Drug resistant tuberculosis in Estonia

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SUMMARY

SETTING: The incidence of drug resistant tuberculosis in Estonia has increased rapidly during the last five to six years.

OBJECTIVE: To investigate the drug resistance patterns of Mycobacterium tuberculosis isolated from tuberculosis patients in Estonia.

RESULTS: In 1994, 623 cases of tuberculosis were diagnosed in Estonia, 518 new cases with no previous history of tuberculosis, and 105 with a history of previous treatment for tuberculosis. All pulmonary M. tuberculosis isolates from 1994 were analysed for drug susceptibility. Of the 302 new cases (58.3%) that were culture verified, 28% had isolates resistant to one or more of the four drugs tested (isoniazid, rifampicin, streptomycin, ethambutol), and 9% had multi-drug resistant (resistant to at least isoniazid and rifampicin) strains.

CONCLUSION: The incidence of drug resistance in M. tuberculosis is high in Estonia.

KEY WORDS: tuberculosis; drug resistance; Estonia

AS ESTIMATED by the World Health Organization (WHO) about one third of the world’s population is infected with organisms of the Mycobacterium tuberculosis complex, about ten million cases of active disease are estimated to occur each year, and annually three million people die of tuberculosis.1 Multidrug resistant tuberculosis (MDRTB) is becoming an increasing threat in many parts of the world, including the USA.2 6 In many countries with a high incidence of tuberculosis (TB) the situation in terms of drug resistance is not known; consequently, there is scarce information on drug resistance in countries of the former Soviet Union, including the Baltic countries.7

As a part of a long-term commitment, a collaboration was initiated by the Karolinska Institute and the Swedish Institute for Infectious Disease Control (SIIDC) in Stockholm, Sweden, and the Tartu University Lung Hospital, Tartu, Estonia, to investigate the drug resistance pattern of M. tuberculosis strains isolated from TB patients in Estonia. In most published studies an increase in tuberculosis incidence has been observed in combination with a simultaneous increase in the human immunodeficiency virus (HIV) infection in the studied population. In Estonia, with a population of 1.5 million, however, the incidence of HIV infection is still low. According to the Estonian Ministry of Social Affairs only 61 individuals were reported HIV positive up to 1996. In this first study, we report on the drug resistance patterns of M. tuberculosis isolates from all patients in Estonia with culture verified tuberculosis during 1994, and the restriction fragment length polymorphism (RFLP) characteristics of some of the isolates.

PATIENTS

In Estonia the majority of TB patients are diagnosed and treated at either of two lung clinics, Tartu University Lung Hospital in Tartu (catering for South Estonia, approximately 500 000 population) or Kivimae Hospital in Tallinn (catering for a population of approximately 1 million). Culture for mycobacteria is performed at both clinics. The TB laboratory in Tartu University Lung Hospital serves as the reference TB laboratory for Estonia. Notification of tuberculosis is compulsory, and is done by the attending physician. Reports are registered in the National TB Register located at the Kivimae hospital.

The number, sex and age of patients notified in the Estonian TB register during 1994 were analysed. Patients with culture-verified tuberculosis were further analysed about previous history of tuberculosis by checking the patients against the tuberculosis register and by interviewing the attending physicians. An analysis of the reported incidence of initial drug resistance against isoniazid (INH), rifampicin, streptomycin and ethambutol in the Tallinn area between 1983 and 1994 was also performed.

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Characterisation of mycobacterial isolates

*M. tuberculosis* complex isolates from patients with culture-verified tuberculosis presenting at the Lung Hospitals of Tartu and Tallinn were characterized in terms of biochemical heterogeneity and drug resistance. Culture was performed on Löwenstein Jensen (LJ) medium: the specimens were inoculated in one tube containing LJ medium and in one containing LJ medium with 0.6% pyruvate. The tubes were incubated in 37°C for seven weeks and examined weekly for mycobacterial growth. Growth of mycobacteria was confirmed by acid-fast microscopy. Isolates were identified by standard biochemical methods as previously described,8 and by deoxyribonucleic acid (DNA) probe testing using the Accuprobe system (Gen-Probe, San Diego, CA, USA).

Drug susceptibility testing of all isolates was performed both in Estonia and in Sweden, in Estonia by conventional culture on solid media using the proportion method,9 and at SIIDC using radiometric respirometry according to the Bactec system (Becton Dickinson, Sparks, MD).10 This method is in good agreement with the resistance ratio method on LJ medium.11 Drugs tested included streptomycin (4 mg/l), isoniazid (0.2 mg/l), ethambutol (5 mg/l) and rifampicin (2 mg/l). The TB laboratory at SIIDC is part of the network of Supernational Reference Laboratories for drug susceptibility testing of *M. tuberculosis* initiated by the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD),12 and serves as the reference laboratory for the Tartu TB laboratory.

RESULTS

Patients

The incidence of notified tuberculosis cases increased in Estonia during the years 1992–1994, reaching 623 cases in 1994 (Table 1). Of those 623 cases, 347 (55.7%) were culture positive. Of the 15 tuberculosis cases diagnosed outside the Tartu and Tallinn clinics, none was culture verified. Thus the data from the Tartu and Tallinn clinics are in essence representative of the whole country.

Of the 623 cases notified in 1994, 518 were reported as new cases, i.e. with no previous history of tuberculosis (355 men and 163 women, mean age 41 years), and 105 as relapse cases (81 men and 24 women, mean age 56 years). Of the patients with no previous history of tuberculosis, 40 (7.7%) had extrapulmonary tuberculosis; the main clinical forms were 11 with urogenital tuberculosis and 16 with bone tuberculosis. Three of these 40 cases were culture verified on samples from the urogenital tract. Of the 518 new cases, 302 (58.3%) were culture positive for *M. tuberculosis*. Of the 105 cases with a history of previously treated tuberculosis (relapses), 27 (25.7%) had culture-verified tuberculosis.

Characterisation of isolates

All isolates biochemically defined as *M. tuberculosis* complex proved to be positive in the *M. tuberculosis*-specific nucleic acid probe test. All isolates were of the classical human *M. tuberculosis* type, none of the strains conforming to the criteria for *M. bovis*.

Drug resistance

In total 266 of the 302 pulmonary isolates derived from new TB cases, 91 from Tartu and 175 from Tallinn, were tested for drug susceptibility. Of the 266 isolates 75 (28.2%) were resistant to one or more of the drugs tested (Table 2), 27 (10.0%) were resistant to rifampicin, and 24 (9.0%) were resistant to both INH and rifampicin. Thus, 9% had isolates that were multi-drug resistant, i.e. resistant to at least isoniazid and rifampicin. There was no significant difference in susceptibility pattern between the isolates from Tartu and Tallinn (not shown). An analysis of the patients in the Tallinn area showed a steady increase in the reported incidence of initial drug resistance between 1983 and 1994 (Figure). There were 27 culture-positive patients with a history of previously treated tuberculosis: 13 (48%) of the isolates from these patients were INH resistant, 6 (22%) rifampicin resistant, 11 (41%) streptomycin resistant and 6 (22%) resistant to ethambutol; 15% of the strains were multidrug resistant.

DISCUSSION

In a previous preliminary study of drug-resistant tuberculosis in Estonia we found an extremely high incidence of drug resistance.14 This first study was, however, cross-sectional, including patients who had been diagnosed earlier. In this prospective study of drug susceptibility we found a lower, but still high, incidence of initial drug resistance. Initial resistance to

| Table 1 | Incidence of notified tuberculosis cases in Estonia 1992–1994 |
|----------|---------------------|-----------------|-----------------|
|          | 1992     | 1993     | 1994     |
| Cases    |          |          |          |
| New cases| 328      | 441      | 518      |
| Relapses | 75       | 91       | 105      |
| Total number (incidence per 100,000 population) | 403 (25.8) | 532 (35.3) | 623 (41.7) |
| Bacteriologically verified (%) | 219 (54.3) | 303 (57.0) | 347 (55.7) |
Table 2  Drug resistant Mycobacterium tuberculosis strains in 266 new patients in Estonia 1994

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of resistant strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>58 (21.8)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>27 (10.0)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>56 (21.0)</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>19 (7.1)</td>
</tr>
<tr>
<td>One or more drugs</td>
<td>75 (28.2)</td>
</tr>
<tr>
<td>Isoniazid + Rifampicin</td>
<td>24 (9.0)</td>
</tr>
<tr>
<td>All four drugs</td>
<td>12 (4.5)</td>
</tr>
</tbody>
</table>

One or more of the drugs tested was 28.2%, and 9.0% were initially multi-drug resistant (i.e. resistant to at least isoniazid and rifampicin). These figures are similar to those reported from New York in 1992, but are much higher than in neighbouring countries such as Sweden, which had a rate of initial multi-drug resistance of 0.6% in 1994.

The reason for the apparently high transmission rate of drug-resistant strains in the Estonian community is multifactorial. It is generally believed that initial drug resistance reflects the quality and efficacy of treatment programmes. A high rate of initial drug resistance suggests a poor treatment programme, while a low rate indicates a successful programme. The M. tuberculosis isolates have been further characterised by RFLP analysis (manuscript in preparation). When RFLP patterns of the individual isolates were related to the results of drug susceptibility testing, there was a striking difference in homogeneity in RFLP banding patterns between susceptible and resistant isolates. The susceptible M. tuberculosis isolates showed greater heterogeneity, compared to the isolates with different types of drug resistance which mostly belonged to a limited number of clones with patterns that were identical or closely related. The limited number of RFLP patterns among the resistant strains indicates an ongoing spread of multidrug-resistant clones of M. tuberculosis in the Estonian community.

Part of the reason for the present high level of drug resistance in Estonia thus appears to lie in the past. Another important reason may be previous intermittent lack of first line drugs, as well as a lack of alternative second line drugs. The drugs against which the isolates were tested, and to which they were found resistant, are all part of the standard treatment regimen in Estonia.

Another important reason for the high rate of resistance may be lack of compliance with treatment. The typical patient with multidrug resistant TB in Estonia is a male between 25 and 40 years of age, often with social problems, including alcoholism or a history of prison internment. There is at present no public health legislation in Estonia allowing the holding of a contagious patient against his or her will, leading to patients absconding from treatment. At the time of writing (January 1996) there are 103 registered cases of drug-resistant TB in Estonia, some of which have had active smear-positive tuberculosis for a number of years. There is thus a pool of patients with active MDR pulmonary tuberculosis able to spread the disease in the community. To avoid further spread of MDR strains there is thus an urgent need for active identification, isolation and treatment of these patients. Such a programme will require access to alternative drugs, some of which are still unavailable in Estonia, and, most importantly, a steady supply of first line drugs. The high incidence of initial multidrug resistance causes an extraordinary strain on the efforts to establish a working TB programme in Estonia.

Acknowledgements

We thank Toomas Pruunilid for valuable contributions in the initial phase of this study and Solomon Ghebremichael, Lisbeth Klinz and Marianne Ljungström for skillful technical assistance. The study was supported by the Karolinska International Research and Training Programme (KIRT), the Swedish Heart-Lung Association and the Commission of the European Communities, Directorate General XII, Biomedical and Health Research, Biomed 1, Contract #BMH1-CT93-1614.

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14 The Swedish Tuberculosis Index, 1995, Swedish Institute for Infectious Disease Control, Stockholm, Sweden.


RÉSUMÉ

CADRÉ: L’incidence de la tuberculose à germes résistants a augmenté rapidement en Estonie au cours des cinq ou six dernières années.

OBJECTIF: Investigation des types de résistance médicamenteuse de Mycobacterium tuberculosis isolés chez les patients tuberculeux en Estonie.

RÉSULTATS: En 1994, sur les 623 cas de tuberculose diagnostiqués en Estonie, 518 sont des cas « nouveaux », c’est à dire sans antécédents de tuberculose et 105 ont des antécédents de traitement antérieur pour tuberculose. Tous les isolats de M. tuberculosis d’origine pulmonaire réalisés en 1994 ont été soumis à une étude de sensibilité à l’égard des médicaments. Parmi les nouveaux cas, 302 (58,3%) ont été confirmés par la culture, 28% avaient des isolats résistants à un ou plusieurs des quatre médicaments testés (isoniazide, rifampicine, streptomycine, ethambutol) et 9% avaient des souches multirésistantes (c’est à dire résistantes au moins à l’isoniazide et à la rifampicine).

CONCLUSION: L’incidence de la résistance de M. tuberculosis aux médicaments est élevée en Estonie.

RESUMEN

MARCO DE REFERENCIA: La incidencia de la tuberculosis resistente a las drogas en Estonia ha aumentado rápidamente en los últimos cinco a seis años.

OBJETIVO: Investigar los modelos de Mycobacterium tuberculosis resistentes a las drogas aislados de pacientes tuberculosos en Estonia.

RESULTADOS: En 1994, se diagnosticaron en Estonia 623 casos de tuberculosis, de los cuales 518 eran nuevos casos, o sea sin historia de tuberculosis previa, y 105 con una historia de tratamiento previo para la tuberculosis. En todas las muestras aisladas de Mycobacterium tuberculosis desde 1994 se estudió la sensibilidad a las drogas. En los nuevos casos, se efectuó en cultivo en 302 (58,3%) y de ellos el 28% tenían gérmenes resistentes a una o más de las cuatro drogas estudiadas (isoniacida, rifampicina, estreptomicina, etambutol) y 9% tenían cepas multirresistentes (resistente por lo menos a la isonicacida y a la rifampicina).

CONCLUSIÓN: En Estonia la incidencia de la resistencia de M. tuberculosis a las drogas es alta.