Surveillance of anti-tuberculosis drug resistance in Ernakulam District, Kerala State, South India

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SUMMARY

SETTING: This is the first report on drug resistance surveillance (DRS) in Ernakulam District, Kerala, South India, based on a standard protocol from World Health Organization (WHO) guidelines.

OBJECTIVES: To determine the level of drug resistance among smear-positive pulmonary tuberculosis (PTB) patients with no history of previous treatment in Ernakulam District, Kerala State.

DESIgn: Two additional sputum samples were collected from all consecutive new smear-positive PTB cases registered under the revised National TB Control Programme (RNTCP) formulated by the Government of India. The generic protocol developed by the Central TB Division for district level DRS in accordance with WHO guidelines was followed. Training of laboratory staff and other health personnel, periodic monitoring and quality assurance of laboratory work were carried out by the Tuberculosis Research Centre, Chennai.

RESULTS: A total of 305 (88.7%) sputum samples were positive for culture. Resistance to any drug was seen in 27.9% and multidrug-resistant tuberculosis (MDR-TB) was observed in 2%. Monoresistance to rifampicin and streptomycin was observed in respectively 1% and 17% of cases, and 27.1% resistance was observed to any drug in the younger age group.

CONCLUSION: MDR-TB is within expected ranges in Ernakulam District. Further studies that include the private sector are needed in the state among different age groups.

KEY WORDS: TB; drug resistance surveillance; Kerala

THE COMPREHENSIVE third global report on the prevalence of drug resistance in tuberculosis (TB), published in 2004, provided details on levels of drug resistance to isoniazid (INH), rifampicin (RMP), ethambutol (EMB) and streptomycin (SM) for the period 1994–2002 in 90 countries covering 109 surveillance projects.1 However, only 11% of South-East Asia was covered. Available information on drug resistance in TB during the early decades of India’s National Tuberculosis Control Programme (NTCP), which accounts for the largest number of TB patients in the world, was either localised, inaccurate or incomplete.2,3

In view of this deficiency and to obtain reliable and periodic updates on drug resistance prevalence for the entire country, the Tuberculosis Research Centre (TRC), on behalf of the Central TB Division (CTD), Government of India, provided a generic protocol based on the World Health Organization (WHO) guidelines to evaluate drug resistance surveillance (DRS) in any particular district in India.4 DRS studies carried out based on this standardised protocol showed that multidrug resistance (MDR, defined as resistance to at least INH and RMP) ranged from 2.5% to 3.4% and resistance to any drug from 7.9% to 27.1% in two nearby states of South India, Tamil Nadu and Karnataka.3,5,6

Although the NTCP was operational in Kerala State from its inception, TB remained an important public health problem. Despite the clinical and occasionally microbiologically confirmed evidence of drug-resistant tuberculosis (DR-TB), no reports based on well-conducted studies on drug resistance have yet been published from Kerala. During 1996–1999, the District Tuberculosis Centre (DTC) in Ernakulam recorded clinical evidence of DR-TB* in 30 of 2500 (1.2%) TB patients registered. However, none of these cases were bacteriologically confirmed due to lack of drug susceptibility testing (DST) facilities in the state.

* Patients who complete treatment as advised, but who remain acid-fast bacilli sputum-positive

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The new DOTS strategy has been implemented by the Revised NTCP (RNTCP) in Ernakulam District since June 2000. The case detection rate during the study period (20 May–27 October 2004) was 41% and the cure rate was 85%. The present study was carried out utilising funds sanctioned by the CTD in 2003. TRC, Chennai, provided necessary assistance in calculating sample size, training laboratory personnel, monitoring progress and quality assurance of laboratory output.

The aim of the study was to determine the level of drug resistance among new cases of TB in Ernakulam District, Kerala State, and to use the levels of drug resistance as a performance indicator for the RNTCP. We report here DST results for 305 strains of *Mycobacterium tuberculosis* isolated from individual patients from all over the district.

**MATERIALS AND METHODS**

**Case definition**

Case definitions were as per WHO guidelines: new TB patients were individuals who had never received any treatment for TB or who had taken anti-tuberculosis drugs for less than one month.7

**Project area**

The project area included the entire Ernakulam District, with a population of about 3.5 million. It is situated in the central region of Kerala (Figure 1); Kochi is the industrial capital of Kerala. In all, there were seven tuberculosis units (TUs)* and 41 microscopy centres (MCs). Of the 41 MCs, 29 were in the government sector and the rest in the private sector. Figure 1 shows the even distribution of MCs over the entire district, thereby ensuring representativeness of the sample.

**Study design**

The generic protocol for the district, developed by the TRC in accordance with the WHO guidelines, was followed.4

**Sample size calculation**

Sample size was calculated based on the available data on the level of MDR-TB among new cases in India and the availability of external quality assurance (EQA) for smear microscopy. Samples were therefore collected only from the MCs covered by the RNTCP. For an estimated RMP resistance prevalence of 0.5–3.5%, the required sample size for the district was 70 (10% precision, 95% confidence interval [CI]). How-

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* In the RNTCP, for administrative purposes every district is divided into four or five TUs, each generally covering a population of about 500,000 and including at least five microscopy centres.

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ever, a sample size of 320 was estimated based on the expected level of 15–30% overall resistance to any single drug, with 10% precision and 95% CI.5 An additional 10% was added for possible loss due to contamination, etc. This increased the sample size to 352.

**Organisation and intake**

As the Indian RNTCP was expanding rapidly, obtaining rapid, reliable DRS data for new cases at the selected districts in different parts of the country was a priority. At a later stage new and retreatment cases would be included in the state-level surveillance. Two additional sputum samples were collected from all consecutive new smear-positive TB cases registered under the RNTCP during the project period from May to September 2004. Training for medical officers and laboratory personnel was conducted at district level to ensure the collection of good quality sputum samples after proper elicitation of clinical history. A structured intake form duly filled in by the medical officers of the respective MCs gave patients’ previous treatment history.

All the Diagnostic Microscopy Centres (DMCs) were provided with the requisite numbers of sterile McCartney bottles containing 5 ml 1% cetylpyridin-
ium chloride (CPC) and 2% sodium chloride solution for collecting sputum. The samples were collected and transported to the laboratory by the project social worker during prescheduled visits to each of the centres.

Organising the laboratory
A separate mycobacteriology laboratory was established with adequate biosafety measures under guidance from the TRC, Chennai. Culture and DST were carried out at this laboratory. The TRC ensured the quality of the work done for the entire study period by training the technicians, periodic on-site evaluation and EQA of DST.

Bacteriological investigations
Sputum samples were processed for acid-fast bacilli (AFB) smear, culture and DST standard procedures. Centrifuged, washed deposits were inoculated onto plain Löwenstein-Jensen (LJ) medium for primary isolation. *M. tuberculosis* was identified based on absence of growth on LJ medium containing 500 mg/l para-nitrobenzoic acid. All positive cultures for *M. tuberculosis* were subjected to DST by the proportion method. The critical proportion for declaring a strain as resistant to the drug was growth of ≥1%.8

Data processing
The clinical histories of the patients and their culture and sensitivity reports were analysed using Epi Info software (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Quality control for clinical history and follow-up information
To ensure the correctness of data on a patient’s profile, 30% of the patients selected at random were interviewed again using a structured questionnaire. All patients showing concurrent resistance to INH and RMP (MDR-TB) and those with monoresistance to RMP were also interviewed again in depth to elicit information on previous anti-tuberculosis treatment.

RESULTS

Sample characteristics
In all, samples were received from 351 patients. Of 41 DMCs identified for sputum collection, only 20 of the 29 (69%) government MCs and 7 of the 12 (58%) private sector MCs contributed samples. However, mapping (Figure 1) of these centres shows that both government and private DMCs are evenly distributed in each of the TUs throughout the entire district. As samples were collected from patients covering all the TUs, the whole of Ernakulam District was adequately represented. Table 1 shows the distribution of samples across the government and private sector.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>New (n = 305)</th>
<th>Total (n = 312)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age groups</strong></td>
<td></td>
<td></td>
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<tr>
<td>&lt;14</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
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<tr>
<td>14–24</td>
<td>29 (9.5)</td>
<td>29 (9.3)</td>
</tr>
<tr>
<td>25–34</td>
<td>29 (9.5)</td>
<td>31 (9.9)</td>
</tr>
<tr>
<td>35–44</td>
<td>64 (21)</td>
<td>64 (20.5)</td>
</tr>
<tr>
<td>45–54</td>
<td>78 (25.6)</td>
<td>81 (26)</td>
</tr>
<tr>
<td>55+</td>
<td>104 (34.1)</td>
<td>106 (34)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>247 (81)</td>
<td>252 (80.8)</td>
</tr>
<tr>
<td>Female</td>
<td>58 (19)</td>
<td>60 (19.2)</td>
</tr>
</tbody>
</table>

Paired samples
Of the 351 samples, 314 were paired and 37 were single. Many MCs, including the referral laboratories, provided single samples for various reasons, e.g., patients were unwilling to provide a second sample or were begun on treatment before providing the second sample, etc. Of the 314 samples received in pairs, only 225 (71.6%) grew *M. tuberculosis* in pairs. The *M. tuberculosis* isolates grown from the paired samples showed no difference in drug susceptibility patterns.

Patient characteristics
There were three times more male than female patients, and twice as many patients aged ≥35 years as <35 years. The patient characteristics are shown in Table 2.

Samples subjected to DST
Seven of the 351 samples were excluded due to misclassification; 344 samples underwent culture, and *M. tuberculosis* was not isolated in 39 samples. Isolates from 305 samples were subjected to DST. Figure 2 shows a flow chart summarising the details.

Drug susceptibility pattern
Resistance to at least one anti-tuberculosis drug was found in 27.9% and MDR-TB in 2% of samples; 18.8% of isolates with any drug resistance belonged to persons aged ≥35 years. The DST patterns are shown in Table 3. Table 4 gives a detailed analysis of DST by age group.
DISCUSSION
The reliability of any DRS study depends upon the representativeness of the samples collected, proper elicitation of each patient’s previous treatment history and routine practice of a fully functional internal quality control and EQA programme for culture and DST.4 Figure 1 provides evidence that the samples are representative of the entire population of the district. Non-availability of results from 39 (11.3%) samples due to contamination or lack of growth was within the permissible limit of DRS guidelines.4 The male-to-female ratio among the culture positive samples was 4:1; low proportions of females among TB patients have been reported earlier in Ernakulam District.9 Of the 225 sputum samples that showed *M. tuberculosis* growth in pairs, all but one showed identical DST patterns.

The majority of the samples (86.6%) came from the government sector (Table 1), reflecting the low participation of the private sector in the RNTCP. The prevalence of resistance to any anti-tuberculosis drug seen in this study was 27.9% (Table 3). The prevalence of resistance to any drug varied from 7.9% to 27.1% in studies reported from nearby Tamil Nadu state.5 While reviewing the current status of MDR-TB in different parts of India, Rajendra Prasad reported that MDR-TB prevalence ranged from 0.4% to 3.4% among new cases and from 6% to 100% among previously treated cases in the period 1992–2005.10 However, data on acquired drug resistance in all these studies were based on non-representative samples, and were most often from tertiary care centres. An ongoing DRS programme in two large western states of India, Gujarat and Maharashtra, with populations of about 60 and 100 million, respectively, is expected to provide authentic data on the level of drug resistance to both first- and second-line anti-tuberculosis drugs in 2007.

Studies from the National Tuberculosis Institute, Bangalore, showed rates of resistance to any drug of 16.7% in Hoogli, West Bengal, and 5.3% in Mayurbhanj District, Orissa State. MDR-TB was respectively 0.4% and 3% in these two sites.11 The rate observed in the present study was 2%. As has been observed in many studies, all the MDR cases also exhibited resistance to one or more other drugs. Studies from different parts of Tamil Nadu also reported MDR-TB prevalence of 2.5% and 3.4%,5 comparable to the findings of the present study. A summary of studies of initial drug resistance among *M. tuberculosis* isolates in India also showed MDR-TB rates ranging from 0 to 5.3% from 1982 to 2004.3 Resistance to any drug (27.1%), and particularly to SM (22%), was very high among patients aged ≤34 years. MDR-TB was not found in this group. One possible reason for this observation could be the high availability of private medical care and possible indiscriminate use of SM. High drug resistance seen among young patients may reflect exposure of this group in particular to drug-resistant cases in the past two decades. This is an important observation. A

### Table 3 Drug susceptibility patterns

<table>
<thead>
<tr>
<th>New patients</th>
<th>n (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients tested</td>
<td>305 (100)</td>
<td></td>
</tr>
<tr>
<td>Fully susceptible</td>
<td>220 (72.1)</td>
<td></td>
</tr>
<tr>
<td>Resistance to any anti-tuberculosis drug*</td>
<td>85 (27.9)</td>
<td>71.8–98.2</td>
</tr>
<tr>
<td>Any INH resistance</td>
<td>27 (8.8)</td>
<td>21.7–32.3</td>
</tr>
<tr>
<td>Any RMP resistance</td>
<td>11 (3.6)</td>
<td>8.7–13.3</td>
</tr>
<tr>
<td>Any EMB resistance</td>
<td>8 (2.6)</td>
<td>6.3–9.7</td>
</tr>
<tr>
<td>Any SM resistance</td>
<td>72 (23.6)</td>
<td>60.2–73.8</td>
</tr>
<tr>
<td>Monoresistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>To INH</td>
<td>8 (2.6)</td>
<td>6.3–9.7</td>
</tr>
<tr>
<td>To RMP</td>
<td>3 (1)</td>
<td>2.4–3.6</td>
</tr>
<tr>
<td>To EMB</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>To SM</td>
<td>53 (17)</td>
<td>43.7–62.3</td>
</tr>
<tr>
<td>Polydrug resistance†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-drug resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP + INH</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>SM + INH</td>
<td>10 (3.3)</td>
<td>7.91–2.09</td>
</tr>
<tr>
<td>EMB + INH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP + EMB</td>
<td>2 (0.6)</td>
<td>1.6–2.4</td>
</tr>
<tr>
<td>RMP + SM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-drug resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP + INH + SM</td>
<td>1 (0.3)</td>
<td>0.8–1.2</td>
</tr>
<tr>
<td>EMB + SM + INH</td>
<td>3 (1)</td>
<td>2.4–3.6</td>
</tr>
<tr>
<td>RMP + EMB + INH</td>
<td>2 (0.6)</td>
<td>1.6–2.4</td>
</tr>
<tr>
<td>Resistance to all anti-tuberculosis drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH + RMP + EMB + SM</td>
<td>3 (1)</td>
<td>2.4–3.6</td>
</tr>
<tr>
<td>Multidrug resistance‡</td>
<td>6 (2)</td>
<td>4.7–7.3</td>
</tr>
</tbody>
</table>

* With or without resistance to other drugs.
† Resistance to any two or three anti-tuberculosis drugs.
‡ Resistance to at least INH and RMP. These six are also included in the groups with resistance to three and four drugs.

CI = confidence interval; INH = isoniazid; RMP = rifampicin; EMB = ethambutol; SM = streptomycin.
similar observation has been made recently by Sophia et al. in Bangalore, India, and Bloch et al. in the USA.6,12 In India, there are ongoing efforts to ensure standardised treatment regimens for all TB patients to prevent indiscriminate use of drugs.

Compared with earlier studies in Tamil Nadu, a marginal rise in drug resistance levels was observed over a 10-year period.13 The present observation would be helpful to monitor future trends in this setting. Chandrasekaran et al. reported a higher rural prevalence of drug resistance in Karnataka, another neighbouring state of Kerala. This was perhaps due to misclassification of patients, as observed by the authors themselves.14 No such differences in urban and rural settings in patterns of drug resistance were observed in the present study. It was also noted that three of the six MDR-TB patients and all those with monoresistance to RMP responded to Category 1 treatment and remained smear-negative after 24 months of follow-up.

A few observations made in this study were different from studies performed elsewhere: RMP monoresistance was observed among 1% of cases, all of whom were untreated patients. This is not a common occurrence in many DRS studies reported to date.3,15 As resistance to RMP is usually considered a surrogate marker for MDR-TB, this observation is striking, and further molecular and other studies are underway to ascertain the level of resistance and the pattern of rpoB mutations.16 Personal follow-up interviews with these patients did not reveal a history of previous treatment or high-risk behaviours for human immunodeficiency virus transmission, common risk factors among such patients.17 Compared to other studies in India, the high prevalence of SM resistance (23.6%) and SM monoresistance (17%) and the low level of INH resistance (8.8%) were other distinct findings.12,14,15

Limitations of the study
As this is the first study from Kerala based on a standard protocol we faced many operational constraints, including absence of trained staff in some MCs, dysfunctional microscopes in the private laboratories and lack of government coordination in certain instances. Although the role of the private sector in health care is significant in Kerala, it is not reflected in the collection of samples due to the still meagre participation of private practitioners in the RNTCP.

CONCLUSIONS
Although the prevalence of MDR-TB in Ernakulam District was within the expected range, it is still of concern and demands diligent action. The observations of the study emphasise the need to continue RNTCP
and DOTS more vigorously, to improve private sector involvement in the RNTCP and to ensure standardised treatment regimens for all TB patients to prevent indiscriminate use of drugs. To fully understand the true magnitude of drug resistance in Kerala, it is necessary to conduct state-level DRS, including both new and treated cases, involving the private sector to the maximum extent possible. Further studies are needed to understand the high levels of SM resistance among patients of a specific age group.

Acknowledgements
We thank the Director and staff of TRC, Chennai, for their help in organising the laboratory and for carrying out quality control; the State TB Officer, Kerala, DTO, the DMO, Ernakulam, and all the medical officers in the TUs and MCs for their cooperation; Ms Bindhu Kurien, Junior Microbiologist, Ms Sreedhara Kurien, Senior Technician in the laboratory and Ms Sreedhara Paul, Project Coordinator for organising and carrying out laboratory work; all the patients who willingly cooperated with us by providing sputum samples; and the administrative authorities of our institution for generously providing sufficient funds to complete the study.

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References

RÉSUMÉ
CONTEXTE : Il s’agit du premier rapport sur la surveillance de la résistance aux médicaments (DRS) provenant du district d’Ernakulam, Kerala, Inde du Sud, rapport basé sur un protocole standard original conforme aux directives de l’OMS.
OBJECTIFS : Déterminer le niveau de résistance aux médicaments dans les cas de tuberculose pulmonaire (TPB) à bacillecoposie positive sans antécédents de traitement antérieur dans le district d’Ernakulam de l’Etat de Kerala.
SCÉMA : On a prélevé deux échantillons supplémentaires d’expectorations dans tous les nouveaux cas consécutifs de TP à bacillosecopie positive enregistrés au sein du Programme National Révisé de Lutte contre la Tuberculose (RNTCP) élaboré par le Gouvernement d’Inde. On a suivi le protocole original développé par la division centrale contre la tuberculose (CTD) pour le DRS du niveau de district en accord avec les directives de l’OMS. La formation du personnel de laboratoire et des autres personnels de santé, un suivi périodique et un contrôle de qualité du travail de laboratoire ont été menés par le Centre de Recherche de la Tuberculose de Chennai.
RÉSULTATS : Sur les 305 échantillons d’expectorations (88,7%) positifs pour la culture, on a trouvé une résistance à n’importe quel médicament dans 27,9% et une multirésistance (MDR) dans 2%. Des monorésistances à la rifampicine et à la streptomycine ont été observées respectivement dans 1% et 17% des cas. Dans le groupe d’âge plus jeune, on a trouvé un taux de résistance de 27,1% à n’importe quel médicament.
CONCLUSION : La TB-MDR dans ce district se situe dans les marges attendues. Des études complémentaires sont nécessaires au niveau de l’Etat parmi différents groupes d’âge, y compris dans le secteur privé.
RESUMEN

MARCO DE REFERENCIA: Es este el primer informe sobre la vigilancia de la farmacorresistencia en el distrito de Ernakulam, Kerala, en el sur de la India, basado en un protocolo común, estandarizado según las normas de la Organización Mundial de la Salud (OMS).

OBJETIVOS: Determinar el grado de farmacorresistencia en los casos de tuberculosis pulmonar (TBP) bacilífera sin antecedente de tratamiento previo, en el distrito de Ernakulam, del estado de Kerala.

MÉTODOS: Se recogieron dos muestras suplementarias de esputo de todos los casos nuevos de TBP con baciloscopia positiva, registrados en el Programa Nacional Revisado de Control de la Tuberculosis (RNCTP), formulado por el gobierno de la India. Se aplicó un protocolo común de vigilancia de la farmacorresistencia elaborado por la División Central de Tuberculosis, según las normas de la OMS. En el Centro de Investigación en Tuberculosis de Chennai se llevó a cabo la capacitación del personal de laboratorio y demás profesionales de la salud, la supervisión periódica y el control de la calidad del trabajo de laboratorio.

RESULTADOS: Trescientas cinco muestras de esputo (88,7%) dieron cultivos positivos para Mycobacterium tuberculosis. El 27,9% de estos presentó resistencia a alguno de los medicamentos antituberculosos y 2% fueron MDR. Se observó monorresistencia a rifampicina en 1% de los cultivos y a estreptomicina en 17%. En el grupo de pacientes más jóvenes se observó un 27,1% de resistencia a cualquiera de los medicamentos.

CONCLUSIÓN: La incidencia de TB-MDR en el distrito se encuentra dentro de los límites previstos. Se precisan más estudios a escala estatal de los diferentes grupos de edad y que tengan en cuenta el sector privado.