Acquired drug resistance pattern in tuberculosis cases at the State Tuberculosis Centre, Delhi, India

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SETTING: State TB Demonstration Centre, Delhi, India.

OBJECTIVE: To obtain a baseline estimate of the prevalence of multidrug-resistant tuberculosis (MDR-TB) among previously treated tuberculosis (TB) cases at the State Tuberculosis Centre in 2006.

DESIGN: A retrospective study. Drug susceptibility data of 5252 previously treated patients tested at this centre were analysed.

RESULTS: Of 2880 Mycobacterium tuberculosis isolates from previously treated cases, 1498 (52%) were resistant to one or more anti-tuberculosis drugs, of which 47.1% were MDR. Resistance to isoniazid was observed in all resistant isolates, followed by resistance to rifampicin in 1357 (47.1%), streptomycin in 403 (14.2%) and ethambutol in 107 (3.72%). A significantly higher rate of resistance, including MDR, was observed among treatment failures compared to relapses and defaulters.

CONCLUSION: A very high proportion of drug-resistant cases had MDR besides resistance to two or more drugs. This proportion was significantly higher among treatment failures compared to relapses and treatment after default cases, underlining the need for early identification of treatment failure by early referral for culture and drug susceptibility testing, and initiation of appropriate treatment.

KEY WORDS: tuberculosis; drug resistance; previously treated

TUBERCULOSIS (TB) remains one of the main public health problems, particularly in developing countries. India accounts for one fifth of the global TB burden in terms of absolute numbers of incident cases occurring each year.1 The emergence of multidrug-resistant TB (MDR-TB), defined as resistance to isoniazid (INH, H) and rifampicin (RMP, R), the two most potent anti-tuberculosis drugs, has posed additional challenges in controlling TB.

The response of patients with MDR-TB to first-line anti-tuberculosis treatment is poor, with high case fatality rates. Their treatment with expensive and toxic second-line drugs for a longer duration, often requiring hospitalisation for the management of toxic reactions and other complications, puts constraints on meagre health care resources in developing countries.2,3 Furthermore, the recent phenomenon of extensively drug-resistant TB (XDR-TB), defined as resistance to INH and RMP plus resistance to any of the fluoroquinolones and at least one of the three injectable second-line drugs (kanamycin, amikacin or capreomycin),4 virtually making treatment with existing anti-tuberculosis drugs impossible, is a cause for grave concern. There is thus an urgent need to estimate the prevalence of MDR-TB among previously treated TB cases and monitor its trends. Such information would be useful for reviewing the anti-tuberculosis treatment programmes and for taking necessary steps for preventing the emergence of MDR-TB.

Most of the limited number of studies on drug resistance among previously treated cases have been carried out in a small number of cases yielding estimates of prevalence of MDR-TB with poor precision.5–8 A study was therefore undertaken at the New Delhi Tuberculosis Centre, the State Tuberculosis Demonstration Centre as well as the designated Intermediate Reference Laboratory (IRL) of the state of Delhi, to obtain a baseline estimate of the prevalence of MDR-TB among previously treated TB cases.

STUDY POPULATION AND METHODS

The New Delhi Tuberculosis Centre is a referral centre for mycobacterial culture and drug susceptibility testing (DST) for the whole of North India; it has been carrying out DST for more than 40 years.

Study design

During 2006, 5252 previously treated TB cases were referred to the centre for mycobacterial culture and DST. These mainly included patients who had been treated at this centre or other government health...
centres from neighbouring states implementing the DOTS strategy. Culture and DST were also carried out among patients referred by private practitioners.

As this was a retrospective study, ethical approval was not required.

**Treatment regimens**

**DOTS**

Standard treatment regimens given to patients under Revised National Tuberculosis Control Programme (RNTCP) DOTS\(^1\) are the Category I regimen, a four-drug regimen consisting of 2(HRZE)\(_2\)/4(HR)\(_3\),* given to new smear-positive pulmonary TB cases and other newly diagnosed seriously ill patients with severe forms of TB; the Category II regimen, consisting of five drugs, 2(SHRZE)\(_3\)/1(HRZE)\(_3\)/5HRE, which is given to all retreatment cases, i.e., relapses, failures and treatment after default; and the Category III regimen, a 3-drug regimen, 2(HRE)\(_3\)/4(HR)\(_3\), given to new smear-negative pulmonary TB and other newly diagnosed patients not included in Cat I.

**Non-DOTS**

Non-DOTS treatment is a self-administered non-RMP-containing regimen given in exceptional cases to smear-negative TB and extra-pulmonary TB cases. The two regimens are Non-DOTS 1: 2-HSE/10-HE and Non-DOTS 2: 12-HE. Besides the RNTCP, approximately 50% of TB cases first approach private practitioners (PPs) for diagnosis and treatment.\(^9\) The majority of PPs do not follow the standard treatment regimens (Cat I/Cat II) and duration as advocated by the RNTCP, but prescribe individualised regimens.

**Culture and DST**

In the majority of cases referred from government health centres following DOTS, culture and DST were performed as per RNTCP guidelines, i.e., in patients who remained smear-positive after ≥4 months of Cat II treatment.\(^1\) Most of the samples received from the RNTCP were therefore Cat II failures. However, PPs do not follow RNTCP guidelines for DST, and hence patients who had relapsed or defaulted (with a history of previous treatment) or who had remained sputum-positive after ≥5 months of anti-tuberculosis treatment were referred for DST. In relapse cases, sputum culture and DST were performed before starting retreatment. Although the exact breakdown for all the 5252 cases would not be possible, nearly 80–85% cases were failures, 10–15% relapses and the rest were treatment after default.

There were no human immunodeficiency virus (HIV) positive patients in this study. However, the centre has a policy of referring all TB patients with suspected HIV infection to the nearest voluntary counselling and testing centre (VCTC) as per state policy. VCTCs provide confidential counselling and testing services.\(^5\)

**METHODS**

A minimum of two sputum samples from each case were processed for mycobacterial culture by Nsassu’s method. This method utilises 5% oxalic acid and 5% sodium citrate solution for decontamination and is a recommended method for the decontamination of smear-positive samples.\(^10\) Species identification of mycobacterial isolates was undertaken using susceptibility to P-nitrobenzoic acid (PNB), niacin test and catalase activity at 68°C/pH 7.\(^11\)

DOTS was performed using the resistance ratio method for the four first-line anti-tuberculosis drugs INH, RMP, SM and EMB.\(^11\) H37Rv strain of Mycobacterium tuberculosis was used as the control. A strain was considered resistant if the ratio was more than four. The Tuberculosis Research Centre, Chennai, India, which is a World Health Organization (WHO) Supranational Reference Laboratory, has been conducting our proficiency testing since 2001. The 2006 results of proficiency testing of DST show 100% concordance for INH and RMP. Before this period, this laboratory was under the quality control of the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium and the Laboratory Centre for Disease Control, Ottawa, Canada.

**RESULTS**

Of the 5252 cases, M. tuberculosis was isolated from the sputum specimens of 2880 (55%) patients. Of these, 1382 (48%) were sensitive to all four primary drugs and the remaining 1498 (52%) had resistance to one or more anti-tuberculosis drugs; 47.1% of the isolates were MDR. The prevalence of drug resistance, including multidrug resistance, was significantly higher among treatment failure cases compared to relapses (Z = 8.5, P < 0.05) and treatment after default cases (Z = 8.21, P < 0.05). About 50% of the M. tuberculosis isolates from treatment failure cases were MDR; these proportions were respectively 26.8% and 25% for relapse and treatment after default cases. The pattern of resistance by drug and type of case is presented in Table 1. Resistance to INH was most common, including multidrug resistance, was significantly higher among treatment failure cases compared to relapses (Z = 8.5, P < 0.05) and treatment after default cases (Z = 8.21, P < 0.05). About 50% of the M. tuberculosis isolates from treatment failure cases were MDR; these proportions were respectively 26.8% and 25% for relapse and treatment after default cases. The pattern of resistance by drug and type of case is presented in Table 1. Resistance to INH was most common, present in all of the resistant isolates, followed by resistance to RMP in 1357 (47.1%), SM in 403 (14.2%) and EMB in 107 (3.7%) cases.

**DISCUSSION**

In the present study, 47.1% of the isolates were MDR, which is higher than the 33.3% reported in a previous
study in 1990–1991. 5 The lower proportion of MDR isolates during the previous study could be attributed to the fact that RMP-containing short-course regimens were incorporated into the National Tuberculosis Programme (NTP) only in the late 1980s. A high proportion (26.8%) of the relapsed patients were MDR, compared to the 4% reported by Salaniponi et al. among patients treated under routine programme conditions.12 It is understandable to find a high rate of MDR-TB amongst relapse cases in our settings, as this is a referral centre for mycobacterial culture and DST for the whole of North India, and it therefore receives a number of samples from chronic patients, who have had more than one relapse.

In this study, the prevalence of drug resistance, including multidrug resistance, was significantly higher among treatment failures compared to relapses. Resistance to three and four drugs did not occur in the latter. Among retreatment patients, higher resistance has also been reported among treatment failures in Ahmedabad (70%) and Bangalore (78%) compared to relapsed patients (respectively 50.7% and 50%).13,14 Neither study specifies the percentage of MDR-TB in the majority of patients in that study, compared to 4.8% treatment failures, which might explain the higher pan-susceptibility results.14 In the present study, treatment failures constituted 87.1% of the study group, which also explains the higher percentage of MDR (47.1%) isolates as compared to the previous two studies (Table 2).13,14 A study from Vietnam reported 80% of Cat I failure cases with MDR-TB.16 Similarly, a very high proportion of Cat I failures (75%) in Peru were found on DST to have MDR-TB.17 It appears that while the Cat II regimen might still work for relapse and treatment after default patients, it may not for failure cases, as a high proportion of failure patients may be MDR.

Although a well-administered DOTS strategy is the best method of preventing drug resistance and eventual treatment failure, it may not adequately treat resistant cases. There is an urgent need for timely identification of treatment failure on Cat I regimen by early referral for culture and DST for prompt initiation of appropriate treatment to improve outcome as well as to sever the chain of primary transmission. The Indian government has recently introduced DOTS Plus services with the technical support of the WHO, the Green Light Committee (GLC) and other technical agencies in a phased manner to address the needs of MDR patients.18 One of the main limitations in extending DOTS Plus activities to all Cat I failure patients is the lack of quality-assured laboratories with

### Table 1  Drug resistance among M. tuberculosis isolates from previously treated cases by drug and type of case*

<table>
<thead>
<tr>
<th>Cases</th>
<th>MDR cases n (%)</th>
<th>H n (%)</th>
<th>HR n (%)</th>
<th>HS n (%)</th>
<th>HE n (%)</th>
<th>HRS n (%)</th>
<th>HRE n (%)</th>
<th>HRSE n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failures1 (n = 2507)</td>
<td>1387 (55.3)</td>
<td>1258 (50)</td>
<td>129 (5.1)</td>
<td>837 (33.3)</td>
<td>0</td>
<td>0</td>
<td>316 (12.6)</td>
<td>22 (0.8)</td>
</tr>
<tr>
<td>Relapse† (n = 309)</td>
<td>95 (30.7)</td>
<td>83 (26.8)</td>
<td>6 (1.9)</td>
<td>83 (26.8)</td>
<td>4 (1.3)</td>
<td>2 (0.6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treatment after default‡ (n = 64)</td>
<td>16 (25)</td>
<td>0</td>
<td>16 (25)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (N = 2880)</td>
<td>1498 (52)</td>
<td>1357 (47.1)</td>
<td>135 (4.6)</td>
<td>936 (32.5)</td>
<td>4 (0.13)</td>
<td>2 (0.06)</td>
<td>316 (10.97)</td>
<td>22 (0.76)</td>
</tr>
</tbody>
</table>

* Total number of resistant isolates = 1498.
† Resistance to one drug (H only) = 135 (4.6%); two drugs (HR/HS/HE) = 942 (32.7%); three drugs (HRS/HRE) = 338 (11.7%); four drugs (HRSE) = 83 (2.9%).
‡ As per the RNTCP case definitions.1

**Table 2  Recent drug resistance studies in previously treated patients**

<table>
<thead>
<tr>
<th>Study site</th>
<th>Study period</th>
<th>Sample size</th>
<th>INH</th>
<th>RMP</th>
<th>SM</th>
<th>EMB</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delhi11</td>
<td>1990–1991</td>
<td>81</td>
<td>50</td>
<td>33.3</td>
<td>*</td>
<td>*</td>
<td>33.3</td>
</tr>
<tr>
<td>Haryana6</td>
<td>1991–1995</td>
<td>196</td>
<td>72</td>
<td>49</td>
<td>37</td>
<td>6</td>
<td>49</td>
</tr>
<tr>
<td>Rajasthan7</td>
<td>1993–1994</td>
<td>34</td>
<td>61.76</td>
<td>70.59</td>
<td>51.52</td>
<td>39.39</td>
<td>38.2</td>
</tr>
<tr>
<td>Delhi15†</td>
<td>1996–1998</td>
<td>263</td>
<td>21.43</td>
<td>27.03</td>
<td>23</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Bangalore14</td>
<td>1999–2000</td>
<td>226</td>
<td>27.4</td>
<td>15.5</td>
<td>23</td>
<td>6.6</td>
<td>12.8</td>
</tr>
<tr>
<td>Ahmedabad13</td>
<td>2000–2001</td>
<td>822</td>
<td>57.18</td>
<td>37.47</td>
<td>35.58</td>
<td>35.45</td>
<td>37</td>
</tr>
<tr>
<td>Delhi8‡</td>
<td>2001–2002</td>
<td>92</td>
<td>61.95</td>
<td>53.26</td>
<td>35.86</td>
<td>20.65</td>
<td>42.39</td>
</tr>
<tr>
<td>Present study</td>
<td>2006</td>
<td>2880</td>
<td>52</td>
<td>47.1</td>
<td>14.2</td>
<td>3.7</td>
<td>47.1</td>
</tr>
</tbody>
</table>

* Not performed.
† Studies from different institutes in Delhi.

INH = isoniazid; RMP = rifampicin; SM = streptomycin; EMB = ethambutol; MDR = multidrug-resistant.
mycobacterial culture and DST facilities. The programme is currently limiting DST and treatment to only those with the highest risk of MDR-TB, i.e., Cat II patients, who continue to be smear-positive at the end of ⩾4 months of treatment.\(^{18}\) Establishment of a high capacity quality-assured laboratory infrastructure that can detect drug-resistant TB reliably and rapidly is of high priority.

It is interesting to find SM resistance in four relapse cases. These patients may have been treated earlier with SM-based regimens in the private sector or non-DOTS SM-containing regimens in the government sector. Lower levels of drug resistance were observed in the present study to SM (14.2%) and EMB (3.7%) than those reported in previous studies from different parts of the country (Table 2).\(^{5,8,13–15}\) However, drug resistance patterns among previously treated cases reported in earlier studies have shown wide variations and suffer from the limitation that most of these studies have been conducted among small samples, thus making it difficult to draw any meaningful comparisons.

One limitation of this study is that it was conducted in a tertiary care referral centre; the results may therefore not be representative of the community. It is thus important that future studies on drug resistance be carried out among representative samples of all cases registered in a given study area. Furthermore, we were not able to compare drug resistance patterns between the patients treated under routine programme conditions and those treated in the private sector.

**CONCLUSION**

In this study, a high proportion of drug-resistant cases had MDR in addition to resistance to two or more drugs. This proportion was significantly higher among treatment failures compared to relapse and treatment after default cases, underlining the need for timely identification of Cat I treatment failures by early referral for culture and DST and prompt initiation of appropriate treatment with second-line drugs. This study also underlines the importance of continuous monitoring of trends in drug resistance, which would provide useful input for shaping future policies to prevent the emergence and dissemination of MDR.

**References**

les ou davantage. Environ 47,1% des isolats étaient multirésistants. La résistance à l’isoniazide a été observée dans tous les isolats résistants ; elle est suivie par la résistance à la rifampicine, présente dans 1357 cas (47,1%), à la streptomycine dans 403 cas (14,2%) et à l’éthambutol dans 107 cas (3,72%). On a observé une résistance médicamenteuse plus élevée, y compris la MDR, dans les cas d’échec de traitement par comparaison aux rechutes et aux abandons.

CONCLUSION : Dans une proportion très élevée de cas porteurs de germes résistants aux médicaments, à côté de la résistance à deux ou plusieurs médicaments, on a observé une MDR. Cette proportion est significativement plus élevée dans les échecs du traitement par comparaison aux rechutes et aux cas d’abandon, ce qui souligne la nécessité d’une identification précoce des échecs du traitement grâce à une référence précoce pour culture et tests de sensibilité permettant la mise en route d’un traitement approprié.

MARCO DE REFERENCIA : Centro estatal de demostración del tratamiento antituberculoso en Delhi, India.

OBJETIVO : Obtener un cálculo de referencia de la prevalencia de tuberculosis multidrogorresistente (TB-MDR) en los casos de tuberculosis previamente tratados en el centro estatal contra la tuberculosis en el año 2006.

MÉTODOS : Estudio retrospectivo en el cual se analizaron los resultados de farmacosensibilidad obtenidos en este centro en 5252 pacientes tuberculosos tratados previamente.

RESULTADOS : De los 2880 aislados clínicos de *Mycobacterium tuberculosis*, 1498 (52%) fueron resistentes a uno o varios medicamentos antituberculosos. Cerca de 47,1% de los aislados fue MDR. Se observó resistencia a isoniazida en todos los aislados resistentes ; siguieron en frecuencia la resistencia a rifampicina en 1357 casos (47,1%), a streptomicina en 403 (14,2%) y a etambutol en 107 (3,72%). Los casos de fracaso terapéutico presentaron más resistencias, incluida la MDR, que los casos de recaída y abandono, en forma estadísticamente significativa.

CONCLUSIÓN : Una proporción muy alta de casos con farmacorresistencia presentaron MDR además de resistencia a dos o más medicamentos. Esta proporción fue significativamente más alta en los casos de fracaso terapéutico, comparados con los casos de recaída y tratamiento después de abandono, lo cual se destaca la necesidad de una detección precoz del fracaso terapéutico, practicando la remisión para cultivo, pruebas de sensibilidad a los medicamentos y comienzo de un tratamiento apropiado.