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Risk factors associated with drug-resistant *Mycobacterium tuberculosis* in Castilla-la-Mancha (Spain)

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Risk factors associated with drug-resistant Mycobacterium tuberculosis *in Castilla-la-Mancha (Spain). M. Arévalo, J. Solera, D. Cebrian, J. Bartolomé, P. Robles. ©ERS Journals Ltd 1996.*

ABSTRACT: The aim of this study was to investigate the incidence of primary and secondary resistance to antituberculosis drugs and the risk factors associated with the resistance in southeast Spain.

Our research was based on the clinical histories of 276 patients, diagnosed as having tuberculosis by positive culture, who were tested for drug susceptibility between January 1988 and October 1994. The clinical histories were checked by two independent investigators.

Drug-resistant strains were isolated from 24 patients (8.7%). Among the 239 patients without previous tuberculosis therapy, 7 (2.9%) were drug-resistant. Among the 37 patients with previous therapy, 17 (46%) were drug-resistant. Seventeen of the 276 patients (6.2%) had resistance to a single drug. Primary resistance to isoniazid was 1.7%. Previous treatment for tuberculosis (odds ratio (OR) 39.2; 95% confidence interval (95% CI) 10.2–150); hepatic cirrhosis (OR 104; 95% CI 12.8–847); and age 45 yrs and older (OR 4.80; 95% CI 1.15–20.1) were considered risk factors associated with drug-resistant tuberculosis.

We conclude that primary and secondary resistance is low in the southeast of Spain and we recommend that susceptibility testing be performed on initial *M. tuberculosis* isolated from patients with a history of previous tuberculosis therapy, hepatic cirrhosis or more than 44 yrs of age.

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Interest in the study of resistance to antituberculosis drugs has risen in recent years [1–4] due to an increase in tuberculosis (TB) coinciding with the appearance of the acquired immune deficiency syndrome (AIDS) [5, 6]. Resistance to one or more first-line drugs increased in the USA from 6.9% in 1982 to 14.2% in 1991 [7], with an incidence of resistance to isoniazid (INH) and rifampin (R) of 3.5%. Furthermore, outbreaks of multiresistant TB have been reported [8, 9]. As opposed to the USA, in European countries, such as the UK [10] and Spain [11–13], the prevalence of tuberculosis resistant to treatment has not risen in recent years. In Germany, the data from two recent studies are not consistent [14, 15].

Epidemiological studies of the risk factors associated with resistance to antituberculosis drugs has shown that previous antituberculosis treatment [2–4, 10, 16, 17], cavitary pulmonary illness [3, 16], immigration [2, 7, 17, 18], and bilateral illness [10] are the main risk factors. No similar tests have been carried out in Spain.

The aim of this study was to analyse the current situation of primary resistance (PR) and secondary resistance (SR) in Albacete (Spain) and to study the risk factors which predispose to the appearance of strains of *Mycobacterium tuberculosis* resistant to drugs.

Methods

The hospital in Albacete is a 700 bed general hospital, which serves a predominantly Caucasian, rural population, in an area without immigration, of 400,000 inhabitants, situated in southeastern Spain. All patients with a positive culture for *M. tuberculosis* hospitalized from January 1988 to October 1994 were included in the study (n=276). All isolates of *M. tuberculosis* obtained in the microbiology laboratory were sent to the Centro Nacional de Microbiología, Virología, e Inmunología Sanitarias de Majadahonda (Madrid), in order to carry out tests on sensitivity to first-line antituberculosis drugs, isoniazid (INH), rifampin (R), streptomycin (S) and ethambutol (E), using the Canetti proportion method [19].

Many patients included in this report at Albacete Hospital were included in a standard protocol of risk factor for tuberculosis. This protocol prospectively recorded numerous variables, including diseases potentially associated to a higher tuberculosis risk. Our study was exploratory, in the sense that numerous variables were examined for their value in predicting resistance to antituberculosis drugs. Patients were treated with standard antituberculosis regimens [6]. The therapy regimen usually included

isoniazid, rifampin and pyrazinamide; but some patients (*e.g.* human immunodeficiency virus (HIV)-seropositive subjects) also received a fourth drug, ethambutol or streptomycin, until their isolates were confirmed to be fully susceptible to the other drugs.

The clinical histories of the 276 patients were checked by two independent investigators (M.A. and D.C.), who collected data concerning age, sex, localization of the tuberculosis illness, smear positivity, previous TB, previous anti-TB treatment, history of treatment in the Thorax Hospital (Centre for Chronic Respiratory Illnesses), diabetes mellitus, cavitary pulmonary illness, alcoholism, chronic obstructive pulmonary disease (COPD), corticosteroids, neoplasia, addiction to drugs administered parenterally, and hepatic cirrhosis.

A patient was considered to have hepatic cirrhosis if the histological study was compatible or if increased hepatic enzymes plus an episode of hydropic decompensation or hepatic encephalopathy were present. A patient was considered to have received previous antituberculosis treatment when written documentation of such treatment or of INH prophylaxis was recorded. Abuse of alcohol ingestion was defined as the daily consumption of 60 g alcohol or more.

Analysis

Statistical calculations were performed with Epi Info Version 6 [20] and BMDP [21] software. To compare groups, odds ratio (OR), 95% confidence intervals (CI), and uncorrected Chi-squared or Fisher's exact test were calculated. Logistic regression analysis was used to examine the associations between drug resistance and potential risk factors. The dependent variable was the presence of drug resistance; the explicative variables were previous tuberculosis therapy, sex, 45 yrs of age or older, origin from a Chest Hospital, pulmonary tuberculosis, smear positive, diabetes mellitus, cavitary lung disease, alcoholism, COPD, corticosteroids, neoplasia, intravenous drug use and hepatic cirrhosis.

Results

Demographic and clinical data are presented in table 1. Pulmonary TB was diagnosed in 214 cases (78%). Of the 276 patients, 37 had received previous antituberculosis treatment (in three cases with INH prophylaxis) and 239 had not. Overall, *M. tuberculosis* resistant to one or more first-line antituberculosis drugs was isolated in 24 of the 276 cases (8.7%), of which 2.5% showed primary resistance and 6.2% secondary resistance. There were no significant changes in the annual rate of patients with drug-resistant *M. tuberculosis,* during 1988–1994. Of the 37 patients who had received previous antituberculosis treatment, 17 (46%) showed secondary resistance to at least one drug. Primary resistance was observed in 7 of the 239 patients who had

Table 1. – Demographic and clinical characteristics of patients studied

	Resistant (n=24)	Susceptible (n=252)	Total (n=276)
Age yrs*	51±16 53(18–75)	45±20 42(1–90)	46±20 45(1–90)
Sex M/F	16/8	184/68	200/76
Type of hospital:			
Chest	8	38	46
General	16	214	230
Previous tuberculosis	15	37	52
Previous anti-TB TX	17	20	37
Pulmonary tuberculosis	19	195	214
Prison	0	22	22
Diabetes mellitus	3	23	26
Hepatic cirrhosis	5	4	9
Neoplasia	1	21	22
COPD	2	31	33
Alcoholism	8	72	80
IDUs	1	15	16
Treatment with CS	1	10	11
Cavitary lung disease	11	83	94
Smear positive	18	139	157

*: mean±SD, and median with range in parenthesis. COPD: chronic obstructive pulmonary disease; IDUs: intravenous drug users; M: male; F: female; CS: corticosteroids; TB: tuberculosis; TX: treatment

Table 2. – Patients with primary and secondary resistance

Pt No./Sex	Age yrs	Localization	Resistant to	Prior TB TX
1/M†	66	Pulmonary	S	No
2/F	72	Skin	INH	No
3/M†	49	Pulmonary	S	No
4/F	18	Pulmonary	S	No
5/M	54	Pulmonary	INH	No
6/M†	29	Pulmonary	INH	No
7/M	74	Pulmonary	INH	No
8/M	64	Pulmonary	S	Yes
9/M	50	Pulmonary	INH,R,E	Yes
10/M†	59	Pulmonary	INH,R,E	Yes
11/F	25	Pulmonary	INH	Yes‡
12/F	55	Pulmonary	INH,R,S,E,PZ	Yes
13/F	59	Pulmonary	R,E	Yes
14/F	45	Genitourinary	INH	Yes
15/M	65	Osteoarticular	INH,E	Yes
16/M	75	Pleural	INH	Yes‡
17/M	39	Pulmonary	INH	Yes
18/F	31	Pulmonary	INH	Yes
19/M	52	Pulmonary	INH	Yes‡
20/F	70	Osteoarticular	INH	Yes
21/M	47	Pulmonary	INH	Yes
22/M†	55	Pulmonary	INH	Yes
23/M	52	Pulmonary	INH,R,S,PZ	Yes
24/M	25	Pulmonary	R,E,PZ	Yes

Pt: patient; M: male; F: female; INH: isoniazid; R: rifampin; S: streptomycin; E: ethambutol; PZ: pyrazinamide. †: hepatic cirrhosis; ‡: previous INH chemoprophylaxis. Prior TB TX: previous tuberculosis treatment.

not been treated previously (2.9%) (table 2). Resistance to one drug was present in 17 cases (6.2%), and resistance to more than one drug in seven cases (2.5%). This means that, of the 24 patients showing resistance to drugs, 71% showed resistance to only one drug.

Resistance to INH was most common, occurring in 18 of the 276 patients (6.5%). Resistance to R, S and E was 2.2% in each case. The seven cases of PR only occurred with one drug: in four cases with INH and three with S, there being no cases of multiple resistance. The PR to INH was 1.7% and to S was 1.3%. Of the 17 cases of SR, only seven were resistant to two or more first-line anti-tuberculosis drugs (table 2).

Hepatic cirrhosis was present in nine patients; five with alcoholic cirrhosis, three with alcoholism and postviral cirrhosis (hepatitis B and C virus) and one patient with postviral cirrhosis. Among these nine patients, five were resistant to antituberculosis drugs and four responsive. Of the five cases showing resistance, two had SR and three PR. The univariate analysis showed that the patients with PR had a 42 times higher risk of being cirrhotic (OR 42.5; 95% CI 5.22–378.9).

Univariate analysis showed that the risk factors which predispose the presence of resistance to antituberculosis drugs were: previous treatment (OR 28.2; 95% CI 9.46–87.0); hepatic cirrhosis (OR 16.2; 95% CI 3.36–81.4); age >44 yrs (OR 3.3; 95% CI 1.18–9.65) and history of internment in a hospital specializing in chronic respiratory illnesses (OR 2.82; 95% CI 1.02–7.62) (table 3).

Multivariate analysis showed that the only risk factors which independently influence the presence of resistance to antituberculosis drugs were age >44 yrs (OR 4.80; 95% CI 1.15–20.1), previous antituberculosis treatment (OR 39.2; 95% CI 10.2–150); and hepatic cirrhosis (OR 104; 95% CI 12.8–847).

Table 3. – Risk factors associated with drug-resistant tuberculosis

Risk factors	Crude OR (95% CI)	Adjusted OR (95% CI)
Age >44 yrs	3.30 (1.18–9.65)	4.80 (1.15–20.1)
Sex (female=1)	1.35 (0.50–3.58)	2.89 (0.68–12.2)
Chest Hospital	2.82 (1.02–7.62)	1.12 (0.27–4.61)
Pulmonary TB	1.11 (0.37–3.57)	1.48 (0.27–8.01)
Smear positive	2.24 (0.80–6.63)	3.38 (0.63–18.2)
Previous anti-TB	28.2 (9.46–87.0)	39.2 (10.2–150)
Cavitary lung disease	1.72 (0.68–4.35)	0.84 (0.17–4.00)
Diabetes mellitus	1.42 (0.31–5.62)	0.38 (0.04–3.39)
Alcoholism	1.24 (0.46–3.27)	1.38 (0.35–5.45)
COPD	0.64 (0.10–3.07)	0.15 (0.01–2.39)
Corticosteroids	1.05 (0.00–8.74)	0.59 (0.02–17.0)
Neoplasia	0.48 (0.02–3.66)	2.39 (0.15–36.9)
IDUs	1.04 (0.00–9.61)	0.42 (0.28–6.22)
Hepatic cirrhosis	16.2 (3.36–81.4)	104 (12.8–847)
Constant		0.007 (0.001–0.04)

OR: odds ratio; 95% CI: 95% confidence interval. For further abbreviations see legend to table 1.

Discussion

The global rate of resistance to antituberculosis drugs of 8.7% obtained in our study is similar to data published recently in other developed countries [10, 22]. In the USA, however, the reported rate was 14.2% in 1991 [7] and 13.2% in 1992 [23], although the figure varies from one state to another. These differences are due, above all, to the number of immigrants living in each study area, and of HIV-positive patients [3, 4, 7, 16, 23].

The PR rate of 2.9% obtained in our study is the lowest of all those published in Spain in the last 10 yrs. The rate oscillated between 7 and 10% in the 1980s [11, 12, 24], whilst the studies published in the last 2 years give rates near 5% [13, 25, 26]. This shows that, at this moment, a downward trend in the rate of PR exists in Spain despite the high prevalence of TB. We believe that our low rate is due to the predominantly rural population of this environment, without immigration and with a low incidence of HIV-positive patients.

In the USA, the situation is different, with an increase in the PR. KELLY *et al.* [1] reported a PR rate of 6.9% between 1975 and 1982, inferior to the 8.6% of KOPANOFF *et al.* [27]. Nonetheless, the Centers for Disease Control (CDC) reported an incidence of 13.4% in the first 3 months of 1991 [7], with 3.5% of PR to INH and R, superior to the 1.6% suggested by CHAWLA *et al.* [4], and which continued through 1992 [23]. In New York, the rate of MDR-TB is 12.9%, with no differences compared to other states as far as foreign birth (immigration) or history of recurrent illness is concerned, although the number of patients who complete the treatment is inferior to the US average [28].

The majority of the PR patients are only resistant to one drug, principally to INH or S, as is the case in our study. The 1.7% of our PR patients resistant to INH, and the 1.3% of those resistant to S constitute, as mentioned above, a lower rate than figures published recently in Spain [12, 13, 25, 26], and there have been no cases of multiresistance. Our data permit us, we believe, to continue the treatment of TB with three drugs within a limited period of 6 months.

The 46% of patients previously treated for TB presented SR, a rate which is similar to other studies [3, 4], but which is slightly higher than recent investigations in Spain [24, 29]. This fact may be due to more exhaustive data collection or to more precise registration. Furthermore, the majority of our patients showing SR suffered chronic, long-term TB and had received different treatments, which favours the appearance of SR. It must also be mentioned, that 59% of our SR (10 cases) were only resistant to one drug, nine to INH and one to S. Of the nine patients with SR to INH, three had undergone prophylaxis with INH (table 2).

As in other studies [2–4, 10, 16, 17], history of treatment with anti-TB drug was the most important risk factor. However, the limitations of the investigative work should be highlighted. The data were collected retrospectively, which could produce differences in

the register of antituberculosis drugs between cases and controls, provoking biased results. Nonetheless, the data-collection was carried out by two independent investigators to avoid possible bias.

The age of the patients and previous history of hepatic cirrhosis were also independent risk factors in the presence of resistance to antituberculosis drugs. SCALCINI *et al.* [30], in Haiti, described an increase in resistance with age, although other writers have not observed this relationship [2, 4, 16]. In our study, those patients who were 45 or more years of age presented a risk almost four times higher than those of less than 45 yrs, even adjusting for other potential risk factors.

In the literature available, we have not found reference to hepatic cirrhosis being an independent risk factor associated with resistance to antituberculosis drugs, and we do not know a putative mechanism. In our study, this risk is 104 times higher in subjects with hepatic cirrhosis. Nonetheless, the 95% CI are very wide due to the small number of existing cases. A full interpretation of this observed association requires the careful consideration of several issues. Firstly, because this was a retrospective study, the evaluations of hepatic cirrhosis were not performed in a standardized fashion; thus, it is possible that some patients who were considered not to have hepatic cirrhosis were in fact cirrhotics, which could produce biased results. Secondly, the presence of hepatic cirrhosis may simply reflect a surrogate for heavy alcohol drinkers and higher exposition to patients noncompliers of TB treatment, therefore with a higher hazard of drug resistance. It should be emphasized, however, that this study did not address the association of hepatic cirrhosis and resistance to antituberculosis drugs, so that our suggestion regarding this association is only speculative. Finally, although multivariate analytical models are powerful tools, the observed association of hepatic cirrhosis and resistance to antituberculosis drugs may simply reflect a Type I error (a significant association may be postulated when none actually exists). In any event, before this association is accepted it should be corroborated by further research.

The correlation with admission to the Chest Hospital merits further clarification. Although univariate analysis showed that admission to the Chest Hospital was associated with resistance to antituberculosis drugs (OR 2.82; 95% CI 1.02–7.62), in the final logistic regression model this factor was unrelated to resistance (OR 1.12; 95% CI 0.27–4.61). Thus, admission to the Chest Hospital can be a surrogate of other factors associated with resistance to antituberculosis drugs, since subjects admitted to this hospital were more probably advanced patients, alcoholics and homeless.

In summary, resistance to antituberculosis drugs in this area is low and the majority of resistance is to be found in patients with previous antituberculosis treatment. Fifty nine percent of SR was only to one drug and only four patients showed resistance to isoniazid and rifampin. Those patients with tuberculosis in our area can be identified as potentially resistant to antituberculosis drugs if they are older than 44 yrs, cirrhotic or have received previous antituberculosis treatment.

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