seven years due to efforts of State Government and strict implementation of treatment guidelines of WHO by the physicians. *J Microbiol Infect Dis* 2013; 3(1): 12-17

Key words: MDR-TB, XDR-TB, DOTS, drug-resistance prevalence rate.

Hindistan’ın Nashik şehrinde çoklu ilaç direnci Mycobacterium tuberculosis prevalansının azalması

ÖZET


Bulgular: 260 izolat *M. tuberculosis* olarak tanımlandı, 2000-2004 yıllarında herhangi iki ilaçtan fazla ilaç direnç gösterme oranı ortalama % 45,6 ve ÇİD oranı % 37 iken 2008-2011 yıllarında 305 izolata ortalama direnç oranı % 30,2 ve ÇİD oranı % 25 idi.

Sonuç: Çoklu ilaç direncinin prevalansı oldukça yüksek bulunmasına rağmen, bu oran devletin çabaları ve tedavi kilavuzlarının hekimler tarafından sıkı bir şekilde uygulanması ile son yedi yıl içinde belirgin bir düşüş gösterdi.


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INTRODUCTION

Tuberculosis (TB) is considered as Global Health threat worldwide for multiple reasons; first is treatment time to cure the disease is remarkably longer than required for most of acute infections, second is transmission of the infection continues at increasing rate despite the control efforts and availability of highly effective treatment. The emergence of multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant (XDR-TB) have aggravated the existing health threat of tuberculosis all over the world. It has been established that, more than 400,000 (0.4 million) cases of MDR-TB emerge every year due to poor control of drug sensitive as well as drug resistant TB. MDR-TB or XDR-TB are considered extremely difficult and expensive to treat and cure. In Asia and Africa, the cases of multi-drug resistant tuberculosis are higher due to over-population and poor socio-economic factors. Most important cause of drug resistance in Mycobacterium tuberculosis is error of healthcare workers. To decide the drug regimen correctly, physicians must have correct data of drug susceptibility patterns of Mycobacterium tuberculosis so that the transmission and spread of multidrug resistance tuberculosis is reduced and eventually halted. Therefore, it is necessary to undertake the epidemiological surveillance of drug susceptibility pattern periodically.

Some researchers, recently, undertook the epidemiological surveillance study of MDR-TB in the various cities of the State of Maharashtra, India, however the comparative epidemiological surveillance study has not been conducted by any researcher in this state. Therefore researcher, undertook the comparative epidemiological surveillance of drug resistant tuberculosis in Nashik City, based on the guidelines prescribed by WHO during 1st January 2004 to 31st December 2011 period in order to observe the impacts of various factors on the rate of emergence of MDR-TB and treatment success rate.

METHODS

Sampling

Cross sectional survey among TB patients visiting Dr. Gangurde Hospital and Nashik Civil Hospital was undertaken for this research work. Both these hospitals combined account for 90% of TB cases in Nashik City.

This research study was approved by ethical review committee of Dr. D. Y. Patil University, Pune, Maharashtra, India and above mentioned hospital administration. The drug susceptibility data of M. tuberculosis generated during the years 2000 to 2004 was obtained from the Nashik Civil Hospital and Dr. Gangurde’s TB Hospital, Nashik. From this data, 100 data samples were randomly selected and minimum drug susceptibility prevalence was found out for a single drug and on the basis of this minimum prevalence the sample size was determined for confidence level of 99% using “Epicalc” in R statistical analysis software. Same procedure was used while determining sample size from the drug susceptibility pattern data generated by processing 350 samples in the period between 2008 to 2011 year to find out the drug susceptibility pattern of M. tuberculosis. The sputum samples of suspected TB patients were collected from Dr. Aniket Gangurde’s TB Hospital situated at Ganjmaal in Nashik city during the period of 2008 to 2011. The two consecutive samples were collected from each patient over a period of one week before starting anti-tuberculosis treatment.

The patients were categorized as children below 15 years age and adult above 15 years age, female children, female adults and male adult. On the basis of minimum drug resistance prevalence of 11% for ciprofloxacin in the year 2004, sample size was calculated using “Epicalc” package of “R” statistical analysis software with 99% confidence level; this sample size came as 260 samples. Similarly sample size was estimated for drug resistance prevalence data generated by processing 350 sputum samples; this sample size determined as 305 samples calculated by “Epicalc” packages with 99% confidence level.

Bacteriological investigations

All the bacteriological investigations such as smear, culture preparation, culture identification and drug susceptibility testing were carried out in private laboratory, Super Religare Laboratory and in the Microbiological Laboratory of Dr. D. Y. Patil’s Medical College and Research Center, Pune. The procedure for sputum smear microscopy, strain identification and drug susceptibility testing were carried out as per the accepted standard protocols. Smears were made from all the collected samples and stained using acid fast staining method and observed under microscope. The positive smears were graded as $1^+$, $2^+$, $3^+$. Culture preparation

Sputum samples were decontaminated by sodium hydroxide-N-acetyl-L-cysteine method. 250μL aliquots were inoculated onto each of the two slopes.
of L-J medium. The inoculated L-J media were incubated at 36°C and weekly examined for visible growth till 8th weeks. Culture identification were carried out using isolated colonies from each slope on the basis of cord formation, niacin test, catalase test at 68°C and pH 7.0 and growth on L-J medium with p-nitrobenzoic acid.18

Drug susceptibility testing
The drug resistance is determined by proportion method, the method developed by Pasteur institute in 1961.17 The drug susceptibility testing were carried out using Middlebrook 7H10 media in SL023 Tuberculosis First line and SL024 Tuberculosis second line Kits manufactured by HiMedia Laboratories Pvt. Limited, Mumbai-400 086, India; as per the manufacturer’s protocol which is as follow: A loopful growth of Mycobacterium tuberculosis culture from a L-J slant and aseptically suspended in 1.0 mL of sterile distilled water and homogenized by mixing for about 10 minutes on vortex machine. Opacity of this culture suspension was adjusted to McFarland 0.5 standard with sterile saline and then the suspension was diluted as 1:10,000; this diluted suspension then used as inoculum. 100µL of the inoculum is inoculated on to each tube of the above mentioned antibiotic kit tubes and control media tubes without drugs. All the inoculated tubes were incubated at 35-37°C under 5-10% CO₂ for 2-4 weeks. 1% or more than 1% ratio of colonies on drug containing tube to the colonies on control tube was taken as resistance and less than 1% ratio was taken as sensitivity to that drug.

The drug susceptibility tests were done using following anti-tuberculosis substances: streptomycin (4.0 µg/mL), rifampicin (40.0 µg/mL), isoniazid (0.2 µg/mL), ethambutol (2.0 µg/mL), pyrazinamide (200.0 µg/mL), ethionamide (40.0 µg/mL), amikacin (2.0 µg/mL), kanamycin (30.0 µg/mL), ciprofloxacin (2.0 µg/mL), P-aminosalicylic acid (1.0 µg/mL), roxythromycin (1.0 µg/mL).13 The significance of difference was inferred with the Chi Square test for the drug resistance prevalence values of 2000 to 2004 and that of 2008 to 2011.

RESULTS
The drug susceptibility data of M. tuberculosis was obtained for 260 isolates tested for selected drugs in the years 2000 to 2004 from two hospitals; Dr. Gangurde’s TB hospital and Nashik’s Civil Hospital. All these 260 isolates were identified as M. tuberculosis. The patterns of these 260 isolates were recorded in tabular form as shown in Table 1&2. The drug susceptibility data of the years 2000 to 2004 shows mean drug resistance prevalence of 45.64% for more than any two drugs. In the years 2000 to 2004, the highest drug resistance prevalence was recorded for roxythromycin followed by pyrazinamide, ethionamide and rifampicin in descending order as 82%, 81%, 80% and 58% respectively. The average lowest drug prevalence, in years 2000 to 2004, was recorded for ciprofloxacin followed by amikacin and PAS in ascending order as 11%, 13% and 21% respectively. The mean MDR prevalence, in years 2000 to 2004, was recorded as 37%.

The drug susceptibility data of M. tuberculosis selected randomly for 305 isolates tested for selected drugs during the period 2008 to 2011 are shown in Table 1&2. The drug susceptibility data of the years 2008 to 2011 shows mean drug resistance prevalence of 30.2% for more than any two drugs. The highest drug resistance prevalence in this data was recorded for ethionamide followed by roxythromycin as 68% and 54% respectively. The lowest drug resistance prevalence was observed for pyrazinamide followed by streptomycin and ethambutol as 8%, 19% and 19% respectively. The MDR prevalence was found to be 25% in the years 2008 to 2011.

Table 1. Resistance to the first-line anti-TB drugs

<table>
<thead>
<tr>
<th>Variables</th>
<th>2000-2004</th>
<th>P-Values</th>
<th>2008-2011</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any S resistance (%) (95% CI)</td>
<td>49 (43-55)</td>
<td>0.709</td>
<td>19 (15-23)</td>
<td>&lt; 2.2e-16</td>
</tr>
<tr>
<td>Any R resistance (%) (95% CI)</td>
<td>58 (52-64)</td>
<td>0.009</td>
<td>25 (20-30)</td>
<td>2.2e-16</td>
</tr>
<tr>
<td>Any H resistance (%) (95% CI)</td>
<td>45 (39-51)</td>
<td>0.106</td>
<td>29 (24-34)</td>
<td>1.507e -16</td>
</tr>
<tr>
<td>Any E resistance (%) (95% CI)</td>
<td>25 (20-30)</td>
<td>7.4e-16</td>
<td>19 (15-23)</td>
<td>&lt; 2.2e-16</td>
</tr>
<tr>
<td>Any Z resistance (%) (95% CI)</td>
<td>81(75-85)</td>
<td>2.2e-16</td>
<td>8 (5-11)</td>
<td>&lt; 2.2e-16</td>
</tr>
<tr>
<td>Any PAS resistance (%) (95% CI)</td>
<td>21 (16-26)</td>
<td>&lt; 2.2e-16</td>
<td>35 (30-40)</td>
<td>1.882e-07</td>
</tr>
</tbody>
</table>

S=streptomycin, R=Rifamipicin, H=isoniazid, E=Etambutol, Z=Pyrazinamide, PAS=Para-Amino-Salicylic-acid
Table 2. Resistance to-second-line drugs

<table>
<thead>
<tr>
<th>Variables</th>
<th>2000-2004</th>
<th>P-Values</th>
<th>2008-2011</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Eto resistance (%) (95% CI)</td>
<td>80 (74-84)</td>
<td>&lt; 2.2e-16</td>
<td>68 (62-72)</td>
<td>4.339e-10</td>
</tr>
<tr>
<td>Any Amk resistance (%) (95% CI)</td>
<td>13 (9-17)</td>
<td>&lt; 2.2e-16</td>
<td>29 (24-34)</td>
<td>1.507e-13</td>
</tr>
<tr>
<td>Any Kn resistance (%) (95% CI)</td>
<td>38 (32-42)</td>
<td>0.0001205</td>
<td>22 (17-27)</td>
<td>&lt; 2.2e-16</td>
</tr>
<tr>
<td>Any Cpro resistance (%) (95% CI)</td>
<td>11 (7-15)</td>
<td>&lt; 2.2e-16</td>
<td>24 (19-29)</td>
<td>&lt; 2.2e-16</td>
</tr>
<tr>
<td>Any Rox resistance (%) (95% CI)</td>
<td>82 (76-86)</td>
<td>&lt; 2.2e-16</td>
<td>54 (48-59)</td>
<td>0.1523</td>
</tr>
</tbody>
</table>

Note: Eto=Ethionamide, Amk=Amikacin, Kn=Kanamycin, Cpro=Ciprofloxacin, Rox=Roxythromycin

In the years, 2000 to 2004, drug resistance prevalence was more for second line TB drugs such as roxythromycin and ethionamide. Susceptibility to streptomycin was 51% in the year 2004 which has observed to be increased to 81% in the year 2011. Susceptibility to Rifampicin and Isoniazide were 42% and 55% respectively, in the years 2000 to 2004, which were observed to increase to 75% and 71% respectively in the year 2008 to 2011. The resistance of Rifampicin is closely associated with the resistance of isoniazid. The overall trends of drug resistance prevalence patterns show average 15% decrease during the last 6 years. The drug resistance prevalence for Pyrazinamide, in the year 2011, was observed as 8% which was observed to be remarkably as high as 81% in the year 2004. The statistical analysis on drug resistance prevalence values of 2000 to 2004 and that of 2008 to 2011 showed a significant differences (p<0.001).

DISCUSSION

The resistance to number of second-line anti-tuberculosis drugs was very high during the years, 2000 and 2004 as compared to the same in the year, 2008 to 2011. The important causes of such high resistance to the second-line drug resistance in the period between the years 2000 and 2004 are inadequate, highly variable anti-tuberculosis treatment and indiscriminate use of the second-line drugs by the general practitioners in the private health care setups. Surveillance study conducted recently in number of cities showed that, the prevalence rate of MDR-TB is increasing. In another study conducted by Lagali in Dr. D.Y. Patil’s College and Research Center’s Microbiological Laboratory showed that, the MDR-TB prevalence rate was as high as 17% in the year 2008 (Shilpa Lagali, 2008; unpublished data). The high prevalence of MDR-TB in Nashik region very frequently visit Mumbai for trading and service purposes.

The researcher in this study has compared the prevalence rate of drug resistance in Mycobacterium tuberculosis in two periods i.e. during the years 2000 to 2004 and 2008 to 2011 and found that, the rate of MDR is actually decreasing. The analysis on drug resistance prevalence values of 2000 to 2004 and that of 2008 to 2011 showed significant differences between drug resistance prevalence values of 2000 to 2004 and that of 2008 to 2011.

There is averagely 15% decrease in drug resistance to any two drugs during the last seven years. In the opinions of eminent doctors and medical microbiologists in the Nashik district, the decreasing drug resistance pattern is attributed to decreased prevalence in HIV infection, heightened health awareness among the general population toward the HIV and tuberculosis and effective implementation of DOT in the district. Other important causes of decreasing drug resistance in Mycobacterium tuberculosis are most probably the facts that the INH-resistance strains of Mycobacterium tuberculosis are less infective than INH-sensitive strains and that if the detection rate of TB is 75% and treatment success rate is 85% then the transmission rate of TB decreases by 7 to 11% per year. These various factors contributing to the decreased prevalence of drug resistance in M. tuberculosis is being studied by the researcher. The decreasing drug resistance prevalence in Mycobacterium tuberculosis is a good positive trend and the surveillance of drug resistance should be continued to test the success of anti-tuberculosis treatment for the better management MDR-TB and XDR-TB. The drug regimen guidelines prescribed by WHO should be strictly followed. The all forms of tuberculosis should be treated by expert medical practioners only and treatment compliance rate should be increased to prevent further emergence of MDR and XDR strains of M. tuberculosis.

In this research study, the prevalence rate of primary drug resistance and acquired drug resis-
tance in *Mycobacterium tuberculosis* could not be estimated separately due to lack of such data. The second limitation of this research study was that the researcher could not take into account the 10% of the TB patients who were treated without testing the drug susceptibility patterns for *M. tuberculosis*. These patients were treated by general physician scattered on the peripheral regions of Nashik city, without testing the drug susceptibility. The third limitation of this research study was that the researcher could not take into account the average mortality rate of 5.0% of TB patients reported by the Civil Hospital of Nashik, India, due to inadequate data of drug susceptibility patterns for the deceased patients.

However, even if it is assumed that the 10% unaccounted TB patients, in this study, were susceptible to the anti-tuberculosis drug, then also the average decrease in drug resistance prevalence for any two drugs comes approximately to 13 (considering the additional 1% error in calculation of sample size) in 2011.

However, since the errors of calculating the sample size (1% error) and 10% unaccounted numbers of TB patients were the same during the two time periods i.e., 2000 to 2004 and 2008 to 2011, the conclusion can be clearly drawn that there is decreasing trends in the MDR rate in *M. tuberculosis* isolated from TB patients during the 7 years time period.

In conclusion, the researcher found that, though the prevalence of multidrug resistance (MDR rate) to the drugs tested is remarkably high, it has come down noticeably from 37% in the years, 2000 to 2004 to 25% in the years, 2008 to 2011, during the past seven years due to Health awareness among the people, efforts of State Government of Maharashtra and Government bodies of India and strict implementation of treatment guidelines of WHO by the physicians in Nashik City.

**Authors contributions**

Arun Punaji More has conceived and designed the experiments and also carried out statistical analysis using free software, R-package and “Epicalc” to draw significant conclusions; the authors has no competing interest in this research project.

R.P. Nagdawane has immensely guided throughout this research project helping designing the experiments and writing this research paper.

Aniket Gangurde has contributed extensively to this research project by collecting consecutive sputum samples from suspected TB patients and collecting medical and treatment history of the patients and classifying these data for statistical analysis.

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**REFERENCES**


