Risk factors for extensive drug resistance in multidrug-resistant tuberculosis cases: a case-case study

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Running title: risk-factors for XDR-TB in France

Wordcount: 2383 words

Abstract

Settings. Extensively drug-resistant (XDR) tuberculosis (TB) identification may be delayed because of the lack of availability of molecular testing to second-line drugs. Hence, early suspicion of XDR-TB is necessary to avoid developing further drug resistance.

Objective. We sought to identify the characteristics associated with XDR-TB among multidrug-resistant (MDR) TB cases, before the availability of second-line drug susceptibility testing (DST) results.

Methods. All MDR-TB cases with available second-line DST results recorded in France from 1998 to 2013 were classified as simple MDR (no resistance to fluoroquinolones and second-line injectables), pre-XDR (resistance to fluoroquinolones or second-line injectables), and XDR cases (resistance to both).

Results. A total of 833 MDR-TB cases were analysed, including 168 (20%) pre-XDR and 62 (7%) XDR-TB cases. A prior history of treatment was acknowledged for 41% of the cases, and 12% were HIV-positive. Characteristics independently associated with XDR-TB were foreign birth (odds ratio: 9.5), prior TB treatment (OR: 2.6), smear positivity (OR: 4.5), and ethambutol resistance (OR: 9.1). Characteristics independently associated with pre-XDR-TB when compared to simple MDR-TB cases were male gender (OR: 1.6), birth in Europe (OR: 2.6), and ethambutol resistance (OR: 1.9).

Conclusion. The presence of clinical or bacteriological characteristics associated with XDR-TB should lead to rapid molecular testing for resistance to second-line drugs before starting a tailored treatment.

Résumé

Cadre : L'identification des cas de tuberculose (TB) à bacilles ultra-résistants (XDR) aux antibiotiques peut être retardée par manque d'accès aux tests de sensibilité aux antituberculeux de seconde ligne (S2L). Afin de ne pas risquer d'aggraver l'étendue de la résistance, il est important d'identifier ces cas XDR sur d'autres critères.

Objectif : Identifier les caractéristiques associées à la TB XDR parmi les cas identifiés de TB à bacilles multirésistants (MDR), avant disponibilité des résultats des S2L.

Méthodes : Tous les cas de TB MDR avec des résultats de S2L diagnostiqués en France de 1998 à 2013 ont été classés en cas MDR simple (pas de résistance aux fluoroquinolones ni aux antibiotiques injectables de seconde ligne), pré-XDR (résistance à un seul de ces deux groupe d'antibiotiques), et XDR (résistance aux deux groupes d'antibiotiques).

Résultats : AU total, 833 cas de TB MDR ont été inclus dans l'analyse, dont 168 (20%) cas pré-XDR et 62 (7%) cas XDR. Des antécédents de traitement antituberculeux ont été identifiés chez 41% du total des cas et 12% étaient séropositifs pour le VIH. Les caractéristiques significativement associées à la TB XDR étaient la naissance à l'étranger (odds ratio : 9,5), les antécédents de traitements (OR : 2,6), un examen microscopique positif (OR : 4,5) et la résistance à l'éthambutol (OR : 9,1). Par comparaison au cas MDR simples, les caractéristiques significativement associées à la TB pré-XDR étaient le sexe masculin (OR : 1,6), la naissance en Europe hors de France (OR : 2,6) et la résistance à l'éthambutol (OR : 1,9).

Conclusion : Chez un malade porteur de TB MDR, la présence de caractéristiques cliniques ou bactériologiques associées à la TB XDR doit faire réaliser des S2L sans délai avant de débuter un traitement adapté individuellement.

INTRODUCTION

France is classified as a low incidence country for tuberculosis (TB) (1). Each year approximately 6000 cases of tuberculosis are diagnosed, representing a prevalence rate of 8-9 per 100 000, and between 50 to 100 cases or 1% to 2% of the total are multidrug-resistant (MDR) (i.e., resistant to isoniazid and rifampicin). A striking increase in the number of MDR-TB cases due to migration from Eastern Europe and the Caucasus has been recently reported in France (2,3), while the MDR-TB prevalence had been stable during the previous years (4). This changing epidemiology has been observed in other low-incidence countries in Western Europe, where the phenomenon poses a substantial threat to the World Health Organization (WHO) goal of TB elimination (5). It has been estimated that MDR-TB/rifampicin-resistant cases occurring in the WHO European region, mostly in its Eastern part, account for more than one fifth of the total MDR-TB/rifampicin-resistant cases estimated in the world in 2015 (1).

Among MDR-TB cases recently reported in western European countries, increasing numbers fulfil the definition of extensively drug-resistant (XDR) TB, i.e. MDR isolates with additional resistance to any fluoroquinolone and any second-line injectable drug (SLI). Despite prolonged treatment regimens, which according to WHO recommendations can exceed 20 months (6), treatment success was achieved in only 64% of MDR-TB cases in a large meta-analysis (7). The proportion of successful outcomes was lower for pre-XDR TB, defined as MDR-TB with additional resistance to any fluoroquinolone or any SLI, and further decreased to 40% for XDR-TB cases (7). Moreover, the prognosis of XDR-TB cases has been shown to be worse for patients harbouring isolates with additional resistance to other first- and second-line drugs (8). The mortality associated with XDR-TB varies according to the degree of HIV

prevalence of the population, while in all settings a remarkable number of these subjects are discharged in the community, which contributes to the spread of the disease (9,10).

The increasing burden of XDR-TB, together with its difficult management and unsatisfactory outcome, emphasizes the importance of timely diagnosis and treatment initiation for XDR-TB patients. However, drug susceptibility tests (DST) are not available in all settings worldwide (1). The development of automated molecular assays for DST to first line drugs may facilitate the diagnosis of MDR tuberculosis but is currently not implemented for all TB cases. Rapid molecular testing for second-line drugs is still restricted to selected laboratories, even in high-income countries. It is therefore of interest to identify the characteristics associated with additional resistance in MDR-TB patients for early recognition and better early management in order to avoid increasing the initial burden of resistance. The major objective of our study is to identify epidemiological and bacteriological factors associated with XDR-TB when compared to non-XDR MDR-TB patients diagnosed in France.

METHODS

MDR-TB surveillance in France

Since 1992, the national Reference Centre for Mycobacteria (NRC) pilots a network of 250 laboratories, which includes all centres performing mycobacterial culture in France. Among them, approximately a third are performing drug susceptibility tests (DST) for first line drugs by phenotypic methods and less than 15 (6%) are using molecular biology to test for resistance to second-line drugs. Each year, the laboratories report the number of MDR-TB cases to the NRC, which confirm the MDR status and retrospectively collects additional information on MDR-TB patients by sending a standardized data collection form. Initial clinical and demographical data are collected for each MDR-TB patient, including country of birth, HIV status, previous TB treatment history, and microbiology. Finally, MDR *Mycobacterium tuberculosis* complex isolates are sent to the NRC laboratory in order to perform comprehensive DST for first and second-line anti-TB drugs. From 1992 to 2004, the second-line DST were performed at either Pitié-Salpêtrière Hospital or the Pasteur Institute, in Paris. Since 2005, this task was entirely taken over by the Pitié-Salpêtrière NRC. Phenotypic DST for first- and second-line drugs were performed by using the proportion method on Löwenstein-Jensen medium according to WHO recommendations (11).

Case-case studies

Since DST for fluoroquinolones and SLI started to be routinely performed in 1998, only MDR-TB cases reported from 1998 to 2013 were included in the analysis. Patients reported during multiple years, i.e. culture-positive chronic cases, were considered only at the first occurrence. Duplicates were eliminated before data anonymization. Patients with missing DST result for fluoroquinolones and SLI were excluded from the analysis.

In order to compare characteristics of MDR-TB patients harbouring different resistance patterns to fluoroquinolones and SLI, we performed different nested case-case studies within the cohort. In the main analysis, patients harbouring XDR isolates were compared to all the other cases harbouring non-XDR MDR isolates. Two other case-case analyses were conducted, comparing i) cases with pre-XDR isolates to those harbouring MDR isolates with no additional resistance to any fluoroquinolone and any SLI (defined as simple-MDR), and ii) XDR to pre-XDR patients.

Data analysis

Medians with interquartile ranges (IQR) were calculated for continuous variables, and frequency distributions were tabulated for categorical variables. Groups were compared using the two-sample Wilcoxon-Mann-Whitney test for continuous data and Fisher's exact test for categorical data. In order to assess the independent association of predictive variables, logistic regression modelling was performed. For each case-case analysis, variables associated to a group of patients under a cut-off of p-value <0.2 in univariate analysis were added to a multivariable model. The variables of resistance to ethambutol and streptomycin were considered for multivariable analysis, as they could be of interest to predict additional resistance in MDR-TB cases. Pyrazinamide DST status was not included because of the difficulty to interpret its result and consequently a high number of missing data. Patients with unknown HIV status were considered as HIV-negative and patients with unknown place of birth were classified as foreign-born. With regard to the other variables, patients with missing data were excluded from the analysis. Backward selection procedure was performed, with age and sex included by default in all models. The foreign-born status or WHO regions of birth were tested alternatively in the models. Birth in France was considered as the reference in all models. The best-fitted model was chosen on the basis of epidemiological and statistical

criteria, such as the Hosmer-Lemeshow goodness-of-fit test and the Akaike Information Criterion. Interaction terms were included in the models and dropped if non-significant. Only the model with the least number of variables is reported for each case-case analysis. Odds ratios (OR) were calculated with their 95% confidence intervals (CI). P-values < 0.05 were considered as statistically significant. STATA, version 11.1 (StataCorp, College Station, Texas, USA) was used for statistical analysis.

Ethics

Data were routinely collected during standard care and abstracted from medical files. No personal identifiers were recorded. Therefore, according to French regulations, the study did not require institutional review board or patients' approvals.

RESULTS

Overall, 983 MDR-TB cases have been recorded in France during the 16-year study period, ranging from 31 to 91 per year. Among them, 93 (9.5%) cases were discarded because of duplicate declarations and 57 (5.8%) because DST results were not available (Figure 1). The baseline characteristics of the 833 MDR-TB patients finally included in the study are shown in Table 1. Patients were mostly male (64%), born in Sub-Saharan Africa (30%) or in European countries excluding France (28%). Among the latter, a large majority of patients (91%) were from the 18 high-priority countries (HPC) identified by WHO Regional Office for Europe's Stop TB Strategy.

French-born patients accounted for only 15% of the cases, and the country of birth was unknown for 23 (2.8%) patients. A majority of patients had pulmonary TB (90%), and 56% had positive sputum-smear examination. A total of 41% had a prior history for TB treatment. A total of 12% were HIV-positive, 76% HIV-negative, and 12% had unknown HIV status. The median age at diagnosis was 32 years (IQR: 25-43).

A total of 603 (72%) patients had isolates susceptible to both fluoroquinolones and SLI, 168 (20%) pre-XDR isolates, and 62 (7%) XDR isolates. Additional resistance was frequent for streptomycin (69%) and ethambutol (47%).

Comparison of XDR-TB cases to all other MDR-TB cases

When comparing the 62 XDR-TB to the 771 non-XDR MDR-TB cases, XDR-TB was associated with an age at diagnosis of 35 to 44 years (Table 1). XDR-TB patients were significantly more likely to be born outside France and particularly in Europe (OR: 35.6; 95% CI 13.9-91.6), to have a prior history of TB treatment (OR: 3.7; 2.0-6.9), and to be sputum smear-positive (OR: 3.5; 1.8-6.9). No significant association was observed with sex, disease

localisation and HIV status. XDR isolates were significantly more likely to harbour resistance to streptomycin and ethambutol (Table 1).

After multivariable logistic regression analysis, foreign birth (OR 9.5; CI: 1.2-75.9), sputum smear positivity (OR 4.5; CI: 2.0-10.1), prior TB treatment (OR 2.6; CI: 1.4-5.0), and resistance to ethambutol (OR 9.1; CI: 4.0-20.7) remained independently associated with the risk of XDR-TB (Tables 2, model 1). When replacing foreign birth by regions of birth in a second model (Table 2, model 2), birth in the WHO European region (OR 21.0; CI: 7.0-63.1) was significantly associated with XDR-TB, while no other region of birth remained associated with XDR-TB status.

Comparison of pre-XDR TB cases to simple-MDR-TB cases

In univariate analysis, pre-XDR TB cases were significantly more likely than simple-MDR TB cases to be male, born in the European region, born in Africa excluding the Sub-Saharan region, to have prior TB treatment and to harbour isolates with additional resistance to ethambutol and streptomycin (data not shown). In multivariable analysis, characteristics significantly associated with pre-XDR TB as compared to simple-MDR TB were male gender (OR 1.6; CI: 1.0-2.6), birth in Europe (OR 2.6; CI: 1.3-5.1), and ethambutol resistance (OR 1.9; CI: 1.4-2.7) (Table 3, model 1).

Comparison of pre-XDR TB cases to XDR-TB cases

In univariate analysis, XDR-TB cases were significantly more likely than pre-XDR TB cases to be born in the European region, or in Africa excluding the Sub-Saharan region, to have a positive sputum smear examination, to have prior TB treatment, and to harbour isolates with additional resistance to ethambutol (data not shown). In multivariable analysis, characteristics remaining significantly associated with XDR-TB as compared to pre-XDR TB were birth in

Europe (OR 8.1; CI: 3.1-21.3), sputum smear positivity (OR 5.2; CI: 2.9-9.3), and ethambutol resistance (OR 3.9; CI: 1.7-8.8) (Table 3, model 2).

DISCUSSION

We compared the characteristics of patients diagnosed with MDR-TB or XDR-TB over 16 years in France. The results of our study delineate a few characteristics associated with resistance beyond MDR.

First, foreign birth was associated with XDR-TB, and with higher resistance in both intermediate steps between MDR-TB and XDR-TB. In low TB incidence countries, the predominance of foreign-born patients among MDR-TB cases is a common finding (12–15). In our study, the highest risk group was those of patients born in countries with high incidence of MDR/XDR-TB, such as European HPC. This phenomenon has been previously described in a country-wide cohort of TB patients in Canada (16). Second, our results have shown that previous TB treatment was significantly associated with XDR-TB. This is a well-known risk factor, which has already been described in different settings in association with both MDR-TB (17–20) and XDR-TB (21–24). Third, smear positivity was also independently associated with XDR-TB in our cohort, in the comparison with both MDR-TB and pre-XDR TB strains. This could be linked to the fact that most XDR-TB patients in our cohort had a long previous treatment histories and advanced pulmonary lesions. This finding, however, is not confirmed by other similar studies (24,25). This may also reflect high bacterial load, which is directly linked to a higher likelihood of resistant mutants in the lesions. Fourth, XDR-TB cases were associated with significantly higher rates of ethambutol resistance, and the same finding was reported for more resistant strains in both intermediate analyses. Higher rates of ethambutol resistance have been consistently reported in XDR-TB patients (10,26,27), and may have been a facilitating factor for the progressive selection of resistance to second-line drugs. Hence, rapid availability of ethambutol DST could help in the management of MDR-TB patients. However, the performance of the first version of the most

commonly used line probe assay for the detection of resistance to second-line drugs was suboptimal for ethambutol, probably due to the lack of testing in regions outside *embB* codon 306 (28). Unfortunately, the second version of this test does not include ethambutol testing at all.

In addition, male sex was linked to pre-XDR TB when compared to MDR-TB, as previously reported in a study in the Russian Federation (21), but in contrast to a multicentre study where female sex was linked to XDR-TB (24). Finally, we did not find a significant association between XDR-TB and HIV infection, in concordance with a large study including countries with variable HIV burden (24). In contrast, a link between HIV and XDR-TB has been shown in a high-incidence country like South Africa (17).

Our study has some limitations. Risk factors that have been consistently described in literature, such as alcohol and drug abuse, history of imprisonment, and previous treatment with second-line drugs, were not captured in the routine data collection form and therefore not analysed in our study. In addition, the epidemiological changes that occurred in the MDR-TB population in France over time (2) could have influenced our findings, and only a small proportion (7%) of patients included in the study were XDR-TB. However, both these secular trends and the low rates of XDR-TB cases are common to most low-incidence countries (29), and therefore do not affect the generalizability of our results to these settings. Finally, a limited number of patients had missing data for the key variables (HIV status and country of birth), which were managed by making assumptions.

In conclusion, we identified factors associated with XDR-TB among patients diagnosed with MDR-TB in a low-incidence setting. These characteristics should increase the level of suspicion for XDR-TB among MDR-TB cases and warrant testing with rapid molecular assays for second-line drugs. Indeed, a new version of a line probe assay has been shown to

be improved (30) and has recently been endorsed by the WHO (31). The combination of implementing rapid diagnostic tools and identifying high-risk groups may lead to promptly detect XDR-TB cases and to design effective empirical regimens while waiting for comprehensive DST results. Finally, new shorter MDR-TB treatment regimens should be avoided in patients harbouring such characteristics.

280 **Table 1.** Characteristics of 833 multidrug-resistant tuberculosis (MDR-TB) cases according to

281 extensively drug-resistant (XDR-TB) status.

Categorical variables	All cases *(n=833)* n (%)	XDR-TB isolates		OR (CI 95%)	p
		Yes *(n=62)* n (%)	No *(n=771)* n (%)		
Sex Male	533 (64)	45 (74)	488 (64)	1.6 (0.8-2.9)	0.16
Age*:					
≤14	26 (3)	1 (2)	25 (3)	1	
15-24	167 (20)	9 (14)	158 (20)	1.4 (0.5-4.0)	0.51
25-34	298 (36)	19 (31)	279 (36)	1.7 (0.7-4.2)	0.25
35-44	165 (20)	21 (34)	144 (19)	3.6 (1.5-8.7)	<0.01
45-64	133 (16)	10 (16)	123 (16)	2.0 (0.6-6.4)	0.23
≥65	38 (5)	2 (3)	36 (5)	1.4 (0.3-5.4)	0.64
Foreign-birth or unknown country*	710 (85)	61 (98)	649 (84)	11.5 (4.2-31.6)	0.001
Region of birth*:					
France	123 (15)	1 (2)	122 (16)	1	-
Europe (France excluded)	230 (28)	52 (84)	178 (24)	35.6 (13.9-91.6)	<0.001
North Africa	97 (12)	4 (6)	93 (12)	5.2 (0.9-30.4)	0.06
Sub-Saharan Africa	252 (30)	3 (5)	249 (33)	1.5 (0.3-6.3)	0.61
Others	108 (13)	2 (3)	106 (14)	2.3 (0.6-9.2)	0.24
Pulmonary tuberculosis	747 (90)	60 (97)	687 (90)	3.3 (1.1-10.1)	0.11
Smear positive for pulmonary cases	464 (56)	51 (82)	413 (57)	3.5 (1.8-6.9)	<0.001
HIV-positive*	101 (12)	4 (6)	97 (13)	0.5 (0.1-2.0)	0.22
Prior tuberculosis treatment	343 (41)	43 (73)	300 (42)	3.7 (2.0-6.9)	<0.001
Resistance to:					
Ethambutol	389 (47)	55 (89)	334 (44)	10.2 (3.6-28.9)	<0.001
Streptomycin	577 (69)	53 (85)	524 (69)	2.6 (1.3-5.3)	0.006

282 OR, odds ratio; CI, confidence interval

283 *6 patients with missing age,23 with unknown region of birth, and 97 with unknown HIV status

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Table 2. Multivariable logistic regression analysis assessing characteristics associated with extensively drug-resistant tuberculosis in a cohort of multidrug-resistant tuberculosis cases. The country of birth of patients was entered into the model after categorization according to foreign-born versus French-born (Model 1) or according to the birth in the European region versus birth outside the European region and French-born (Model 2). The European region was defined according to the World Health Organisation; France was excluded from this group.

Categorical variables	**Model 1**		**Model 2**	
	OR (CI 95%)	**p**	**OR (CI 95%)**	**p**
Foreign birth	9.5 (1.2 – 75.9)	0.034	-	
Born in the European region (France excluded)	-		21.0 (7.0 – 63.1)	<0.001
Sputum smear-positivity	4.5 (2.0 – 10.1)	<0.001	3.8 (2.1 – 6.8)	<0.001
Prior tuberculosis treatment	2.6 (1.4 – 5.0)	0.004	2.2 (1.2 – 3.9)	0.018
Additional resistance to ethambutol	9.1 (4.0 – 20.7)	<0.001	6.1 (2.3 – 16.4)	<0.001

OR, odds ratio; CI, confidence interval.

Table 3. Multivariable logistic regression analysis assessing characteristics associated with tuberculosis (TB) due to multidrug-resistant (MDR) isolates with additional resistance to any fluoroquinolone or any second-line injectable drug (pre-XDR) as compared to MDR-TB with no additional resistance to any fluoroquinolone or second-line injectable drug (Model 1) and characteristics associated with extensively drug-resistant TB as compared to Pre-XDR TB (Model 2). The European region was defined according to the World Health Organisation; France was excluded from this group and included as itself in the models.

Categorical variables	**Model 1** **MDR-TB vs. pre-XDR TB**		**Model 2** **pre-XDR TB vs. XDR-TB**	
	OR **(IC 95%)**	**p**	**OR** **(IC 95%)**	**p**
Sex, male	1.6 (1.0 – 2.6)	0.033	-	
Born in the European region (France excluded)	2.6 (1.3 – 5.1)	0.006	8.1 (3.1 – 21.3)	<0.001
Sputum smear-positive pulmonary tuberculosis	-		5.2 (2.9 – 9.3)	<0.001
Strain with additional resistance to Ethambutol	1.9 (1.4 – 2.7)	<0.001	3.9 (1.7 – 8.8)	<0.001
CI, confidence interval; OR, odds ratio.				

Figure 1. Flow-chart of the study

ACKNOWLEDGEMENTS

The authors thank all microbiologists that participated in the NRC Laboratory Network for MDR-TB surveillance and Cristina Gutierrez for data checking during the 1998-2004 period.

We are also thankful to the clinicians who shared information.

JR conceived and designed the study

LG & JR performed the statistical analysis, analysed the results and drafted the manuscript

LG, NV, AA, FB, CB, WS, VJ & JR collected the data, participated in the interpretation of the results, and critically revised the manuscript.

NV, AA, FB, CB & WS were in charge of drug susceptibility tests.

All authors gave final approval of the current version to be published

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