INT J TUBERC LUNG DIS 10(5):554–558

Drug-resistant tuberculosis in Castilla-León, Spain, 1996–2000

A. Alberte-Castiñeiras,* M. F. Brezmes-Valdivieso,† A. Campos-Bueno,‡ I. Montes-Martinez,§ R. López-Medrano,¶ C. Avellaneda,* P. Pérez-Pascual,* P. Della-Latta#

* Departamento de Microbiología, Hospital del Río Hortega, Valladolid, † Departamento de Microbiología, Hospital Virgen de la Concha, Zamora, ‡ Departamento de Microbiología, Hospital General, Soria, § Departamento de Microbiología, Hospital Virgen del Puerto, Plasencia, Cáceres, ¶ Departamento de Microbiología, Hospital del Bierzo, Ponferrada, León, Spain; # Clinical Microbiology Service, Columbia-Presbyterian Medical Center, New York, New York, USA

SUMMARY

SETTING: During 1996–2000, a regional anti-tuberculosis drug resistance survey was conducted in Castilla-León, Spain.

OBJECTIVE: To determine the incidence of drug-resistant tuberculosis (TB) in newly treated human immunodeficiency virus (HIV) negative and HIV-positive TB patients.

DESIGN: Nine hundred and eighty-five *Mycobacterium tuberculosis* strains isolated from HIV-negative (926) and HIV-positive (59) patients were studied (one strain per patient). Univariate and multivariate analyses were used to determine the prevalence of drug resistance in high-risk groups.

RESULTS: Thirty-eight isolates (3.8%) showed resistance to one of the following drugs: streptomycin (S), isoniazid (H), rifampicin (R) or ethambutol (E). Of these, 36 (3.9%) were from HIV-negative and 2 (3.4%) from HIV-positive patients. The rate of drug resistance among HIV-negative patients was 1.2%, 2.0%, 0.3% and 0.8%, respectively, for S, H, R and E, and for HIV-positive patients it was 3.4%, 0%, 0% and 1.7%. Among the HIV-negative patients, monoresistance was observed in 32 (3.4%) strains and resistance to both H and R (multidrug resistance) was detected in one.

CONCLUSION: The incidence of primary drug resistance in the surveyed area was low and increased resistance was not observed in the HIV-positive group ($P = 0.99$). Routine surveillance of drug resistance is recommended by the TB control programme in representative patient populations to optimise treatment regimens.

KEY WORDS: tuberculosis; tuberculosis resistance; primary drug resistance; HIV and tuberculosis; immigrants and tuberculosis

DRUG RESISTANCE, particularly multidrug resistance (MDR), is a substantial threat to tuberculosis (TB) control programmes throughout the world.[1] Patients infected with MDR strains, particularly those infected with the human immunodeficiency virus (HIV), are extremely difficult to cure, and the treatment regimen is expensive and burdened by severe side effects.[2,3] A review of the literature and unpublished reports from the past decade indicate the presence of high levels of drug resistance in certain areas of the world. To determine the incidence of drug resistance among newly treated cases in various regions of Castilla-León, Spain, a collaborative study among hospitals within this community was initiated in 1991.[4] The immigrant population within these regions originated from Latin America, Asia and Eastern European countries, which are at high risk of harbouring MDR strains. A secondary study was conducted to determine the incidence of resistance to the main antituberculosis drugs in newly treated TB patients, evaluating the contribution of high-risk groups (HIV patients) to the epidemiology of drug resistance.

MATERIALS AND METHODS

The present study was designed to fulfill the following principles: 1) the sample size of TB patients must be representative of the area; 2) drug resistance in newly diagnosed cases should be clearly confirmed; 3) the quality of laboratory performance must be assured. No ethical approval was required for the development of this study.

Definitions

Resistance to streptomycin (S, SM), isoniazid (H, INH), rifampicin (R, RMP) or ethambutol (E, EMB) was evaluated. Primary drug resistance (PDR) was defined as resistance to strains of *Mycobacterium tuberculosis* in patients without a history or other evidence of previous treatment. Any drug resistance (ADR) was

Correspondence to: Armando Alberte-Castiñeiras, Departamento de Microbiología, Hospital del Río Hortega, Rondilla de Santa Teresa, 9 E-47010 Valladolid, Spain. Tel: (+34) 9 8342 0400. Fax: (+34) 9 8333 1566. e-mail: aalberte@hurh.sacyl.es
Article submitted 17 June 2005. Final version accepted 5 December 2005.

defined as resistance to any drug. Monoresistance was defined as resistance to only one drug. MDR was defined as resistance to at least INH and RMP.

Data collection and participants

Prospective analysis included all drug susceptibility tests (DSTs) performed on 985 initial isolates of *M. tuberculosis* between 1 January 1996 and 31 December 2000. A total of five medical centres located in the Castilla-León region, serving 860 000 inhabitants (33.8% of the total population of Castilla-León) participated in this cooperative study. The estimated HIV rate of the region is 6 per 100 000 population.[5] The minimum sample size (850 TB cases) was calculated using the following parameters: estimated definite cases in the area during that period = 4155; precision = 3%; single-tailed confidence intervals (CI) = 95%.

Laboratory standardisation

DST was conducted by a national reference laboratory (Dra Marisol Jiménez and Arturo Ortega, Instituto de Salud Carlos III, Madrid, Spain) using the standardised agar proportion method,[6] according to the guidelines of the external quality assurance programme. A median of 15 strains were tested for quality control, with 93.3% agreement. Resistance was defined as at least 1% colony growth at critical concentrations of the drug (i.e., SM [dihydrostreptomycin sulphate] 4 mg/l, INH 0.2 mg/l, RMP 40 mg/l and EMB 2 mg/l).

Data analysis

TB cases with PDR strains were stratified using the following categories: ADR, monoresistance and MDR. The pattern of drug resistance was also stratified by HIV status and year.

Proportions were compared with the uncorrected two-tailed χ^2 test using Epidat (version 2.1 for Windows, Xunta de Galicia-OPS/OMS, Spain). Logistic regression analysis was performed to identify variables independently associated with drug resistance between non-HIV and HIV patients. Relative risk and 95%CIs were calculated. A P value <0.05 was considered statistically significant.

Table 1 Global resistance to first-line drugs in *M. tuberculosis* isolates, 1996–2000

	Strains *n* (%)	95%CI
Number of isolates	985 (100)	
Resistance to any drug	38 (3.8)	3.5–4.1
Monoresistance*	33 (3.3)	3–3.6
Streptomycin: any resistance†	13 (1.3)	1–1.5
INH: any resistance†	19 (1.9)	1.6–2.1
RMP: any resistance†	3 (0.3)	0.2–0.4
Ethambutol: any resistance†	9 (0.9)	0.7–1.1
MDR-TB‡	1 (0.1)	0.05–0.2

* Monoresistance: resistance to only one drug.
† Any resistance: resistance to the drug, with or without resistance to other drugs.
‡ MDR-TB: resistance to at least INH and RMP
CI = confidence interval; INH = isoniazid; RMP = rifampicin; MDR-TB = multidrug resistance.

RESULTS

All isolates

A total of 985 *M. tuberculosis* strains isolated between 1996 and 2000 from 926 HIV-negative and 59 HIV-positive patients with no history of previous treatment were tested. Of these, 38 (3.8%) were resistant to any drug (ADR), 13 (1.3%) to SM, 19 (1.9%) to INH (with or without other resistance), 3 (0.3%) to RMP and 9 (0.9%) to EMB. There was 1 (0.1%) MDR isolate (Table 1).

HIV status

Fifty-nine initial isolates of *M. tuberculosis* (6% of total) were reported between 1996 and 2000 from patients known to be co-infected with HIV. The isolates were not more likely to be either ADR (3.4% vs. 3.9%) or MDR (0% vs. 0.1%) than those from HIV-negative patients ($\chi^2 = 0.37$, odds ratio [OR] = 1.15, $P = 0.99$ [ADR]; $\chi^2 = 0.064$, OR = 0.19, $P = 0.99$ [MDR]) (see Table 2).

Secular trends

No upward trend in the total number of isolates was observed over this time frame. Although the proportion of isolates resistant to INH increased from 1.1% in 1996 to 4.3% in 2000, this difference was not sta-

Table 2 Resistance to first-line drugs in *M. tuberculosis* isolates and HIV status, 1996–2000

	HIV-negative *n* (%)	HIV-positive *n* (%)	*P*	OR (95%CI)
Number of isolates	926	59		
Resistance to any drug	36 (3.9)	2 (3.4)	0.99	1.15 (0.2–7.1)
Monoresistance*	32 (3.4)	1 (1.7)	0.72	2.07 (0.3–41.6)
Streptomycin: any resistance*	11 (1.2)	2 (3.4)	0.39	0.34 (0.06–2.3)
Isoniazid: any resistance*	19 (2)	0	0.53	2.55 (0.1–42.9)
Rifampin: any resistance*	3 (0.3)	0	0.99	0.45 (0.02–8.8)
Ethambutol: any resistance*	8 (0.8)	1 (1.7)	0.99	0.5 (0.06–10.9)
MDR-TB*	1 (0.1)	0	0.99	0.19 (0–4.8)

* See Table 1 for definitions
HIV = human immunodeficiency virus; OR = odds ratio; CI = confidence interval; MDR-TB = multidrug-resistant TB.

Table 3 INH resistance and MDR by year of diagnosis

Year	All isolates	INH resistance		MDR	
		n (%)	95%CI	*n* (%)	95%CI
1996	177	2 (1.1)	0.7–1.7	0	
1997	179	3 (1.7)	1.2–2.3	0	
1998	225	5 (2.2)	1.7–2.8	0	
1999	220	1 (0.45)	0.2–0.8	1 (0.45)	0.2–0.8
2000	184	8 (4.3)	3.6–5	0	
Total	986	19		1	

1999–2000, $P < 0.05$.
INH = isoniazid; MDR = multidrug resistance, defined as resistance to at least INH and rifampicin; CI = confidence interval.

tistically significant ($P = 0.1$, OR 0.25, 95%CI 1.3–0.03). However, a significant increase in INH-resistant strains was observed between 1999 and 2000 ($P = 0.02$, OR 0.1, 95%CI 0.8–0.01) (see Table 3).

DISCUSSION

The Global Project on Anti-Tuberculosis Drug Resistance Surveillance[3] recommends that drug resistance patterns of *M. tuberculosis* strains within geographic regions be monitored on a routine basis. Although drug-resistant TB in Western Europe is not currently a major public health problem, the increase in migration of individuals from areas endemic for drug-resistant strains necessitates routine trend surveys. This collaborative study analyses drug resistance patterns of *M. tuberculosis* isolates from patients within the community of Castilla-León over a 5-year period. The data compiled from this survey will have an impact on the TB control programme and treatment schedules in our region. In a report that evaluated the effectiveness of TB control programmes in Spain,[7] the region of Castilla-León ranked comparatively highly.

The overall level of drug resistance in the population studied in Castilla-León was consistently low, as exemplified by the fact that only one MDR isolate was obtained from an HIV-negative patient. These results are similar to those reported for the 1991–1995 period (ADR 4.1%, SM 2.6%, INH 1.8%, RMP 0.2%, EMB 0.6%, MDR-TB 0%),[4] significantly lower than the prevalence found in a city-wide cluster in Barcelona between 1995 and 1996[3] and the multicentre study in the same area,[8] and similar to those of Marín Royo[9] in Castellón (3.9% and 0.2% for ADR and MDR, respectively). Table 4 shows rates of primary drug resistance in some areas of Spain, including data from the United Kingdom for comparison.[2]

The slight increase in the proportion of INH-resistant isolates between 1996 and 2000 was not statistically significant, except in 1999. With regard to HIV-infected patients and drug resistance, our results are similar to those of Martín-Casabona,[8] and no association was observed. However, to ensure that there is no association, further studies should be performed. At present no association of HIV infection with drug resistance has been proven, and we conclude that in our region HIV infection is not associated with drug resistance. This may be due to the early diagnosis of new cases, the low ongoing transmission of drug resistance within this population, the rapid availability of anti-tuberculosis drugs in the national anti-tuberculosis programmes and good patient adherence.

To expedite prompt and appropriate treatment, rapid molecular techniques to detect RMP resistance should be considered. The analysis of drug-resistant isolates determines the MDR problem in our area and the need for continuing surveillance.[10–12]

Immigration of individuals from areas endemic for drug-resistant TB is steadily increasing[13] in various Spanish cities.[14,15] The impact of increasing populations of patients with TB on the prevalence of disease in different regions needs to be studied further. Although the number of immigrants with TB evaluated in this paper is too low for statistical analysis (3% of TB patients), the data support a recommendation that TB screening tests be required for recent immigrants at risk for infection with drug-resistant strains.

Table 4 Comparative primary drug resistance in different series

Investigator	Alberte-Castiñeiras[4]	Marín Royo[9]	Djuretic[2]	Pablos-Méndez[3]	Martín Casabona[8]	Present report
Period	1991–95	1992–98	1993–99	1995–96	1995–97	1996–2000
Country	Spain (Castilla-León)	Spain (Castellón)	UK	Spain (Barcelona)	Spain (Barcelona)	Spain (Castilla-León)
Cases, *n*	825	461	25 217	218	1535	985
ADR, %*	4.1	3.9	6.2†	9.6†	5.7†	3.8
Monoresistance, %*	2.9	3	5.2	8.7	4.4	3.3
Streptomycin: any resistance, %*	2.6	1.5	5.9	4.6	1.3	1.3
Isoniazid: any resistance, %*	1.8	2.4	5.7	3.2	2.5	1.9
Rifampin: any resistance, %*	0.2	0.6	1.3	0.9	0.07	0.3
Ethambutol: any resistance, %*	0.6	0.2	0.6	1.8	0	0.9
MDR-TB, %*	0	0.2	1.2	0.5	0.9	0.1

* See Table 1 for definitions
† $P < 0.05$.
ADR = any drug resistance; MDR-TB = multidrug-resistant tuberculosis, defined as resistance to at least isoniazid rifampicin.

In conclusion, surveys are vital in assessing trends in resistance. This study reports a low rate of drug resistance, including MDR-TB, even among HIV-positive patients living in the Castilla-León region of Spain.

Acknowledgements

We are indebted to Dr M González-Sagrado (Research Unit, Hospital R Hortega, Valladolid, Spain) for helping in the implementation of this paper.

References

1 Migliori G B, Fattorini L, Besozzi G, et al. Prevalence of resistance to antituberculosis drugs: results of the 1998/99 national survey in Italy. Int J Tuberc Lung Dis 2002; 6: 32–38.
2 Djuretic T, Herbert J, Drobnieewski F, et al. Antibiotic resistant tuberculosis in the United Kingdom: 1993–1999. Thorax 2002; 57: 477–482.
3 Pablos-Mendez A, Raviglione M C, Laszlo A, et al. Global surveillance for antituberculosis drug resistance, 1994–1997. N Eng J Med 1998; 338: 1641–1649.
4 Alberte Castiñeiras A, Pérez Pascual P y Grupo de Microbiologos de Castilla y León. Resistencias primarias de *Mycobacterium tuberculosis* en diez hospitales de la Comunidad de Castilla y León. Rev Clin Esp 1999; 199: 132–135.
5 Redondo Martín S, Morquecho Gil M, Berbel Hernández C, Viña Simón M C. [Requesting and performing HIV testing in primary health care. Study of the Sentinel Physician Network of Castilla y León, 1990–1996]. In Spanish. Gaceta Sanitaria 2002; 16: 114–120.
6 Canetti G, Fox W, Kohmenko A, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Organ 1969; 41: 21–43.
7 Rodrigo T, Caylá J A. Efectividad de los programas de control de la tuberculosis en España. Med Clin (Barc) 2003; 121: 375–377.
8 Martín-Casabona N, Alcaide F, Coll P, et al. Farmacorresistencia de *Mycobacterium tuberculosis*. Estudio multicéntrico en el área de Barcelona. Med Clin (Barc) 2000; 115: 493–498.
9 Marín Royo M, González Morán F, Moreno Muñoz R, et al. Evolución de las resistencias a *Mycobacterium tuberculosis* en la provincia de Castellón. Años 1992–1998. Arch Bronconeumol 2000; 36: 551–556.
10 Irish C, Herbert J, Bennett D, et al. Database study of antibiotic resistant tuberculosis in the United Kingdom, 1994–1996. BMJ 1999; 318: 497–498.
11 Ausina Ruiz V, Lonca Gimenez J. Resistencia a los fármacos antituberculosos en las cepas de *M tuberculosis* aisladas en España. Rev Clin Esp 1999; 199: 121–125.
12 Casal M, Gutierrez J, Ruiz P. Situación actual de la multirresistencia en tuberculosis. Rev Esp Quimioter 2000; 13: 167–170.
13 Rusch-Gerdes S. Epidemiology of resistant tuberculosis in Europe. Infection 1999; 27 (Suppl 2): S17–S18.
14 Vallés X, Sanchez F, Pañella H, García de Olalla P, Jansá J M, Caylá J A. Imported tuberculosis: an emerging disease in industrialised countries. Med Clin (Barc) 2002; 118: 376–378.
15 Salinas Solano C, Altube Urrengoetxea L, España Yandiola P P, Capelastegui Saiz A, Quintana López J M. Tuberculosis en la población inmigrante de Bilbao. Arch Bronconeumol 2002; 38: 506–510.

RÉSUMÉ

CONTEXTE : Au cours de la période 1996–2000, une enquête régionale sur la résistance aux médicaments antituberculeux a été menée en Castille-León, Espagne.
OBJECTIF : Déterminer l'incidence des tuberculoses (TB) à germes résistants aux médicaments antituberculeux chez les patients nouvellement traités pour TB, qu'ils soient séronégatifs ou séropositifs pour le VIH.
SCHÉMA : On a étudié 985 souches de *Mycobacterium tuberculosis*, dont 926 isolées chez des patients séronégatifs pour le VIH et 59 chez des patients séropositifs (une souche par patient). On a utilisé des analyses uni- et multivariées pour déterminer la prévalence de la résistance médicamenteuse dans les groupes à risque.
RÉSULTATS : On a observé une résistance à l'égard d'un des médicaments suivants dans 38 isolats de patients (3,8%) : streptomycine (S), isoniazide (H), rifampicine (R) ou éthambutol (E). Parmi ceux-ci, 36 (3,9%) provenaient de patients séronégatifs pour le VIH et 2 (3,4%) de patients séropositifs pour le VIH. Le taux de résistance parmi les patients séronégatifs pour le VIH été respectivement de 1,2%, 2,0%, 0,3% et 0,8% pour S, H, R et E et chez les patients séropositifs, respectivement de 3,4%, 0%, 0% et 1,7%. Parmi les patients séronégatifs pour le VIH, il y avait une monorésistance dans 32 souches (3.4%) et une résistance à l'égard de H et R (multirésistance) dans une souche.
CONCLUSION : Dans la zone ayant fait l'objet de l'enquête, l'incidence d'une résistance primaire aux médicaments est faible et on n'a pas noté d'augmentation dans le groupe séropositif pour le VIH ($P = 0,99$). La surveillance de routine de la résistance des isolats aux médicaments est recommandée par le programme de lutte contre la TB dans des populations représentatives de patients afin d'optimiser les régimes thérapeutiques.

RESUMEN

SITUACIÓN : Durante el periodo 1996–2000 se llevó a cabo un estudio de resistencia a los antibióticos antituberculosos de primera línea, en Castilla-León, España.
OBJETIVO : Determinar la incidencia de resistencia antibiótica en casos nuevos de tuberculosis (TB) tanto en pacientes con VIH negativo como positivo.
DISEÑO : Se estudiaron 985 cepas de *Mycobacterium tuberculosis* procedentes de 926 pacientes con VIH negativo y 59 de pacientes con VIH positivo, utilizándose análisis uni y multivariado para determinar la prevalencia de la resistencia a las drogas antituberculosas en los grupos de riesgo.
RESULTADOS : La tasa global de resistencias fue del 3,8% (38/985), de las cuales 36 (3,9%) procedían de los pacientes con VIH negativo y 2 (3,4%) de aquellos con VIH positivo. En los pacientes con VIH negativo, el

1,2% eran resistentes a estreptomicina, el 2% a isoniacida (INH), el 0,3% a rifampicina (RMP) y el 0,8% a etambutol. En los casos con VIH positivo las resistencias fueron respectivamente de 3,4%, 0%, 0% y 1,7%. Se observó monorresistencia en 32 (3,4%) de los pacientes con VIH negativo, presentándose, solamente en este grupo, un caso (0,1%) de resistencia a INH y a RMP (multirresistencia).

CONCLUSIÓN: La incidencia de resistencia primaria a los antibióticos en el área estudiada es baja, no constatándose un incremento significativo ($P = 0,99$) en el grupo de pacientes con VIH positivo. Es recomendado por los programas de control de la TB realizar estudios representativos de resistencia a los antibióticos para optimizar las pautas de tratamiento.