Original article:

Drug resistance patterns in smear positive pulmonary tuberculosis at a tertiary health care centre in Pune , Western Maharashtra

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Abstract:

Background: Multidrug resistant(MDR) tuberculosis continues to harbour in the community, inspite of aggressive TB control measures. Continuous monitoring of the drug resistance patterns is necessary establish the efficacy of public health interventions . We, therefore, sought to determine the drug resistance patterns in our setup.

Material & methods: Of a total of 687 patients clinically suspected of pulmonary tuberculosis, 100 AFB positive cases were included.52 were newly diagnosed(Cat I) and 48 were previously treated (Cat II).DST done by 1% economic variant of proportion method on LJ medium.

Results: Only 26% were sensitive to all isoniazid ,rifampicin ,ethambutol and streptomycin.51% were resistant to isoniazid,53% were resistant to rifampicin,40% were resistant to ethambutol and 42% were resistant to streptomycin.40% strains were MDR.

Conclusion: High levels of resistance even after implementation of DOTS is alarming . However, a large scale epidemiological surveys are need of the hour for uniform and accurate assessment of the drug resistance scenario in India.

Introduction

Tuberculosis control continues to be a major challenge despite significant advances in the diagnosis and treatment. India harbours about a fourth of the global burden of TB and has the highest number of newly diagnosed cases, despite nation-wide intense efforts by RNTCP and the DOTS therapy.(1)

The emergence of drug resistant TB has added a new dimension in the management of the disease. Resistance to one of the first line anti TB drugs, streptomycin was recognised way back in 1993, but was considered to pose only a modest risk .(2) It is multidrug resistance (MDR) i.e., resistance to at least to Rifampicin and Isoniazid, the core and the most effective drugs of the RNTCP's DOTS regimen that created a major setback in the gains achieved by NTP's over the years . Emergence of additional resistant forms like extended drug resistance(XDR)& the so called extensively drug resistant(XXDR)) /Totally drug resistant(TDR) have compounded the problem manifold. (3)

The success of the national TB control programme is gauged not only by the number of cured cases but also those reported resistant to anti TB drugs. Regular surveillance data on drug resistance , therefore are needed to monitor the effectiveness and impact of TB prevention and control. It would primarily enable formulation of optimum second line drug regimens and track the current epidemiological trends which could allow rapid outbreak response for drug-resistant TB. (4)

However, data from India on multi-drug resistant TB are still scanty and irregular (4, 5) Unlike countries like China (that contributes more than 50% of world's MDR cases) which has produced reliable data since 2007 (4).

This study was, therefore, initiated with the objective to examine the patterns of drug resistance to anti tuberculosis drugs at B.J.Medical College & Sassoon General Hospital, a large tertiary health care centre in Pune, Western Maharashtra.

Material and Methods:

This was a cross sectional, descriptive study conducted over a 18 month period (January 2011 to July 2012) at Department of Microbiology, B.J. Government Medical College, Pune. The study was approved by the Institutional Ethics Committee. Both inpatients and outpatients attending the OPD were examined and referred to our laboratory for further microbiological testing. A total of 687 clinically suspected pulmonary tuberculosis patients were included in the study. A detailed history including that of treatment was recorded. We classified the patients according to standard definitions into newly diagnosed and previously treated patients as per the WHO/IUATLD for the purpose of surveillance (6,7). Early morning sputum samples & BAL were first screened for Acid fast bacilli(AFB) by Ziehl Neelson staining. Specimens positive for AFB were processed by NaLc-NaOH(N-acetyl L cysteine-sodium hydroxide) digestion and decontamination method (8) and inoculated onto Lowenstein-Jenson media solid media slopes. Specimens positive for AFB were processed by NALC-NaOH digestion and decontamination method (8) and inoculated onto Lowenstein-Jenson (LJ) media. These LJ media were followed up weekly for growth. Any growth detected was further confirmed to be *M.tuberculosis* (MTB) by Niacin accumulation test, Nitrate utilisation test and Susceptibility to paranitrobenzoic acid (PNB). Drug susceptibility testing on the isolates confirmed to be MTB, was done by 1% economic variant of proportion method for primary line drugs. (9). Concentrations of drugs used in the LJ medium were Rifampicin (RIF) 40µg/ml , Isoniazid (INH) 0.2µg/ml, Ethambutol (EMB) 2µg/ml & Streptomycin (STR) 4µg/ml, as recommended in the standard operating procedure of RNTCP (9).Standard strain of H37Rv was used as quality control for each batch.

Results

Of the total 687 samples that were screened, 100 were found positive for acid fast bacilli (AFB) by smear microscopy and grew on culture. Of these, 52 were from newly diagnosed and 48 from previously treated patients (Table I,III). When drug susceptibility test was done, of the total 100 isolates , only 26 were found susceptible to INH,RIF,EMB and STR - 20 from newly diagnosed and 6 were from previously treated patients . There were 74 patients who showed resistance to at least a single drug. Single drug resistance was found in 74 newly diagnosed patients as INH (32.6%), RIF(36.5%), EMB (34.6%) and STR (28.8%).In previously treated patients, the pattern was 70.83%, 70.83%, 45.8, 56.2% for INH,RIF,EMB and STR resistance respectively(Table I). Highest drug resistance was recorded for RIF in both the groups of patients. Multidrug resistance (MDR)(defined as resistance to at least rifampicin and isoniazid) , was seen in 21.15% of all newly diagnosed cases and and 60.4% of previously diagnosed cases. (Table II)

Discussion

This study was taken up to understand the extent of prevailing anti-tuberculosis drug resistance at B.J.G. Medical College and Sassoon General Hospital, which is the prime tertiary care centre of Pune and one the major public sector hospitals in Maharashtra.

Our data show that among the 100 newly diagnosed TB patients reported at our Hospital, 61.5% were resistant to one or more drugs. These are much higher than the reported prevalence range of 7.9%-27.1% in surveys done from Tamilnadu,

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Maharashtra and West Bengal (5,10-12.) Resistance in previously treated patients to one or more drugs was 87.5% and lies within the range of 25-100% in various studies from Delhi, Tamilnadu, Gujarat, Haryana and Mumbai (2) (5) (13) (14) (18).Our data of 53% overall resistance is less than 74.4% reported by by Menon et al from Mumbai in 2010(19) and 68% reported by Pradhan et al 2009 from Pune (20).

On evaluation of individual drug resistances, resistance to rifampicin was found to be the maximum. Resistance to rifampicin has been steadily increasing over the years. In new cases, it was 36.5% (15-17) which is higher and in previously treated patients , it was 70.83% ,which correlates well with other Indian studies(2), (5) (18).

Resistance to Isoniazid has been observed to be the commonest amongst of all the anti TB drugs. However, in our present study, it was lesser as compared to resistance to rifampicin. Our data of 32.6% resistance in newly diagnosed patients and 70.83% in previously treated patients lies well with reported data (2) (5) (21) .The overall resistance of 51% from our study correlated well with earlier reports from Mumbai (19,22).

Streptomycin resistance in new cases was 28.5% and previously treated cases was 56.2% and overall resistance of 42% also lie within known ranges.(17)(23)(24),(5))Streptomycin resistance was the first ever to be recorded and continues to be high. Overall it is 42%,which correlates with 52% as seen by Pradhan et al,Pune(20)

Ethambutol resistance surveillance begun only in the 1990's as it was introduced later into NTP'S drug regime. Resistance to ethambutol has always been low. But, when compared with the published data (5) (16) (17), 34.6% resistance in new cases and 45.8% in previously treated cases is quite high .

Therefore, overall the trends of individual drug resistance as noted in the present study are high.

MDR tuberculosis is the major obstacle to tuberculosis control. High prevalence of MDR was observed in both new and previously treated patients in the present study. 21.5% resistance in newly diagnosed patients was higher than other reported studies and median global prevalence of (0- 14%). It was also higher than 14.8% reported by Johnson et al in 2001 from Pune . In accordance with the observation that , drug resistance is always higher in previously treated than in new patients ,in this study as well,60.4% previously treated patients were MDR .This is comparable with various Indian studies and also lies within global median prevalence(0-48%)(2) (10) (13) (16) (17) (23) (25) (26) (27)(28) (29), (30), (31) . Overall MDR prevalence was 40% which is similar to earlier reports of 53% MDR by Pradhan et al, B.J.M.C, Pune , 47.54% by Menon et al & 51% by Almeida et al from Mumbai. This highlights the problem of inadequate TB control in these major cities of Maharashtra.

Similar trends reported by us have also been observed by other high burden countries also. Like high MDR rates have been observed in Russian Federation (Murmansk oblast, 28.9%) and the Republic of Moldova (65.1%), respectively.(4).

Implications of all the above findings are that-

- In new cases, high levels of resistance is an epidemiologic indicator of continuous transmission of drug resistant strains in Pune and surrounding areas.
- High acquired resistance point towards inadequate TB control arising from various factors like overcrowding ,HIV epidemic, erroneous private practise and overburdened public health setup.(6)
- We have compared our findings with various Indian studies and although there

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seems to be a clear increase in levels of resistance in present study, it is still obvious that studies from Maharashtra as well as other parts of India, are insufficient , irregular and report variable levels of resistance. This makes comparison and commenting on resistance and its time trends, a difficult task . The reasons for the variation could be improper selection of study group and quality of questionnaire used,extent of drug misuse & inadequate laboratory support and reporting systems. (31)

- Most of the published data till date, including the present study, are from large tertiary health care centres which makes interpretation of drug resistance scenario biased. Urban areas usually record higher resistance levels as overworked healthcare setup may be unable to monitor DOTS efficiently. On the contrary, Rural areas may seem to be less plagued by MDR as result of factors like supervised DOTS, lower transmission due to low population density and more consistent treatment due to lesser access to multiple doctors. (6)
- WHO/IUATLD conducts periodic surveillance on the extent of drug resistance and prepares guidelines on recommendations for management of drug resistant TB also, emphasises on the establishment of nationwide surveillance systems, with greater urgency in the highest burden settings. There are countries which have done well like China, the only high burden country which has produced reliable data since its first nationwide survey in 2007. India on the contrary has produced only subnational data , from selected few parts of the country (4). A national wide continuous large scale and small scale epidemiological studies are need of the hour. RNTCP establishing its own surveillance network would be highly ideal .Also, it would be ideal to have a standard quality control in the microbiology laboratories all over the country to make comparative variations more reliable.(7)
- A high prevalence of MDR, highlights the need of timely drug sensitivity testing, for prompt second line treatment and preventing amplification of resistance in community. Newer molecular diagnostic tests like Line probe assays & GeneXpert have revolutionised TB diagnostics.(32,33)They have been endorsed by the WHO and are being considered for implementation all over the country by the RNTCPs current plan .

Conclusion:

High levels of individual drug resistance as well as MDR are present in the patients in Western Maharashtra. In spite of certain limitations of the study, it still is an obvious indicator that the situation of drug resistance is not only distressing, but is being ignored and underreported, which has serious implications on implementation of RNTCP .Large and small scale epidemiological studies, uniformly covering all parts of the country, are need of the hour to establish a reliable anti-mycobacterial resistance surveillance network & to understand the true magnitude of MDR .

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Table I

	New cases(H/o treatment < 1 month)		Previously treated cases(H/O treatment >1 month)		Total	
	N	%	N	%	N	%
Total no.of strains tested	52	52%	48	48%	100	100
Susceptible to all drugs	20	38.4	6	12.5	26	26
Any resistance	32	61.5	42	87.5	74	74
Isoniazid(H)	17	32.6	34	70.83	51	51
Rifampicin(R)	19	36.5	34	70.83	53	53
Ethambutol(E)	18	34.6	22	45.8	40	40
Streptomycin(S)	15	28.8	27	56.2	42	42

	N	%	N	%	N	%
Monoresistance	13	25	12	25	25	25
Isoniazid(H)	2	3.8	6	12.5	8	8
Rifampicin(R)	3	5.7	3	6.25	6	6
Ethambutol(E)	5	9.6	3	6.25	8	8
Streptomycin(S)	3	5.7	0	0	3	3

Table II

	New cases(H/o treatment < 1 month)		Previously treated cases(H/O treatment >1 month)		Total	
	N	%	N	%	N	%
Multi drug resistance (resistant to	11	21.15	29	60.4	40	40

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atleast H& R)						
H+R	3	27.27	3	10.3	6	15
H+R+E	0	0	0	0	0	0
H+R+S	1	9.09	7	24.1	8	2
H+R+E+S	7	63.63	19	65.51	26	65
Other resistance patterns						
H+E	0	0	0	0	0	0
H+S	1	1.9	0	0	1	1
H+E+S	1	1.9	0	0	1	1
R+E	0	0	0	0	0	0
R+S	2	3.8	2	4.16	4	4
R+E+S	1	1.9	1	2.08	2	2
E+S	1	1.9	0	0	1	1

Table III

	New cases(H/o treatment < 1 month)		Previously treated cases(H/o treatment >1 month)		Total	
Susceptibility profile according to Number of drugs	N	%	N	%	N	%
Susceptible to 4 drugs	20	38.4	6	12.5	26	26
Resistant to 1 drug	13	25	6	12.5	26	26
Resistant to 2 drugs	7	13.4	5	10.4	12	12
Resistant to 3 drugs	3	5.7	8	16.66	11	11
Resistant to 4 drugs	7	13.4	19	39.5	26	26

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