RESEARCH PAPERS
Publications and Presentations
NON-TUBERCULOUS LUNG MYCOBACTERIOSIS IN GUJARAT†

S.S. TRIVEDI*, S.G. DESAI**, and S.B. TRIVEDI***

SUMMARY: The isolation rate of mycobacteria other than Mycobacterium tuberculosis and their species, from patients admitted in the K.J. Mehta Tuberculosis Hospital, Amargadh, Gujarat, from July 1983 to June 1985, was studied.

The isolation rate of disease-associated non-tuberculous mycobacterial strains was estimated as 0.15%, with the back-ground isolation rate (for casual isolates) as 0.25%. The former consisted of Mycobacterium kansasii and Mycobacterium fortuitum only.

Introduction

Mycobacterium tuberculosis is the most common mycobacterial pathogen isolated from pulmonary lesions in India. Some other mycobacteria, known as atypical, anonymous or unclassified mycobacteria, have also been isolated frequently. We know now that these species are not atypical of the genus mycobacterium, but have reproducible characteristics that are used for identification. The terms anonymous or unclassified are no longer acceptable. Most appropriate and least objectionable terms proposed are 'non-tuberculous mycobacteria' (NTM) and MOTT bacilli—mycobacteria other than tubercle bacilli. (Wolinsky, 1979; Good, 1979).

A study is being undertaken at Tuberculosis Research Centre, Amargadh (Gujarat) to determine the frequency of lung diseases due to NTM within the state and to identify their species.

The study is still continuing and the present report deals with the mycobacteria isolated from July, 1983 to June, 1985.

Material and Methods

The study is based on the patients admitted in the K.J. Mehta Tuberculosis Hospital, Amargadh (Gujarat). Only residents of Gujarat were included and recent migrants to Gujarat from other states were excluded. Out-patients were not included. Relapses, if admitted again in study years, were included only if NTM were isolated, but if tubercle bacilli were isolated again, the patient was not included in the study. When several specimens of sputum from the same patient were examined, the organisms from the first culture were included. Thus, sputum specimens from a total of 2,945 patients were examined.

Sputum specimens were collected in September, December, March and June of the study year, so as to get a good number of newly admitted patients and also enough time to proceed for the screening and identification of NTM.

Isolation: Isolation of mycobacteria was carried out as follow:

Sputum (mix. 4 ml.) was collected in a sterile wide mouth screw capped bottle and equal volume of 4% NaOH was added. The mixture was shaken for 5 minutes to homogenize the specimen and then left at room temperature for 15 minutes. This was then centrifuged at 3,000 r.p.m. for 15 minutes and supernatant was discarded. Sterile distilled water was added to the sediment and centrifuged at 3,000 r.p.m. for 10 minutes. Supernatant was discarded. Sediment was inoculated with 4.0 mm. loop into 2 slopes of L.J. medium and incubated at 37° C. Slopes were observed daily for the first week to pick up any rapidly growing strain and then twice a week for eight weeks before discarding them as negative.

Screening: Acid-fast organisms isolated were examined microscopically using the Ziehl-Neelsen method of staining. Screening for non-tuberculous mycobacteria was carried out by three tests (Tsukamura, 1981 a): (i) niacin production test (Konno, 1956), (ii) test for growth on PNB medium—L.J. medium containing 0.5 mg. of p-nitrobenzoic-acid/ml. (Tsukamura and Tsukamura, 1964) and (iii) test for growth on Hydroxylamine medium—L.J. medium containing 0.125 mg. of hydroxylamine/ml. (Tsukamura, 1965 a, b).
As shown in Table 1, if the test organisms showed negative niacin production and growth on the PNB and/or Hydroxylamine medium, organisms were considered to be suspect of NTM, and were subjected to further identification test.

**Table 1**

*Screening of Non-tuberculous Mycobacteria*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Niacin test</th>
<th>Growth on PNB-medium</th>
<th>Growth on Hydroxylamine-medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Other Mycobacteria</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Identification:** Identification of mycobacteria was made following the system of successive differentiation with the use of dichotomous keys. At every step, at least three key characteristics were used and differentiation was decided by taking two or more fitnesses for these characteristics. Final identification was decided by comparison of overall similarity of the characteristics of our isolates with the key characteristics of named species (Tsukamura, 1967; Tsukamura, 1975; Tsukamura, 1981b). Identification tests were monitored by including standard mycobacterial cultures as known positive and negative controls. A group of standard mycobacterial cultures was obtained from Trudeau Mycobacterial Culture Collection, National Jewish Hospital and Research Centre, Denver, Colorado, U.S.A.

The following tests were performed to identify the strains: (Tsukamura, 1975; Vestal, 1975).

1. Growth at 28° C and 45° C.
2. Growth within 7 days.
3. Photochromogenicity.
4. Growth on hydroxylamine medium (0.25 mg and 0.5 mg/ml).
5. Catalase test (Semi-quantitative and at pH 7/68° C).
6. Growth on 5% NaCl medium.
8. Arylsulfatase test (3 days and 2 weeks).
11. Tween hydrolysis (7 days and 14 days). 12. Tellurite reduction test
13. Peridinomycin test
14. Urease test

**Definition of lung disease due to non-tuberculous mycobacteria**

Occasional isolation of these organisms from sputum in the absence of related disease or without any association with the disease, may occur due to temporary colonization in the respiratory tract. These organisms were referred to as *casual isolates*.

A definite diagnosis of lung disease due to NTM was based on the criteria published by American Thoracic Society. (American Thoracic Society, 1974).

i) Presence of radiographic abnormalities indicating active disease.

ii) Isolation of the same mycobacterial strain repeatedly from the sputum in the absence of other pathogens.

**Results**

Out of a total 2,945 sputum specimens, 2,016 (68.4%) were culture positive for acid-fast bacilli. Among these, 8 strains (0.4%) were screened out as non-tuberculous mycobacteria. All others were identified as *M. tuberculosis*. The species of NTM isolates are shown in Table 2. Three NTM strains, 2 of *M. kansasii* and 1 of *M. fortuitum*, were found to be associated with disease, giving an isolation rate of 0.15%. The other 5 strains were found to be...

**Table 2**

*Kind of Non-tuberculous Mycobacterial species isolated during the study*

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease associated</th>
<th>Casual isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. kansasii</em></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><em>M. xenofaciens</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>M. gordonae</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>
casual isolates, giving a background isolation rate of 0.25%.

Discussion

Increasing interest of mycobacteriologists in the non-tuberculous mycobacteria has improved our knowledge about the incidence of lung disease due to NTM. Data on the frequency of lung disease due to NTM are available for several countries. The frequency is 4.6% in Western Australia (Carruthers and Edwards, 1955), 0.1% in South Africa (Stottmeir, Kleeberg and Blockbergen, 1966), 2.8% in Canada (Gale, 1976), 1.7% in Japan (Tsukamura et al., 1981), 3.3% in Rhodesia (Tsukamura et al., 1972), 1 to 30% in various locations of United States (Wolinsky, 1979).

In India, the isolation rate varies from 0.7 to 34% (Thomas et al., 1961; Patel, D’Souza and Sayed, 1966; Choudhri et al., 1979; Ramakrishnan, 1981; Kotian et al., 1981; Das et al., 1982; Hardas and Jayaraman, 1984; Paramesivan et al., 1985). However, most of the reports from India range from less than 1.0% to as high as 13.1%, except one, which reports 34% isolation rate (Patel, D’Souza and Sayed, 1966), which was unusually high. In many reports, there is no distinction between casual isolations and actual cases of disease, or mycobacterial strains were not identified up to the species-level. Merely, the report of isolation rate or number of mycobacterial isolates does not give an exact idea of the clinical significance of various species of potentially pathogenic non-tuberculous mycobacteria.

In the present study, an isolation rate of 0.15% has been obtained, which is quite low, when compared with other reports. Temporary colonization of NTM in the respiratory tract is not uncommon, and about 5% of the healthy individuals were found to have such colonization (Kotian et al., 1983). In the present study, all the patients were known cases of pulmonary diseases and were taking anti-tuberculous chemotherapy and this may be the reason for the suppression of temporary colonization and hence, very low casual isolation rate of 0.25% only.

Geographic differences in the occurrence of disease due to a particular non-tuberculous mycobacterial species have been reported. In Europe and the United States, about 50% of all isolates of non-tuberculous mycobacteria obtained from the patients in Tuberculosis Hospital are M. kansasii. In contrast, M. avium-intracellulare strains are in the majority among isolates in Japan, Rhodesia and Australia (as quoted by Tsukamura et al., 1981). In the present study, the number of cases of non-tuberculous mycobacteriosis was too small to indicate the prevalence of particular type of species. However, M. kansasii and M. fortuitum were found to cause the disease, and it was noticeable that no M. avium-intracellulare strain was isolated. In other parts of India, however, isolation of M. avium-intracellulare from sputum has been reported (Kotian et al., 1981; Das et al., 1982; Paramesivan et al., 1985).

In United States and Japan, it has been noticed that as the number of cases of tuberculosis declines, disease due to other mycobacterial species increases (Good, 1979; Mycobacteriosis Research Group of the Japanese National Chest Hospital, 1983). In Gujarat, with a relatively high morbidity of tuberculosis (about 1.5%—unpublished data from Tuberculosis Research Centre, Amargadh), the prevalence of lung disease due to NTM appears to be very low.

Acknowledgement

We are grateful to Dr. J. Kenneth McClatchy, Ph.D., Curator, Trudeau Mycobacterial Culture Collection, National Jewish Hospital and Research Centre, Denver, Colorado, U.S.A for providing us standard mycobacterial strains, and to Mr. S.D. Andharia, senior laboratory technician, Tuberculosis Research Centre, Amargadh, for his help throughout the study.

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PYRAZINAMIDASE ACTIVITY OF MYCOBACTERIUM TUBERCULOSIS—A TEST OF SENSITIVITY TO PYRAZINAMIDE

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Summary
Pyrazinamidase activity has been found to correlate with pyrazinamide sensitivity in strains of Mycobacterium tuberculosis. In vitro sensitivity to pyrazinamide in acidified Löwenstein-Jensen medium, and pyrazinamidase activity by the Wayne method, were determined in 378 clinical isolates of M. tuberculosis. A close correlation was observed between the results of both tests. This method of detecting pyrazinamidase activity was found to be a rapid, simple and reliable substitute for pyrazinamide sensitivity testing, and it overcomes the difficulty of growing M. tuberculosis at pH 5.5, as required in the standard method.

Resume
Une corrélation a été constatée entre l’activité de la pyrazinamidase et la sensibilité des souches de Mycobacterium tuberculosis vis-à-vis du pyrazinamide. La sensibilité in vitro au pyrazinamide en milieu acidifié de Lowenstein-Jensen et l’activité de la pyrazinamidase par la méthode de Wayne ont été déterminées pour 378 isolats cliniques de M. tuberculosis. Les auteurs ont observé une corrélation étroite entre les résultats de ces deux tests. Cette méthode pour mesurer l’activité de la pyrazinamidase s’est avérée être un substitut rapide, simple et fiable pour tester la sensibilité vis-à-vis du pyrazinamide; de plus, elle résout la difficulté de croissance que rencontre M. tuberculosis au pH 5.5 requis par la méthode standard.

Resumen
Se constató una correlación entre la actividad de la pirazinamidasa y la sensibilidad de cepas de Mycobacterium tuberculosis a la pirazinamida. Se determinó la sensibilidad in vitro a la pirazinamida en medio acidificado de Lowenstein-Jensen y la actividad de la pirazinamidasa por el método de Wayne para 378 aislados clínicos de M. tuberculosis. Se observó una correlación estrecha entre los resultados de estas dos pruebas. Este método para medir la actividad de la pirazinamidasa se mostró como un substituto rápido, simple y fiable para determinar la sensibilidad a la pirazinamida; además resuelve la dificultad de crecimiento que encuentra M. tuberculosis en ph 5.5 requerido por el método estándar.

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Introduction

It has become important to determine the in vitro sensitivity of Mycobacterium tuberculosis to pyrazinamide (Pz), because of its increasing clinical use in short course regimens for the treatment of tuberculosis. The difficulty in performing sensitivity tests is that Pz is only active against M. tuberculosis in an acid environment, so that the culture medium needs to be of low pH [1]. Pz has maximal anti-tuberculosis activity in vitro at pH 5.5 [2], but many strains of M. tuberculosis grow poorly or not at all in an acid medium [2, 3, 4, 5].

It has been reported that Pz-sensitive strains of M. tuberculosis produce an enzyme, pyrazinamidase (Pzase) that metabolizes Pz to pyrazinoic acid, while resistant strains have lost this enzyme activity [6]. Detection of Pzase activity has been recommended, therefore, as a useful screening method for determining sensitivity to Pz [3, 5], although its reliability is questionable [7].

The purpose of the present study is to compare the results of Pz sensitivity testing and Pzase activity in clinical isolates of M. tuberculosis and to assess the reliability of the enzyme test.

Materials and methods

The study was performed on 378 strains of M. tuberculosis which were isolated from patients admitted to the K. J. Mehta Tuberculosis Hospital, Amargadh, Gujarat, India. Information about previous treatment was obtained from all the patients.

Pz—sensitivity test

The sensitivity tests to Pz were performed by the proportional method on Löwenstein-Jensen (L.-J.) medium adjusted to pH 5.5, to which Pz was added at a final concentration of 100 μg/ml. The results were read after 6 weeks of incubation at 37 °C. Any strain showing 10 % or more of resistant bacilli was classified as resistant [8].

Level of Pz resistance

One hundred and eighty-three Pz-resistant strains, including Pz-resistant mutants from predominantly Pz-sensitive cultures, showing growth on L.-J. medium containing 100 μg of Pz per ml, were further inoculated on the medium containing 200 μg and 300 μg of Pz per ml to isolate mutants with the highest levels of Pz resistance.

Pyrazinamidase test

Pzase activity was determined by the method described by Wayne [9]. Several loopsfuls (5–10 mg wet weight) of each strain of M. tuberculosis were obtained from L.-J. medium, placed on to the surface of Wayne Pz medium and incubated at 37 °C. After 4 days, 1.0 ml of freshly prepared 1 % ferrous ammonium sulphate was added to each tube. The tubes were refrigerated for 4 h and then examined. The presence of a pink band (any degree of pink colour) indicates Pzase activity.

Results

Out of the 378 clinical isolates of M. tuberculosis tested, 77 strains (20.4 %) would not grow satisfactorily when subcultured in the acid L.-J. medium. Results of the Pzase test and the Pz sensitivity test were obtained on 301 strains and are presented in Table 1.
Table I. Results of Pyrazinamidase test and Pyrazinamide (Pz) sensitivity test.

<table>
<thead>
<tr>
<th>Pzase test</th>
<th>Pz sensitivity test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td></td>
<td>(0–1 %)</td>
<td>(2–9 %)</td>
</tr>
<tr>
<td>Positive</td>
<td>101</td>
<td>23</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>177</td>
</tr>
</tbody>
</table>

Predictive value of positive Pzase test: \( \frac{124}{131} \times 100 = 94.6 \% 

Predictive value of negative Pzase test: \( \frac{170}{170} \times 100 = 100 \% 

Table II. Results of Pzase test and levels of Pz resistance

<table>
<thead>
<tr>
<th>Number of strains tested</th>
<th>Level of Pz resistance (µg/ml)</th>
<th>Pzase test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
<td>120</td>
</tr>
<tr>
<td>38</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>183</td>
</tr>
</tbody>
</table>

**Pzase test and Pz sensitivity**

One hundred and seventy strains found to be negative for Pzase activity were resistant to Pz. All these strains were isolated from cases of treatment-failure or relapse. Of the 131 strains found to be positive for Pzase activity, 124 (94.6 \%) were sensitive to Pz; 72 of them were isolated from patients claiming to have had no previous treatment. Seven other Pzase positive strains, isolated from patients having a definite history of previous treatment with Pz, were classified as Pz-resistant (10–50 \%).

**Pzase test and level of Pz resistance**

Results obtained on 183 Pz-resistant mutants are presented in Table II. No mutant with a high level of Pz resistance was found to retain Pzase activity.

**Discussion**

In recent years determination of Pzase activity has received more attention for its possible use as a screening method for determining the sensitivity of *M. tuberculosis* to Pz. This overcomes the difficulty of growing the organisms at pH 5.5, as required in the standard method of Pz sensitivity testing. The Wayne method, in particular, because of its simplicity and rapidity, is a useful test for determining Pzase activity, but its reliability and correlation with Pz sensitivity require confirmation.

Our study showed a 100 \% predictive value for the negative Pzase test, since all the Pzase negative strains were found to be Pz-resistant. Pzase positivity showed a predictive value of 94.6 \%, as seven out of 131 Pzase positive strains were classified as Pz-resistant. These seven Pz-resistant strains, however, were a mixed population and contained 50–90\% of...
sensitive organisms. When Pz-resistant mutants were isolated from these cultures, they were found to be Pzase negative.

Thus, our findings are in agreement with those of McClatchy et al [3] and Kantor et al [5]. In the former study, pyrazinonic acid production was also detected by the thin-layer chromatography, which was found to be more sensitive than the Wayne method in detecting small quantities of pyrazinoic acid. The Wayne method requires a large inoculum to be used or false-negative readings may result. Kantor et al [5] reported an 81% predictive value for the negative Pzase test, as 15 out of 80 Pzase negative strains were sensitive to Pz. In the present study, no Pzase negative strain was found to be Pz-sensitive. It has previously been reported that strains highly resistant to Pz are not always Pzase negative [7], but we could not confirm this observation.

We, therefore, conclude, that the Pzase test is a simple, rapid and fairly reliable substitute for the qualitative determination of Pz sensitivity of M. tuberculosis.

Acknowledgements

We are grateful to Dr S. B. Trivedi, Director, Tuberculosis Research Centre, Amargadh-364210, Gujarat, India, for his kind co-operation throughout the study. Thanks are due to Mr S. D. Andharia for his technical help and to Mr B. B. Bhatt for typing the manuscript.

References

PRIMARY ANTITUBERCULOSIS DRUG RESISTANCE AND ACQUIRED RIFAMPICIN RESISTANCE IN GUJARAT, INDIA

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Summary

The prevalence of primary antituberculosis drug resistance in Gujarat, as studied between 1983 and 1986, was found to be significantly high, especially for isoniazid (13.9 %) and streptomycin (7.4 %). Primary rifampicin and pyrazinamide resistance were not detected in any strain. The prevalence of rifampicin resistance among treatment failure and relapse cases of pulmonary tuberculosis increased significantly from 2.8 % in 1980 to 37.3 % in 1986. In about 95 % of the rifampicin resistant strains there was also resistance to isoniazid or streptomycin or both; resistance to isoniazid was detected in more than 90 %.

Résumé

La prévalence de la résistance primaire aux médicaments antituberculeux à Gujarat étudiée pendant les années 1983 et 1986 s’est montrée élevée, spécialement pour l’isoniazide (13,9 %) et al streptomycine (7,4 %). Aucune souche n’a montrée de résistance primaire vis-à-vis de la rifampicine ni du pyrazinamide. La prévalence de la résistance vis-à-vis de la rifampicine parmi les cas d’échec au traitement et les cas de rechute de tuberculose pulmonaire a augmenté de façon significative : de 2,8 % en 1980 à 37,3 % en 1986. Pour environ 95 % des souches résistantes à la rifampicine, on a constaté également une résistance à l’isoniazide ou à la streptomycine ou à ces deux médicaments; la résistance à l’isoniazide a été détectée dans plus de 90 % des souches.

Resumen

La prevalencia de la resistencia primaria a los medicamentos antituberculosos en Gujarat, estudiada durante los años 1983 y 1986 se mostró elevada, especialmente para la isoniaicida (13,9 %) y la estreptomicina (7,4 %). Ninguna cepa mostró resistencia primaria a la rifampicina ni a la pirazinamida. La prevalencia de la resistencia a la rifampicina entre los casos de tuberculosis pulmonar cuyo tratamiento ha fracasado o que han presentado recaídas, aumentó de manera significativa: de 2,8 % en 1980 a 37,3 % en 1986. En alrededor de 95 % de las cepas resistentes a la rifampicina se constató igualmente una resistencia a la isoniaicida o a la estreptomicina o a ambas; la resistencia a la isoniaicida se detectó en más del 90 %.

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Introduction

The prevalence of pulmonary tuberculosis is fairly high in Gujarat, India. The disease occurs in about 1.5% of the general population (unpublished data from Tuberculosis Research Centre, Amargadh, Gujarat). Mass antituberculosis chemotherapy is being implemented by 19 Government District Tuberculosis Centres as well as by about 11 voluntary tuberculosis organisations of the state.

The occurrence of primary antituberculosis drug resistance may be taken as an index of the effectiveness of the therapeutic methods adopted on a large scale in a given area [1, 2]. Further, the emergence of resistance to rifampicin needs serious consideration, as its use as a first line drug has increased considerably following the introduction of successful short-course chemotherapy. There have been reports from some parts of the world of wide scale indiscriminate use of rifampicin and, in consequence, of high levels of initial rifampicin resistance, in addition to high levels of isoniazid and streptomycin resistance [3].

It was therefore decided to undertake a study to determine the prevalence of primary antituberculosis drug resistance in Gujarat as well as the prevalence of rifampicin resistance among treatment failure and relapse cases of pulmonary tuberculosis, irrespective of previous chemotherapy.

Materials and methods

The study was conducted at K. J. Mehta Tuberculosis Hospital, Amargadh, a voluntary organisation with 747-bed capacity—the biggest one in Gujarat—located in a rural area. About 3000 individuals are treated here as in-patients and over 30 000 are examined in the out-patients' department every year. The patients come from different regions of Gujarat as well as from other states of India.

The study was carried out from January 1983 to December 1986. The data on rifampicin resistance among eligible patients were also analysed for the previous 3 years.

Criteria for eligibility of patients

Patients of all ages were considered eligible for the study if they were residents of Gujarat—recent migrants to Gujarat from other states were excluded—and were attending K. J. Mehta Tuberculosis Hospital, Amargadh, for the first time, because of symptoms, and had radiographic evidence suggestive of tuberculosis. For studies on primary drug resistance only those patients who had not received any antituberculosis chemotherapy previously were included. Patients were excluded if they had histories of previous antituberculosis chemotherapy even for a short duration or, for rifampicin-resistance studies, had received any type of regimen of antituberculosis chemotherapy elsewhere for not less than 3 months. Care was taken to avoid the re-inclusion of the same patient on his next visit to the hospital. Finally, only those excreting Mycobacterium tuberculosis were included. If the organisms were identified as other mycobacteria the patients were excluded from the study.

Bacteriological procedures

Isolation and identification of mycobacteria were carried out according to the procedures described elsewhere [4]. Tests of sensitivity to streptomycin, isoniazid, ethambutol, thiacetazone, rifampicin and pyrazinamide were performed on Lowenstein-Jensen medium.

The strains were considered resistant to the respective antituberculosis drugs if the growth was observed at the following concentrations (based on Canetti et al [1] and
Tsukamura [5]. streptomycin 8 μg/ml, isoniazid 0.2 μg/ml, ethambutol 4 μg/ml, thiacetazone 2 μg/ml, rifampicin 50 μg/ml, pyrazinamide 100 μg/ml

**Results**

**Primary drug resistance**

A total of 570 *M. tuberculosis* strains, isolated from an equal number of eligible patients.

Table I. Patterns of primary drug resistance in 570 strains of *M. tuberculosis*

<table>
<thead>
<tr>
<th>Total number of strains tested</th>
<th>Number of strains resistant to</th>
<th>Total resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 drug</td>
<td>2 drugs</td>
</tr>
<tr>
<td>570</td>
<td>H 45</td>
<td>SH 19</td>
</tr>
<tr>
<td></td>
<td>S 18</td>
<td>HE 4</td>
</tr>
<tr>
<td></td>
<td>E 14</td>
<td>HT 6</td>
</tr>
<tr>
<td></td>
<td>T 3</td>
<td></td>
</tr>
</tbody>
</table>

*Figures in brackets indicate percentage.
H isoniazid, S - streptomycin, E - ethambutol, T - thiacetazone.

**Figure.** Levels of drug resistance to isoniazid (INH), streptomycin (SM) and rifampicin (RMP) among treatment failure and relapse cases of pulmonary tuberculosis, 1980-1986
were tested for drug resistance patterns. As shown in Table I, 114 (20\%) of the 570 strains were resistant to one or more drugs. The majority of them (14\%) were resistant to only one drug, more than half of these strains being resistant to isoniazid only. Multiple drug resistance was less frequent: 5.1\% strains were resistant to two drugs and 0.9\% strains were resistant to three drugs. Isoniazid resistance was detected among all multiple resistant strains. Rifampicin and pyrazinamide resistance were not detected in any strain. Total resistance to isoniazid was 13.9\%, to streptomycin 7.4\%, to ethambutol 4.0\% and thiacetazone 1.5\%.

No association was found between primary resistance and age or sex of the patients.

Drug resistance among treatment-failure and relapse cases

The Figure shows the levels of drug resistance to isoniazid, streptomycin and rifampicin among treatment-failure and relapse cases of pulmonary tuberculosis during 1980–1986. The data are shown in Table II.

There was a marked increase in isoniazid resistance from 34.5\% in 1980 to 55.8\% in 1986, while streptomycin resistance showed a slight fluctuation around 26\% throughout the study period. Rifampicin resistance, however, increased significantly from 2.8\% in 1980 to 37.3\% in 1986, and there was a rapid increase during 1982–1983.

Table II. Resistance to isoniazid, streptomycin and rifampicin, among treatment failure and relapse cases, 1980–1986.

<table>
<thead>
<tr>
<th>Year</th>
<th>Isoniazid R/N %</th>
<th>Streptomycin R/N %</th>
<th>Rifampicin R/N %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>37/107 34.5</td>
<td>29/110 26.3</td>
<td>3/104 2.8</td>
</tr>
<tr>
<td>1981</td>
<td>41/110 37.3</td>
<td>22/97 22.8</td>
<td>7/97 7.2</td>
</tr>
<tr>
<td>1982</td>
<td>32/90 35.6</td>
<td>22/90 24.4</td>
<td>30/90 32.7</td>
</tr>
<tr>
<td>1983</td>
<td>130/305 42.6</td>
<td>65/259 25.0</td>
<td>87/305 28.5</td>
</tr>
<tr>
<td>1984</td>
<td>217/404 53.7</td>
<td>108/404 26.7</td>
<td>131/404 32.4</td>
</tr>
<tr>
<td>1985</td>
<td>149/298 50.2</td>
<td>78/298 26.2</td>
<td>101/290 34.8</td>
</tr>
<tr>
<td>1986</td>
<td>145/260 55.8</td>
<td>70/260 26.9</td>
<td>97/260 37.3</td>
</tr>
</tbody>
</table>

R = Number of drug resistant strains.
N = Number of total strains tested.

Table III. Rifampicin (RMP) resistance, combined with resistance to either isoniazid (INH) or streptomycin (SM) or both. 1983–1986.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total RMP-resistant strains</th>
<th>Number of strains resistant to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>INH · RMP</td>
</tr>
<tr>
<td>1983</td>
<td>87</td>
<td>35 (46.2)</td>
</tr>
<tr>
<td>1984</td>
<td>131</td>
<td>54 (41.2)</td>
</tr>
<tr>
<td>1985</td>
<td>101</td>
<td>47 (46.5)</td>
</tr>
<tr>
<td>1986</td>
<td>97</td>
<td>48 (49.5)</td>
</tr>
<tr>
<td>Total</td>
<td>416</td>
<td>184 (44.2)</td>
</tr>
</tbody>
</table>

Figures in brackets indicate the percentage of the total RMP resistant strains.
Drug resistance patterns of rifampicin resistant strains

Rifampicin resistance, combined with resistance to either isoniazid or streptomycin, or both, during 1983–1986 is shown in Table III. Almost 90% of rifampicin-resistant strains were also resistant to isoniazid, with or without streptomycin resistance, while only 3.4% of rifampicin resistant strains were resistant to streptomycin only.

Discussion

The present study clearly indicates a fairly high prevalence of primary antituberculosis drug resistance among newly diagnosed patients from Gujarat. There are several reports of a higher prevalence of primary resistance in young persons and children in other regions [6, 7]. In the present study, no association was found between primary drug resistance and the age or sex of patients, which is similar to the findings of ICMR studies [8, 9].

Since primary and acquired drug resistance are major causes of treatment-failure and relapse, a significantly high prevalence of drug resistance is expected in this group. Because there is no prevalence of primary rifampicin (RMP) resistance in Gujarat, it can be presumed that resistance to this drug is almost entirely acquired among treatment-failure and relapse cases. A significant rise in the rifampicin-resistant strains occurred during 1980–1983, followed by a relatively slow but steady rise over the next 3 years. This rise clearly indicates the increasing use of this drug in the chemotherapy of tuberculosis. In many cases, though, it must be used with other ineffective drugs which subsequently leads to the emergence of rifampicin-resistance, because the pattern of resistance development of tubercle bacilli to rifampicin is an 'obligatory single step' pattern [5] and resistance to rifampicin occurs rapidly at 2–3 months if it is used alone or with other drugs which are ineffective against M. tuberculosis [10, 11].

Isoniazid (INH) and streptomycin (SM) are the two drugs most widely used in first line treatment. Prevalence of primary resistance to INH and SM in Gujarat was found to be 13.9% and 7.4% respectively. In about 95% of rifampicin resistant strains, RMP-resistance was combined with resistance to either INH or SM or both. This could mean that the high prevalence of primary INH and SM resistance might be responsible for the emergence of rifampicin resistance.

Further, in the present study, INH-resistance, with or without resistance to SM, was detected in more than 90% of the RMP-resistant strains, while strains resistant to both SM and RMP but still sensitive to INH were seldom isolated. Siddiqui et al [12] reported that all RMP-resistant strains tested were also resistant to INH. Thus, initial resistance to INH, the drug which is included in all regimens, seems to provide the basis for the emergence of RMP-resistance.

The absence of primary rifampicin resistance, or a very low incidence, was reported by some authors [2, 13, 14]. The most significant finding of the present study is that there is no prevalence of primary rifampicin resistance, even though there is a considerable amount of acquired rifampicin resistant strains in the community. This fact supports the view of Daddi et al [2] that one of the characteristics of rifampicin resistant strains is loss of virulence.

The greater the level of drug resistance in a community the greater the number of drugs which should be used in first-line therapy [15]. An important point is that primary resistance to either rifampicin or pyrazinamide is very rare. Both these drugs can therefore be expected to be fully active in all countries, unless initial drug resistance to them becomes a problem, as a result of their misuse either by prescription of inadequate drug combinations, or because of poor patient supervision and compliance [3]. It is very important to avoid the careless use of rifampicin-containing regimens.
Acknowledgements

We are grateful to Dr S. B. Trivedi, Director, Tuberculosis Research Centre, Amargadh, for his kind co-operation throughout the study and for his valuable suggestions during the preparation of the manuscript. Thanks are also due to the Medical Officers of the K. J. Mehta Tuberculosis Hospital, Amargadh, for their help in selecting the eligible patients, to Mr S. D. Andharia for the technical help and to Mr B. B. Bhatt for typing the manuscript.

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