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A Case-Control Study for Multidrug-Resistant Tuberculosis: Risk Factors in Four European Countries

M. CASAL,[1] M. VAQUERO,[1] H. RINDER,[2] E. TORTOLI,[3] J. GROSSET,[4] S. RÜSCH-GERDES,[5] J. GUTIÉRREZ,[1] and V. JARLIER[4]

ABSTRACT

The aim of this study was to detect risk factors for multidrug resistance in patients with pulmonary tuberculosis in four European Union countries: France, Germany, Italy, and Spain. A prospective epidemiological case control study was conducted, made up of patients with clinically diagnosed and microbiologically confirmed pulmonary tuberculosis in the four countries between 1997 and 2000. A total of 138 cases and 276 controls were studied. Considering the four countries as a whole, the most statistically significant risk factors were as follows: intravenous drug use (OR 4.68); asylum-seeker support (OR 2.55) as income factor; living in a nursing home (OR 2.05); previous tuberculosis (OR 2.03) with pulmonary location; prison (OR 2.02); known tuberculosis contacts (OR 2.01); immunosuppression other than human immunodeficiency virus (HIV) (OR 1.96); acquired immunodeficiency syndrome (AIDS) (OR 1.96); current tuberculosis with pulmonary location (OR 1.77); and health-care worker (OR 1.69). These risk factors will have to be taken into account in the European Union as a whole, as well as in each individual country, to establish a health policy of monitoring and control for these cases of multidrug resistance. Although rare, their seriousness makes them particularly important.

INTRODUCTION

MULTIDRUG-RESISTANT TUBERCULOSIS (MDR-TB) initially emerged as a public health problem in European countries. MDR-TB, associated with high death rates of 50–80%, has a relatively short time span from diagnosis to death (4–16 weeks). Delayed recognition of drug resistance, resulting in delayed initiation of effective therapy, is one of the major factors contributing to MDR-TB outbreaks, particularly in health-care facilities.

In Europe, the percentage of isolates displaying drug resistance has been found to vary widely between countries. Two groups have been defined as a function of the proportion of MDR isolates among all culture-positive patients.[12,14] In eight countries—Denmark, Finland, Iceland, Netherlands, Norway, Slovenia, Sweden, and Switzerland—MDR ranges from 0% (Iceland) to 1.1% (Sweden and Switzerland). In Romania and Estonia, MDR accounts for 3.4% and 13.0%, respectively.

In the first group, a larger proportion of foreign-born patients had resistant isolates than did nationals. The proportion of isolates with isoniazid resistance was higher in patients from Asia (9.2%), Africa (9.6%), or other European countries (3.7%) than in nationals (2.3%). A similar observation was made for streptomycin resistance (10.1%, 12.9%, 2.8%, and 2.0%, respectively) and for MDR (1.3%, 1.3%, 0.6%, and 0.1%, respectively). These differences by geographic origin were seen in all countries except for Slovenia, and applied both to treatment-naive and to previously treated in-patients.

All countries except Finland reported both patients previously treated and patients only diagnosed for tuberculosis. The proportion of drug-resistant isolates was higher among patients previously treated than among treatment-naive patients.

The multiresistance rate of *Mycobacterium tuberculosis* in Spain was 8.6%.[9] In France, two studies have been carried out on risk factors in MDR-TB. Factors related to a poorer outcome

[1]Mycobacteria Reference Center, Faculty of Medicine, University of Córdoba, Spain.
[2]Department of Infectious Diseases and Tropical Medicine, Klinikum Innenstadt, University of München, Germany.
[3]Regional Mycobacteria Reference Center, Microbiology and Virology Laboratory, Careggi Hospital, Firenze, Italy.
[4]Pitie Salpetriere School of Medicine, University of Paris, France.
[5]National Reference Centre for Mycobacteria, Börstel, Germany.

were HIV co-infection, treatment with less than two active drugs, and known MDR at the time of diagnosis.[6] A case control study in Italy showed a multidrug-resistance rate of 2.5%.[11]

However, there has as yet been no broad epidemiological case control study at the European Union level to establish the risk factors related to these processes of multidrug-resistant tuberculosis. This project analyzed the factors related to MDR-TB cases reported in four major European Union countries: France, Germany, Italy, and Spain.

MATERIALS AND METHODS

A prospective case control study design, unmatched with two controls to each case, was used to determine the risk factors associated with MDR-TB. A total of 138 cases and 276 controls were reported in the period from June 30, 1997, to June 30, 2000. All cases were collected within the area of influence of the Reference Centers of participating countries (Table 1). For sample size calculations,[7] the following were considered: controls per case = 2; odds ratio worth detecting = 2; percentage exposure among controls = 20%; power = 80%; confidence level = 95%.

Clinical cases of tuberculosis formed the source of cases and controls[3] using the following criteria: a positive tuberculin skin test; signs and symptoms consistent with tuberculosis (*e.g.*, abnormal chest radiographs) or clinical evidence of current disease; treatment with two or more antituberculosis drugs, and completed diagnostic evaluation. All cases and controls were culture positive for *M. tuberculosis*.

The techniques used for drug susceptibility testing were the proportion method in solid media[2] and the BACTEC system.[15] Mycobacterial diseases other than those caused by the *M. tuberculosis* complex were not included.

The term CASE MDR-TB was used to define tuberculosis patients with culture-proved *M. tuberculosis* resistant to both isoniazid (INH) and rifampicin (RIF) (with or without resistance to other drugs). The term CONTROL was used to define patients with culture-proved *M. tuberculosis* that was either not resistant to antituberculosis drugs or resistant to only one antituberculosis drug.

Three groups of variables were considered: patients and TB-related data, environmental and co-morbidity data, and current TB episode data. Patients and TB-related data included age, sex, foreign-born (no, unknown; yes, country and years), known TB contacts (no, unknown; yes, household, workplace, other), previous TB (receiving >1 month of treatment before the specimen was collected for susceptibility testing), type (pulmonary and/or extrapulmonary), therapy, and drugs used and duration. Environmental and co-morbidity data included living situation (family/individual home, nursing home, prison, health care worker, homeless/no permanent address, other), income (employed, retired, unemployment benefit, asylum-seeker support, welfare, other, unknown), intravenous drug use (no, yes, unknown), immunosuppression other than HIV (yes, no, unknown), illness (diabetes, gastrointestinal symptoms, transplant, other), and HIV status (HIV seronegative, AIDS, unknown). Current TB episode included TB site (pulmonary, extrapulmonary, both pulmonary and extrapulmonary), current therapy, drugs, and start therapy.

The Spanish Reference Center in Cordoba received individual data sheets of cases and controls, and analyzed the epidemiological data.[16] For statistical and epidemiological analysis, Mantel–Haenszel's corrected chi square (χ^2) test or Fisher's exact test was used to analyze the statistical significance of the difference in the amount of exposure in cases when compared to controls.[7] To estimate the magnitude of the association between suspected risk factors and MDR-TB, the odds ratio (OR) was used.[13]

A logistical regression model was used for multivariate analysis to identify, among a group of independent variables, those most significantly associated with MDR-TB. MDR-TB represented the dependent variable, whereas age, sex, birth place, TB contacts, previous tuberculosis, intravenous drug use, income, living situation, immunosuppresion other than HIV, and HIV status were the independent variables. For each variable a coefficient of logistical regression (cr) was obtained, from which its natural antilogarithm was calculated. This value corresponded to the odds ratio,

$$OR_{MDR\text{-}TB} = e^{cr}$$

A bivariate analysis was performed to determine the significance of the risk factor,

$$OR_{MDR\text{-}TB} = \frac{\%\text{ exposure of the considered variable, given MDR-TB}}{\%\text{ exposure of the considered variable, without MDR-TB}}$$

Confidence Intervals (CI) were considered, with an alpha error of 5%, in order to establish the interval of the chi-square and OR. OR values expressed the difference in risk associated with exposure to ascertain whether the variable in question was or was not a possible cause of multidrug-resistant TB.

Epidemiological data were analyzed using the EpiInfo program (version 6.04 c 1999, CDC, Atlanta) and SPSS for Windows (v. 9.0 1999, SPSS Latin America, Chicago).

RESULTS

Patient characteristics

Patient mean age was 40.6 years (range 16–77 years); 109 patients (78.9%) were male. Seventy-three patients (52.9%) were born outside the country where TB was diagnosed. Thirty-one (22.5%) had known TB contacts. Forty-seven cases (34.1%)

Table 1. Countries Are Arranged by Size

	Germany	*Spain*	*Italy*	*France*	*Total*
Number of cases	51	45	34	8	138
Number of controls	102	90	68	16	276

had previous tuberculosis. Most patients lived with the family or in an individual home (79.7%). A total of 36.9% of MDR cases were employed. Nineteen (13.7%) were HIV co-infected and 91 (65.9%) had seronegative HIV status. Ten percent of the cases had immunosuppression other than HIV. Thirteen patients with MDR-TB (9.4%) were intravenous drug users.

In most cases, the tuberculosis site was pulmonary (90.6%). Of the 138 cases of MDR-TB, 120 (86.9%) were receiving therapy with antituberculosis drugs: isoniazid (38.3%), rifampicin (37.5%), streptomycin (18.3%), ethambutol (70.8%), pyrazinamide (75.8%), and other drugs (54.1%) such as ciprofloxacin, amikacine, rifabutin, and prothionamide. The patients received a regimen that included at least two drugs.

Of the 138 patients resistant to isoniazid and rifampicin, the following percentages were also resistant to other drugs: streptomycin (54.3%), ethambutol (48.5%), and pyrazinamide (35.5%).

Case control study

This study is the first attempt to produce a coordinated assessment of the contribution of risk factors to MDR-TB in Europe. The logistic regression model used for multivariate analysis yielded significant differences for age, TB contacts, previous TB, intravenous drug use, and income (Table 2). The age variable yielded an OR <1, thus, decreasing the likelihood of MDR-TB. Known contacts, previous TB, intravenous drug use, and income all yielded OR >1, thus increasing the likelihood of MDR-TB.

In comparison with control patients, similar results were obtained in all four countries (Table 3) for foreign-born status, previous illness, living with family/in individual home and homeless, HIV status (seronegative and seropositive not AIDS), and current therapy.

However, MDR-TB cases were significantly more prone than control patients to the following risk factors: age 40–59 years (OR = 1.62), male sex (OR = 1.56), known TB contacts (OR = 2.01), previous tuberculosis (OR = 2.03) in pulmonary location (OR = 1.80), living in nursing home (OR = 2.05), prison (OR = 2.02), health-care worker (OR = 1.69) or asylum-seeker support (OR = 2.55) as income factor, immunosuppression other than HIV (OR = 1.96), intravenous drug use (OR = 4.68), AIDS (OR = 1.96), and pulmonary site of current tuberculosis (OR = 1.77).

In bivariate analysis, the variables associated with MDR-TB were age (40–59 years), male sex, known TB contacts; previous TB (more than 1 year without therapy), living situation (nursing home, prison, health-care worker), income (asylum-seeker support); therapy and duration of previous TB, intravenous drug use, illness, immunosuppression other than HIV, and TB in pulmonary location. The factors of illness, immunosuppression other than HIV, non-AIDS HIV infection, and current therapy were not associated with MDR-TB.

Table 2. Risk Factors for MDR-TB in Four European Countries: Factors in the Logistical Regression Model

	OR	*CI 95%*
Age	0.98	0.97–0.99
Known TB contacts	1.92	1.07–3.47
Previous TB	2.65	1.59–4.41
Income	10.36	2.35–45.70
Intravenous drug use	4.70	1.67–13.17

The results from the three Mediterranean countries (France, Italy, and Spain) were similar to previous results, but not identical. The age group most strongly associated with MDR-TB was 40–59 years (OR = 3.10). Other risk factors were known TB contacts (OR = 3.52), duration of previous TB longer than 30 days (OR = 2.50), living in a nursing home (OR = 5.24), prison (OR = 2.01) and health-care worker (OR = 3.07), and income, particularly asylum-seeker support (OR 3.15).

Unlike the combined results for European countries, intravenous drug use and AIDS were risk factors in MDR-TB for the three Mediterranean countries, with OR = 6.15 and OR = 2.59, respectively. Not being currently in treatment was also a factor associated with MDR (OR = 2.37).

When considered independently, the results from each country also showed differences. France showed MDR following tuberculosis risk factors in age (patients $>$ 60 years, OR = 4.20), sex (female, OR = 11.67), living in family or individual home (OR = 5.44), income variable (employed or self-supported, OR = 4.33), and intravenous drug use (OR = 3.18).

The results from Germany showed, for MDR-TB patients, that the age group with the highest risk factor was 20–39 years (OR = 4.85). Foreign-born status was an important risk factor (OR = 32.26), the countries of origin being Russia (OR = 8.81) and Kazakhstan (OR = 7.89). Other risk factors were prison as living situation (OR = 2.62) and asylum-seeker support and unemployment benefit as income variable (OR = 5.10 and OR = 2.16), intravenous drug use (OR = 2.04), HIV unknown status (OR = 2.40), and pulmonary (OR = 9.30) site–current therapy tuberculosis (OR = 3.15).

The results for Italy show that age (40–59 years, OR = 3.59), and male sex (OR = 2.25) were significantly associated with MDR-TB. Other associated variables were nursing home and health-care workers (OR = 4.19 and OR = 3.19), asylum income situation (OR = 6.48), intravenous drug use (OR = 8.93), known immunosuppression status other than HIV (OR = 6.48), other illness (carcinoma OR = 1.68) and AIDS (OR = 4.64).

For Spain, the related risk factors were age group 40–59 years (OR = 4.00), male sex (OR = 1.78), not foreign-born (OR = 1.45), known TB contacts (OR = 3.32), same work place (OR = 2.24); nursing home (OR = 4.14) and prison (OR = 2.02) and homeless (OR = 2.07) as living situation, income variable (unemployment benefit OR = 4.14 and asylum, OR = 4.14), intravenous drug use (OR = 5.34), immunosuppression other than HIV (OR = 2.29), HIV status AIDS (OR = 2.19), and no current TB therapy (OR = 2.13).

DISCUSSION

A series of socioeconomic factors have been associated with TB in Western European countries. Factors identified by our survey include immigration, aging, HIV infection, population mobility, living in communities, and drug abuse. These factors may affect the appearance of several illnesses, including MDR-TB.

Table 3. Risk Factors for MDR-TB in Four European Countries

	MDR-TB (n = 138)	*Non-MDR-TB (n = 276)*	*OR*	*CI 95%*
Age				
0–1	9	82	30.68	0.3–1.6
20–39	66	113	1.32	0.9–2.0
40–59	46	65	1.62	1.0–2.5
+60	18	75	0.40	0.2–0.7
Sex				
Male	109	195	1.56	0.9–2.5
Female	29	81	0.64	0.4–1.0
Born outside country				
Yes	73	120	1.46	0.9–2.2
No	1	40	0.68	0.4–1.0
TB known contacts				
Yes	31	32	2.21	1.3–3.8
No	107	244	0.45	0.3–0.8
Previous TB				
Yes	47	56	2.03	1.3–3.2
Pulmonary	42	54	1.80	1.1–2.9
No	96	222	0.56	0.3–0.9
Therapy	35	46	1.70	1.0–2.8
No + unkn	103	230	0.59	0.4–0.9
Living situation				
Family/individual home	110	227	0.85	0.5–1.4
Nursing home	7	7	2.05	0.7–5.9
Prison	3	3	2.02	0.4–10.1
Health-care worker	5	6	1.69	0.5–5.6
Homeless	5	13	0.76	0.3–2.2
Unknown	8	20	0.79	0.3–1.8
Income				
Employed	51	117	0.80	0.5–1.2
Retired	17	55	0.56	0.3–1.0
Unemployed benefits	14	21	1.37	0.7–2.8
Asylum	21	18	2.55	1.3–4.9
Welfare	7	18	0.77	0.3–1.9
Unknown	23	41	1.15	0.7–2.0
Other	5	6	1.69	0.5–5.6
Intavenous drug use				
Yes	13	6	4.68	1.7–12.6
Immunosuppression other HIV				
Yes	14	15	1.96	0.9–4.2
No + unknown	124	261	0.51	0.2–1.1
HIV status				
HIV seronegative	91	172	1.17	0.8–1.8
HIV seropos. Non-AIDS	5	11	0.91	0.3–2.7
Unknown	28	78	0.65	0.4–1.1
AIDS	14	15	1.96	0.9–4.2
TB localization				
Pulmonary	125	233	1.77	0.9–3.4
Current therapy				
Yes	120	238	1.06	0.6–1.9

The key to controlling tuberculosis is the rapid detection and cure of infectious cases[18]; the identification of risk factors through epidemiological studies is thus of major importance.

An effective strategy of TB control is based on passive case-finding through microscopic examination of sputum from individuals presenting themselves to diagnosis centers with a cough of at least 3 weeks' standing. In DOTS (Directly Observed Treatment, Short-Course), the detection of new cases is followed by active case management, supervision of the intensive phase, and efficient monitoring of treatment outcomes. This strategy is capable of curing the large majority of cases and preventing the development of chronic cases that may continue to spread infection in the community, leading to a greater chance of developing drug resistance.[8]

In western Europe, the incidence of tuberculosis has decreased over the years as a result of economic and social development. But the phenomenon of MDR has reportedly increased in developed countries such as the United States and constitutes a latent but very real danger in places with ready access to antituberculosis drugs, where indiscriminate use and interruption of treatment provide the selective pressure for emergence of resistance. Outbreaks of MDR, determined by clinical and/or microbiological examination of isolates, have been reported in several countries. The experience from a number of successful national control programs assisted by WHO or IUATLD shows that a well-conducted national tuberculosis control program reduces the incidence of TB in just a few years; the proportion of 'old cases' decreases to 10–20% of all pulmonary cases.[5]

The rate of acquired resistance is around 20% among 'old cases' (previously treated patients); among these patients, the rate of MDR-TB is 4–10%. Primary MDR-TB arises in settings where antituberculosis chemotherapy has been applied inappropriately for several years. In these settings, the rate of primary MDR cases may be as high as 7.5% of all new cases. In contrast, in settings where programs have delivered chemotherapy effectively for several years, the primary MDR rate is very low (typically 1% or less) in new patients. The highest rates of MDR-TB have been reported in Nepal (48%), India (33.8%), New York City (30.1%), and Korea (14.5%).[4]

The results show that MDR-TB is likely to be linked to immigration, as has been seen in previous studies in countries such as France, Italy, and The Netherlands.[6,10,11] The countries that proved to be the most significant reservoirs of infection as far as immigrants are concerned were Brazil, Russia, and Kazakhstan.

Other statistically-significant risk factors appeared to be previous TB, living situation, income, and TB site. The antimicrobial drugs most frequently found to be ineffective in cases of MDR-TB were streptomycin (54.3%), ethambutol (48.5%), and pyrazinamide (35.5%); these findings are similar to the results reported in studies by Grosset[6] and Nuntini *et al.*[11]

At the European level, the following did not prove to be statistically significant risk factors: age, sex, known TB contact, therapy and duration of previous TB, intravenous drug use, illness, immunosuppression other than HIV, HIV status, and current therapy. Risk factors reportedly important in other countries, for example the United States, include race (= OR 2.30–4.00),[1] recurrent TB (= OR 3.10),[1] and HIV seropositivity (= OR 2.34).[17] In the present study, risk factors for single countries included age over 60 in France, foreign-born status in Germany, and intravenous drug use in Italy and Spain. When only the three Mediterranean countries were considered, significant differences were found for age (40–59 years), known TB contacts, duration of previous therapy, intravenous drug use, HIV status, and no current therapy.

The small number of MDR cases in France is also noteworthy, when compared with other study countries. In Germany, additional significant factors were diabetic status and welfare income, whereas in Italy, further risk factors were known TB contacts and HIV status. In Spain, previous TB and immunosuppression other than HIV were additional factors.

In conclusion, given the serious nature of the problem, it is essential to widen epidemiological research to include the largest possible number of MDR patients in study countries, and to cover other EU countries not involved in the present study.

It would also be advisable to establish an ongoing control program for multiple TB resistance in EU countries, with a view to monitoring MDR-TB risk factors both independently in individual EU countries and globally throughout Europe.

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Address reprint requests to:
Dr. Manuel Casal
Mycobacteria Reference Center
Faculty of Medicine
14004 Córdoba, Spain

E-mail: mi1carom@uco.es