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Multidrug-resistant tuberculosis in the Florence province from 1992 to 1995

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SUMMARY

SETTING: Epidemiological data on the frequency of drug-resistant tuberculosis is not available in Italy.

OBJECTIVES: Evaluation of the rate of multidrug-resistant tuberculosis in the Province of Florence, Italy.

DESIGN: Retrospective analysis of all sensitivity tests performed with the Bactec method on initial mycobacterial isolates, from 1 January 1992 to 31 December 1995, in the Province of Florence.

RESULTS: The following rates of resistance were found in the 433 samples tested: isoniazid + rifampicin 2.5%, at least one drug 13.8%, isoniazid 10.6%, rifampicin 3.6%, streptomycin 3.6%, pyrazinamide 1.7% and ethambutol 0.6%. Resistance was higher in foreign-born individuals from high prevalence countries than in the Italian-born population, whereas resistance to streptomycin was more frequent in the latter. The yearly rates of resistance showed no significant variation in the period examined. Clinical data were available in 231 patients: the rate of resistance to at least one drug and to isoniazid + rifampicin were 10.8% and 0%, respectively, in never treated patients, and 28.5% and 7.1%, respectively, in previously treated patients.

CONCLUSION: These data show higher multidrug resistance rates than those found in other European countries such as England and Wales, France and Switzerland. This result suggests the need to establish official guidelines for the correct treatment of tuberculosis in Italy, in order to prevent the onset of drug resistance, and to establish a national surveillance system for mycobacterial resistance.

KEY WORDS: multidrug-resistant tuberculosis; surveillance

TUBERCULOSIS due to mycobacteria resistant to at least isoniazid (H) and rifampicin (R) (multidrug-resistant tuberculosis, MDR-TB[1]) is a serious problem. Whereas current chemotherapy regimens can treat virtually 100% of forms caused by sensitive or only H-resistant strains of *Mycobacterium tuberculosis*,[2] multidrug resistance is associated with a low percentage of cures and a high death rate.[3] In recent years a substantial increase in MDR-TB has been registered in the United States, especially in patients co-infected with the human immunodeficiency virus (HIV), rising from 0.5% in the period 1982–1986 to 3.1% in 1991 in subjects never previously treated for tuberculosis, and from 3% to 6.9% in previously treated individuals. In New York City, 13.9% of all cases registered in 1991 were diagnosed with MDR-TB.[4]

MDR-TB epidemics have been reported in the USA in institutional settings: in one study over 90% of subjects who contracted MDR-TB were HIV seropositive and the mortality rate was 70-90%.[5]

The problem appears to be more contained in Europe than in the United States. Although available data are scarce, surveys involving microbiological laboratories have reported rates of multidrug-resistant strains of 0.5% in France in 1992, 0.6% in England and Wales between 1982 and 1991, and 1.7% in Switzerland between 1995 and 1996.[6–8] In Italy no nation-wide data are available on mycobacterial drug resistance and few clinical studies have been performed.[9–12]

The aim of the present study was to evaluate the frequency of drug-resistant and multidrug-resistant tuberculosis in the Province of Florence (1 180 728 inhabitants) between 1992 and 1995.

MATERIALS AND METHODS

All the sensitivity tests performed on initial mycobacterial isolates in the Province of Florence between 1 January 1992 and 31 December 1995 were evaluated. 'Initial isolate' refers to the first positive culture for *M. tuberculosis* of each patient notified. Sensitivity tests were routinely performed on all initial isolates. Strains were isolated from clinical samples obtained from the Pulmonology Centre of the Province of Florence (a 96-bed centre with 20 beds exclusively allocated to tuberculosis patients), from all the other medical and surgical divisions of the Ospedale di Careggi (a 1900-

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bed general hospital), and from the various microbiological laboratories in the Province of Florence. Mycobacterial cultures were performed on both solid and liquid media using Lowenstein-Jensen and Bactec (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA). The isolates were identified to the species level using deoxyribonucleic acid (DNA) probes (AccuProbe, Gen-Probe, San Diego, CA, USA) with the addition of conventional biochemical tests.

Susceptibility testing was performed for isoniazid (H), rifampicin (R), streptomycin (S), ethambutol (E) and pyrazinamide (Z) using the Bactec method. The susceptibility test for Z was not performed during 1992.

In this study, multidrug-resistant tuberculosis refers to tuberculosis caused by germs resistant to at least H and R.[1]

The χ^2 test was performed for statistical analysis.

RESULTS

From 1 January 1992 to 31 December 1995, a total of 433 sensitivity tests were performed on initial isolates corresponding to as many patients with tuberculosis, 286 male (66%) and 147 female (34%). The distribution of samples over the years studied was as follows: 96 in 1992, 104 in 1993, 108 in 1994, and 125 in 1995.

There were 72 (16.6%) foreign-born patients from countries with a high prevalence of tuberculosis. The percentage of foreign-born patients among the total number of patients examined steadily increased during the study period from 8% (8/96) in 1992 to 12.5% (13/104) in 1993, 25.6% (28/109) in 1994 and 18% (23/125) in 1995. The country of origin was North Africa for 15 patients (20.8%), Central Africa for 13 patients (18%), China for 10 patients (13.8%), South-East Asia for four patients (5.5%), South America for 14 patients (19.4%), and Eastern Europe for 16 patients (22.2%). The time since arrival in Italy was known in 17 of the 72 patients (23.6%); it was ≤2 years in 10 patients (58.8%), 3–4 years in six (35.2%), and >4 years in one (5.8%).

Data relative to HIV status were available only for 26 patients who were known to be HIV positive. Data concerning previous antituberculosis treatment were available for 231 of the total 433 patients (53.3%); among these 175 (75.7%) had never been treated previously and 56 (24.3%) had.

Samples were obtained from the following material: sputum (n = 208, 48%), bronchial aspirate (64, 15%), urine (23, 5.3%), pleural fluid (21, 5%), lymph node aspirate (17, 4%), pus (17, 4%), other (26, 6%), and unspecified material (57, 13%).

With regard to the institutions from which the specimens were reported, 185 (42.7%) came from the Pulmonology Centre, 103 (23.7%) from other medical and surgical wards of the Azienda Ospedaliera di Careggi, and 145 (33.4%) from other hospitals and laboratories of the Province of Florence.

Frequency of drug resistance

The frequency of drug resistance in all the samples tested is indicated in Table 1. Of the 433 isolates examined, 13.8% (60/433) showed resistance to one or more drugs. Resistance to H was present in 10.6% (46/433), to R in 3.6% (16/433), to S in 3.6% (16/433), to Z in 1.7% (6/337) and to E in 0.6% (3/433). Resistance to H + R was present in 2.5% (11/433) of samples examined. Resistance to H + R was associated with resistance to Z in one case, to S in two cases and to S + E in two further cases. Of the 11 subjects with MDR-TB, eight (72.7%) were male, and four (36.3%) were immigrants from high prevalence countries (two from China, and two from North Africa).

The frequency of drug resistance in the Italian-born and foreign-born groups is indicated in Table 1.

Of the foreign-born patients from high prevalence countries, 19.4% (14/72) showed resistance to at least one drug. Resistance to H was found in 19.4% (14/72), to R in 5.5% (4/72), to S in 2.7% (2/72), and to Z in 1.5% (1/64). No resistance to E was found. Resistance to H + R was observed in 5.5% (4/72) of cases.

In the Italian-born population, the frequency of resistance to at least one drug was 12.7% (46/361), resistance to H 8.8% (32/361), to R 3.3% (12/361), to S 3.8% (14/361), to Z 1.8% (5/273) and to E 0.8% (3/361). Resistance to H + R was found in 1.9% (7/361).

Table 1 Resistance to antituberculosis drugs, 1992–1995

	All cases			Italian-born population			Foreign-born		
Drug	Tested	Resistant	Rate (%)	Tested	Resistant	Rate (%)	Tested	Resistant	Rate (%)
Isoniazid	433	46	10.6	361	32	8.8	72	14	19.4
Rifampicin	433	16	3.6	361	12	3.3	72	4	5.5
Streptomycin	433	16	3.6	361	14	3.8	72	2	2.7
Ethambutol	433	3	0.6	361	3	0.8	72	0	0
Pyrazinamide	337	6	1.7	273	5	1.8	64	1	1.5
H + R	433	11	2.5	361	7	1.9	72	4	5.5
At least one drug	433	60	13.8	361	46	12.7	72	14	19.4

H + R = isoniazid + rifampicin

Of the 26 samples from HIV-positive patients, the following drug resistance rates were found: to at least one drug 15.3% (4/26), to H 7.6% (2/26), to R 3.8% (1/26), and to Z 3.8% (1/26). No resistance was found to S, E or H + R.

In the subgroup of 231 patients for whom data concerning previous treatment were available the rates of resistance were the following: 10.8% (19/175) to at least one drug, 8% (14/175) to H, 1.7% (3/175) to R, 2.8% (5/175) to S, 0% (0/175) to H + R in never treated patients; 28.5% (16/56) to at least one drug, 23.2% (13/56) to H, 10.7% (6/56) to R, 3.5% (2/56) to S, 7.1% (4/56) to H + R in previously treated patients. The four patients with MDR-TB in this subgroup were all previously treated Italian-born patients.

Drug resistance trend over time

Although the frequency of resistance in the total number of samples increased during the period examined, it did not show any significant differences ($P > 0.05$). Resistance to at least one drug increased from 13.5% in 1992 to 14.4% in 1995, whereas resistance to specific drugs rose from 9.3% to 12% for H, from 4.1% to 5.6% for R, from 5.2% to 4% for S, and from 2.1% to 4% for H + R. Figure 1 shows the trend of drug resistance rates during the period examined in the entire population tested. No noteworthy variation of drug resistance rates was found in the period studied ($P > 0.05\%$), even when the samples from foreign-born patients from high prevalence countries were excluded from the analysis, as indicated in Figure 2.

Comparison with other studies

The rates of resistance obtained in this study and in other Italian studies[9–11] are reported in Table 2.

DISCUSSION

The data from the present study show that: 1) the rate of resistance to at least one drug was 13.8% during the period examined; although not statistically significant, the percentage of drug resistance was higher in the foreign-born (19.4%) than in the Italian-born population (12.7%); 2) resistance to H and to R was higher in the foreign-born than in the Italian-born population, while resistance to S was found to be higher in the Italian-born population; 3) the rate of multidrug resistance in all the cases examined was found to be 2.5%, with a higher prevalence in the foreign-born than in the Italian-born population; 4) the trend of drug resistance during the period studied did not show any significant variations; 5) in the subgroup of 231 patients in whom data about previous treatment were available, the rate of resistance to at least one drug and to H + R were 10.8% and 0%, respectively, in never treated patients and 28.5% and 7.1%, respectively, in previously treated patients.

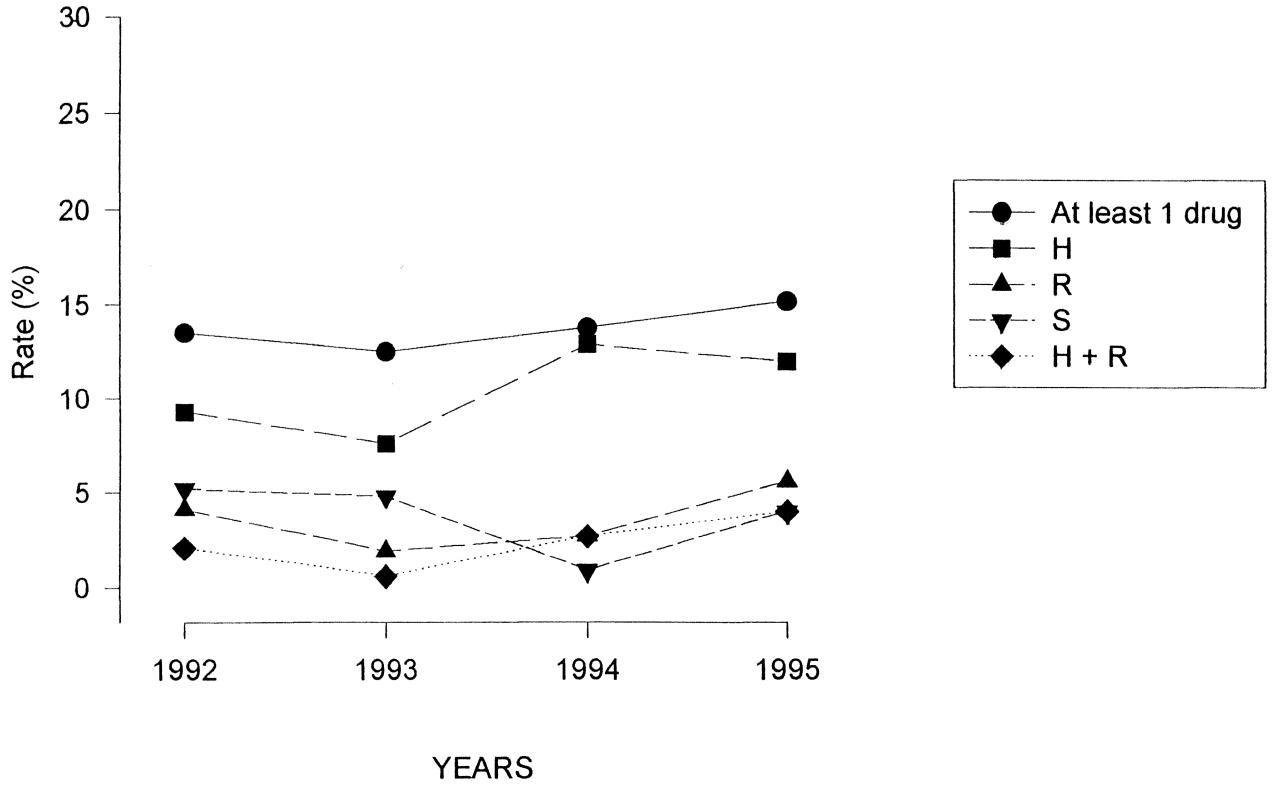

Figure 1 Annual proportion of isolates resistant to: at least one drug, isoniazid (H), rifampicin (R), streptomycin (S), isoniazid + rifampicin (H + R).

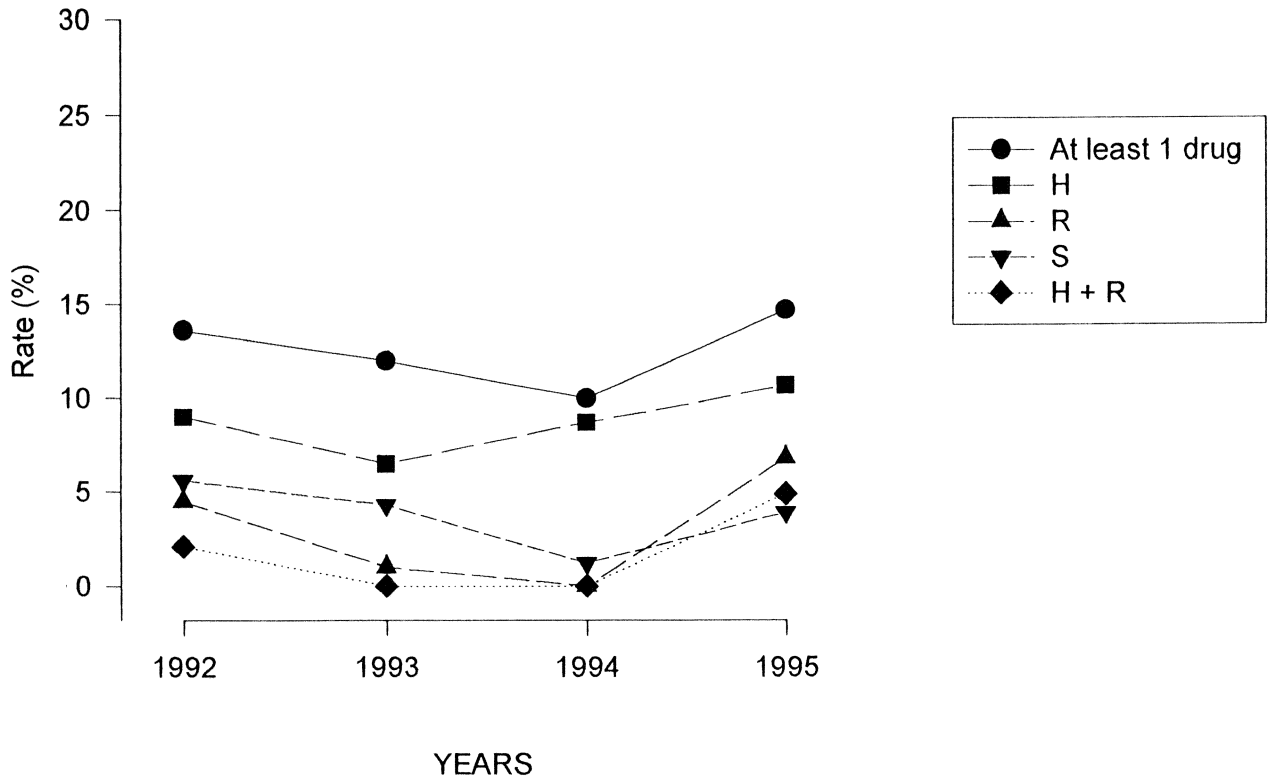

Figure 2 Annual proportion of isolates in the Italian-born population resistant to: at least one drug, isoniazid (H), rifampicin (R), streptomycin (S), isoniazid + rifampicin (H + R).

In the USA, a rate of resistance to at least one drug of 14.4% was reported in 1991,[4] whereas lower rates were reported in other European countries: 9.8% in England and Wales for the period 1982–1991,[7] 10% in Germany for the period 1991–1992,[13] and 6.7% in Switzerland for the period 1995–1996.[8] The data from the present study highlight a similar rate of drug resistance to that of the USA and a higher rate than those reported in European studies. The frequency of resistance to at least one drug, to H and to R, was found to be higher in foreign-born patients from high prevalence countries than in the Italian-born population, but only H showed a statistically significant difference ($P < 0.02$). These data are in agreement with those reported in other countries,[4,14] and by another Italian study by Berozzi et al.[9] An interesting finding in our study is the higher rate of resistance to S in the Italian-born than in the immigrant population. These data confirm the situation reported by Besozzi et al.,[9] and can presumably be correlated to the past—and current—widespread use of streptomycin in Italy.

With regard to the problem of multidrug resistance, strains resistant to at least H + R were found in 2.5% of samples examined. This frequency is lower than that reported in the USA in 1991 (3.5%),[4] but higher than those reported in France (0.5%), England and Wales (0.6%) and Switzerland (1.7%).[6–8] The frequency of multidrug resistance was higher in the foreign-born (5.5%) than in the Italian-born population (1.9%), although this difference was not statistically significant ($P > 0.05$). Whereas in recent years a progressive increase in drug resistance and multidrug resistance has been found in the USA, our data is in agreement with those reported in England and Wales,[7] showing no significant variation during the period examined ($P > 0.05$).

Table 2 Rates of resistance in different geographic areas of Italy

Reference	Geographic area	Years	Cases	Resistance to at least one drug	Resistance to H + R
Besozzi et al.[9]	Milano (Northern Italy)	1989–1994	436*	16.9%	2.06%
Girardi et al.[10]	Roma (Central Italy)	1990–1992	407†	26.0%	5.7%
Montesano et al.[11]	Matera (Southern Italy)	1989–1992	83‡	14.5%	2.0%
Nutini et al.	Florence (Central Italy)	1992–1995	433‡	13.8%	2.5%

* Newly diagnosed pulmonary TB in Italian and foreign-born patients
† Hospitalized tuberculosis patients
‡ Data from microbiological laboratories

The main limitations of our study are the following: 1) the restricted geographical area examined, 2) the limited number of samples examined compared to laboratory studies performed in other countries, and 3) clinical information such as data concerning previous treatment with antimycobacterial drugs and HIV status was available only in 53% of cases.

Previous badly managed antituberculosis treatment has been reported in the literature as a factor favouring drug resistance.[15,16] The data from the subgroup of 231 patients with clinical information show a higher rate of resistance in previously treated than in never treated patients, and multidrug resistance was present only in four previously treated patients. No firm conclusion could be drawn from these data, given that clinical information were available only in 53% of the patients examined.

HIV infection has been also reported as another factor favouring drug resistance.[16] However, the resistance rates found in the 26 samples from HIV-infected subjects were similar to the rates in those for whom no data on HIV status were available. It is therefore possible to assume that the HIV factor does not substantially influence our results. On the other hand, our study can provide some useful indications given the absence in Italy of a drug resistance surveillance system and, therefore, the lack of information on mycobacterial drug resistance rates on a national level.

The drug resistance and multidrug resistance rates reported in our study are similar to those reported in other studies performed in different geographical areas of Italy. Although not alarming, these rates are higher than those reported by other European countries. One of the main causes of drug resistance is the poor management of tuberculosis patients, particularly with regard to the choice of chemotherapy regimen and follow-up.[16] Inadequate treatment leads to the selection of resistant mutants—naturally present in a mycobacterial population—which, by spreading into the community, determine the onset of new cases of tuberculosis with primary resistance to drugs.

Following the abolition of antituberculosis associations in Italy in 1978, the specific expertise of this sector disappeared, and for many years little attention has been paid to tuberculosis. Although some progress has recently been made in the attempt to re-organise health activities in this sector,[17] there are still no official national guidelines for the choice of chemotherapy regimens or for case-holding in Italy. It is therefore possible that the treatment delivery process (TDP), defined as the sum of the different processes involved in the prescription and delivery of antituberculosis drugs to the diagnosed tuberculosis patient in a specific area,[18] has not always been managed correctly.

A recent study involving 182 pulmonology centres in Italy has shown that treatment is not always prescribed in accordance with modern international guidelines: pyrazinamide is used in only 30.8% of cases, whereas streptomycin is used in 40.7%; short-term treatment is not prescribed by the majority of centres, and the total duration of treatment is often unnecessarily long.[19] Furthermore, the growing number of foreign-born patients from high prevalence countries among the cases of tuberculosis reported in Italy[20] could account for the higher frequency of drug-resistant tuberculosis.

As prevention of drug resistance is largely identified with the prevention of inadequate treatment, it is important to re-define guidelines regarding chemotherapy regimens and case-holding methods to be adopted on a national level, so as ensure that all cases of tuberculosis are treated correctly. The creation of a national surveillance system to monitor resistance rates can provide useful indications as to what chemotherapy regimens to adopt and the quality of the TDP being provided.[15]

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RÉSUMÉ

CADRE : Des données épidémiologiques sur la fréquence de la tuberculose à germes résistants ne sont pas disponibles en Italie.
OBJECTIF : Evaluation du taux de tuberculose à germes multirésistants dans la Province de Florence, Italie.
SCHÉMA : Analyse rétrospective de tous les tests de sensibilité pratiqués avec la méthode Bactec sur des isolats mycobactériens initiaux entre le 1er janvier 1992 et le 31 décembre 1995 dans la Province de Florence.
RÉSULTATS : Sur les 433 échantillons testés, les taux suivants de résistance ont été observés : isoniazide + rifampicine 2,5%, au moins un médicament 13,8%, isoniazide 10,6%, rifampicine 3,6%, streptomycine 3,6%, pyrazinamide 1,7% et ethambutol 0,6%. La résistance est plus fréquente chez les sujets nés dans les pays à haute prévalence que dans la population native d'Italie où la résistance à la streptomycine est la plus fréquente. Les taux de résistance ne montrent pas de variation annuelle significative pendant la période examinée. Les données cliniques furent disponibles chez 231 patients. Chez les sujets n'ayant jamais été traités, les taux de résistance à au moins une drogue et à isoniazide + rifampicine sont respectivement de 10,8% et 0%, alors que chez les sujets traités précédemment, ils sont respectivement de 28,5% et de 7,1%.
CONCLUSION : Les données de notre étude montrent des taux de multirésistance plus élevés que ceux trouvés dans d'autres pays européens, comme l'Angleterre et Pays de Galles, la France et la Suisse. Ce résultat suggère la nécessité d'établir en Italie des directives officielles pour le traitement correct de la tuberculose afin de prévenir le développement de la résistance aux médicaments, et d'établir un système de surveillance nationale des résistances mycobactériennes.

RESUMEN

MARCO DE REFERENCIA : No existen en Italia datos epidemiológicos sobre la frecuencia de tuberculosis con bacilos resistentes a las drogas.
OBJETIVOS : Evaluación sobre la tasa de tuberculosis multirresistente en la Provincia de Florencia, Italia.
MÉTODO : Análisis retrospectivo de todos los tests de sensibilidad efectuados por el método Bactec en aislados iniciales de micobacterias, desde el 1 de enero de 1992 al 31 diciembre de 1995, en la Provincia de Florencia.
RESULTADOS : En las 433 muestras examinadas se hallaron las siguientes tasas de resistencia : isoniacida + rifampicina 2,5%, por los menos a una droga 13,8%, isoniacida 10,6%, rifampicina 3,6%, estreptomicina 3,6%, pirazinamida 1,7% y etambutol 0,6%. La resistencia fue mayor en los extranjeros nacidos en países con alta prevalencia que entre los nacidos en Italia, aunque la resistencia a la estreptomicina fue más alta en estos últimos. Las tasas anuales de resistencia no mostraron variaciones significativas en el período de estudio. Se obtuvieron datos clínicos en 231 pacientes : las tasas de resistencia a una droga y a isoniacida + rifampicina fueron de 10,8% y de 0%, respectivamente, en los pacientes sin tratamiento previo y de 28,5% y 7,1%, respectivamente, en los ya tratados.
CONCLUSIÓN : Las cifras de nuestro estudio muestran tasas de multirresistencia más elevadas que las halladas en otros países europeos como Inglaterra y País de Gales, Francia y Suiza. Estos resultados sugieren la necesidad de establecer normas oficiales en Italia para el tratamiento correcto de la tuberculosis con el objetivo de prevenir la aparición de la resistencia a las drogas y de establecer un sistema nacional de vigilancia de la resistencia de las micobacterias.