Effect of Drug Resistance on the Generation of Secondary Cases of Tuberculosis

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Background. The results of animal studies suggest that isoniazid-resistant strains of Mycobacterium tuberculosis are less pathogenic than isoniazid-susceptible strains. Here, we assess the relative pathogenicity of drug-resistant and drug-susceptible strains, in a human population.

Methods. We linked IS6110 genotype patterns of M. tuberculosis strains with drug-susceptibility test results and epidemiologic information for 85% of culture-positive incident cases of tuberculosis (TB) in San Francisco during 1991–1999. We assumed that drug-susceptible and drug-resistant strains were transmitted to secondary case patients if the drug-resistance and genotype patterns were identical. We calculated the number of secondary cases for each drug-resistance pattern and determined the relative secondary-case rate ratio (SR) of drug-resistant TB to drug-susceptible TB.

Results. There were 1800 patients with culture-positive TB, drug-susceptibility test results, and genotyping results. The overall SR of drug-resistant to drug-susceptible TB cases was 0.51 (95% confidence interval [CI], 0.37–0.69). The SR was 0.29 (95% CI, 0.15–0.57) for isoniazid-resistant strains, 0.10 (95% CI, 0.02–0.42) for strains resistant to both isoniazid and streptomycin, and 0.88 (95% CI, 0.53–1.47) for streptomycin-resistant strains. There were no secondary cases caused by multidrug-resistant (MDR) TB. The SR for rifampin-resistant cases was 2.33 (95% CI, 1.04–5.25). Seventy-eight percent (7/9) of the patients with rifampin-resistant secondary cases of TB were seropositive for human immunodeficiency virus.

Conclusion. In the context of an effective TB program in San Francisco, strains that were resistant to isoniazid either alone or in combination with other drugs were less likely to result in secondary cases than were drug-susceptible strains. In this setting, isoniazid-resistant and MDR TB cases were not likely to produce new, incident drug-resistant TB cases.

Serious concern has been expressed that dissemination of Mycobacterium tuberculosis strains that are resistant to antituberculosis drugs could undermine global efforts to control tuberculosis (TB) [1, 2]. However, there is considerable uncertainty about the potential magnitude of the global epidemic of drug-resistant TB. Two sequential global surveys have shown that the prevalence of drug-resistant TB is influenced by the quality of TB control programs and by local epidemiologic circumstances [3, 4]. In addition, experimental data and mathematical models suggest that the reduction in bacterial fitness imposed by antimicrobial resistance could influence the frequency of drug-resistant TB in a population [5–7]. Thus, it is important to determine whether drug-resistant and drug-susceptible strains of M. tuberculosis have an equal likelihood of spreading in the community [8].

The spread of M. tuberculosis involves a 3-step process: transmission of bacteria, establishment of infection, and progression to disease. A previous study that used tuberculin skin testing to diagnose infection found an equal prevalence of infection among contacts exposed to patients with active TB caused by both drug-resistant and drug-susceptible strains [9]. In contrast, animal studies have shown that isoniazid-resistant strains caused significantly less disease in guinea pigs than drug-susceptible strains [10–12]. In addition, molecular epidemiologic studies observed that cases of TB caused by drug-resistant
strains were less likely to be in clusters, inferring that drug-resistant strains were less likely to be transmitted and/or to cause active TB [13–15].

The present study was conducted over the course of a 9-year period in the context of an effective TB control program in San Francisco [16] and tracked specific strains of *M. tuberculosis* as they spread in the community. To assess the effect of resistant strains on the incidence of TB disease, we quantified the number of secondary cases generated by drug-resistant versus drug-susceptible strains and calculated the relative secondary-case rate ratio (SR) of the drug-resistant strains.

**PATIENTS AND METHODS**

The study population included all patients with culture-confirmed, incident cases of TB in San Francisco, from 1 January 1991 through 31 December 1999. Data were collected as part of an ongoing study of the molecular epidemiology of TB in San Francisco [17]. The study protocols and procedures for the protection of human subjects were approved by Stanford University and the University of California, San Francisco.

All patients suspected of having active TB had sputum specimens obtained for microscopy for acid-fast bacteria (AFB) and mycobacterial culture, and most of them were treated with a regimen of isoniazid, rifampin, ethambutol, and pyrazinamide. All patients with positive cultures had initial drug-susceptibility testing performed for the above 4 drugs and for streptomycin. Drug-susceptibility test results became available 4–6 weeks after initiation of treatment. Strains found to be multidrug resistant (MDR; resistant to at least isoniazid and rifampin) underwent repeat testing for susceptibility to first-line drugs and additional testing for susceptibility to second-line drugs. Patients found to have MDR TB received individualized therapy, on the basis of test results. Contact tracing occurred before drug-susceptibility test results became available, and all case patients with TB and their contacts were evaluated in the same manner, regardless of their drug-susceptibility test results.

The microbiological laboratory followed standard testing guidelines, with ongoing quality control procedures. AFB smears, mycobacterial cultures, and drug-susceptibility testing were performed in licensed laboratories, by use of standard methods [18]. Drug concentrations used to determine susceptibility were as follows: isoniazid, 0.1 μg/mL; rifampin, 2.0 μg/mL; ethambutol, 2.5 μg/mL; and streptomycin, 2.0 μg/mL, according to the standard BACTEC methods [19]. Drug-susceptibility test results were confirmed using culture on solid media (Lowenstein-Jensen or Middlebrook 7H10/7H10S agar by plates). Pyrazinamide-susceptibility test results were considered to be unreliable and were excluded from the analysis.

Genotyping was performed with both IS6110 and the polymorphic guanine-cytosine–rich sequence (PGRS), by use of standardized methods. Isolates with >5 copies of IS6110 were considered to be clustered if at least 1 other genotype pattern was identical. Isolates with ≤5 copies of IS6110 were further genotyped with PGRS and were considered to be clustered if the PGRS and IS6110 genotype patterns were identical [20].

To evaluate the generation of secondary cases of TB, we chronologically ordered the cases in each cluster on the basis of the date that the first culture-positive specimen was obtained for each patient. For the primary analysis, the first case in a cluster was assumed to be the source case patient, and subsequent cases (secondary cases) were presumed to be the result of transmission from the putative source case patient. A patient whose isolate was resistant to ≥1 drug was considered to be the source case patient for any subsequent cases with the same resistance pattern in the same cluster. Clusters were categorized according to whether they had ≥2 cases with the same drug-resistance pattern, 1 drug-resistant case in the cluster (i.e., no transmission of drug-resistant TB was detected), or no drug-resistant cases.

We used univariate analyses and multivariate logistic-regression modeling to identify the host and microbiologic factors that were independently associated with drug resistance and with the development of resistant secondary cases. Statistical analysis was done with Stata software (version 6; Stata). We used the χ² test of proportions or Fisher’s exact test to compare categorical variables. The Shapiro Wilk W test was used to determine whether continuous variables approximated a normal distribution [21], and continuous variables were compared by use of Student’s *t* test or the nonparametric Wilcoxon rank-sum test statistic [22]. We used the Bonferroni test to determine the probability of erroneously concluding that a difference exists at least once when making multiple comparisons [23].

We then estimated the relative rate of generating a secondary drug-resistant case by calculating the individual rate for both drug-resistant and drug-susceptible cases that arose from all drug-susceptible and drug-resistant cases [8, 24]. The relative SR of cases of drug-resistant TB was estimated according to the following formula:

\[
SR = \frac{n_r/(N_r - n_r)}{n_s/(N_s - n_s)}
\]

where *n*<sub>*r*</sub> represents the number of drug-resistant secondary cases, *n*<sub>*s*</sub> represents the number of drug-sensitive secondary cases, *N*<sub>*r*</sub> represents all drug-resistant cases, and *N*<sub>*s*</sub> represents all drug-susceptible cases. If SR = 1, drug-resistant strains and drug-susceptible strains are spreading at the same rate in the population. If SR < 1, drug-resistant strains are spreading at a slower rate than drug-susceptible strains. If SR > 1, drug-resistant strains are spreading at a faster rate than drug-susceptible strains. This formula was repeated for every drug-resistance pattern. **6110**
pattern detected in the study population, to estimate the relative SR and the 95% confidence intervals (CIs). We also stratified and repeated the above analyses by human immunodeficiency virus (HIV) status, place of birth, and site of disease.

We performed several different sensitivity analyses to assess potential biases. Unidentified case-ascertainment bias could have occurred; a TB case could be missing from our data because it belonged to the 14.7% of patients without genotyping results, because it preceded the date we began the study (1 January 1991), or because it was diagnosed somewhere other than San Francisco County. To determine whether case-ascertainment bias was important, we compared the characteristics of patients whose initial isolate did and did not have a genotyping result available and used the \( \chi^2 \) test of proportions. The potential effect of missing TB cases or partial sampling was assessed by progressively removing the largest clusters (with 16–38 persons each), to assess whether our SR estimates were biased by the occurrence of large clusters. The potential effect of extrapulmonary TB was assessed by removing TB cases that had extrapulmonary TB only and recalculating our SR estimates.

**RESULTS**

From 1991 through 1999, there were 2498 incident cases of TB reported in San Francisco; *M. tuberculosis* was isolated from 2142 (85.8%) of them. Genotyping results were available for 1828 (85.3%) of the culture-positive cases. Twenty-six cases (1.4%) met predefined criteria for laboratory cross-contamination; drug-susceptibility testing was not performed for 2 (0.1%) cases, so they were excluded from analyses (figure 1). In a multivariate analysis, patients whose initial isolate was genotyped were more likely than patients whose isolate was not genotyped to have pulmonary TB (\( P < .001 \)), to have an initial chest radiograph showing cavitation (\( P < .001 \)), and to be \( \geq 25 \) years old at the time of diagnosis (\( P < .001 \)).

Of the 1800 cases in the analysis, 1118 (62.1%) had a non-clustered genotype, and 682 (37.9%) were in 171 clusters. There were 1520 isolates that were fully susceptible to isoniazid, rifampin, ethambutol, and streptomycin; 280 isolates (18.4%) were resistant to \( \geq 1 \) drug. Ninety-six (34%) of the 280 drug-resistant cases were in clusters. There were 290 (16.1%) cases of TB with
extrapulmonary disease only. Demographic characteristics of the patients are available in an Appendix published only in the electronic edition of the *Journal* (http://www.journals.uchicago.edu/JID/journal/).

To make the inference of transmission and generation of a secondary case more specific, we examined the occurrence of drug-resistant strains from an individual putative source case to other secondary cases within a cluster. The different types of clusters are depicted in figure 2. Comparisons of both drug-resistant with drug-susceptible putative source cases and drug-resistant with drug-susceptible secondary cases showed no significant differences in known risk factors associated with clustering. These comparisons are shown in a table available in an Appendix published only in the electronic edition of the *Journal* (http://www.journals.uchicago.edu/JID/journal/). When we compared all source cases with their secondary cases, the latter were more likely to be US born \( (P < .001) \), of black race \( (P = .009) \), and HIV seropositive \( (P = .021) \). Overall comparisons of drug-resistant and drug-susceptible cases showed that patients with drug-resistant isolates were more likely to be younger \( (P = .002) \), US born \( (P = .007) \), and AFB-smear positive \( (P = .021) \) than were patients with drug-susceptible TB.

The overall relative SR for all drug-resistance patterns of *M. tuberculosis* was 0.51 (95% CI, 0.37–0.69). When we controlled for HIV-seropositive status, the relative SR of drug-resistant strains remained significantly \(<1\). The SR was 0.45 (95% CI, 0.30–0.69) for HIV-seronegative patients and 0.49 (95% CI, 0.30–0.80) for HIV-seropositive patients (table 1). When we controlled for place of birth, the relative SR of drug-resistant strains remained significantly \(<1\) (0.41) for the foreign-born patients (95% CI, 0.24–0.70). For US-born patients, the relative SR was \(<1\) (0.66), but with a wide confidence interval (95% CI, 0.43–1.02).

We further examined the SR of drug-resistant strains according to resistance patterns. The SR was 0.29 (95% CI, 0.15–0.57) for isoniazid-resistant strains and 0.10 (95% CI, 0.02–0.42) for strains resistant to both isoniazid and streptomycin. There were no secondary cases associated with MDR strains (table 2). The relative SR for rifampin-resistant strains was 2.33 (95% CI, 1.04–5.25). Secondary cases caused by rifampin-resistant strains occurred mainly among patients with HIV infection; 77.8% \((7/9)\) of the patients with secondary rifampin-resistant cases were HIV seropositive. The SR for streptomycin-resistant strains was 0.88 (95% CI, 0.53–1.47).

In the sensitivity analysis, when we progressively removed the largest clusters in the study population, the SR increased. The sensitivity analysis is available in an Appendix published only in the electronic edition of the *Journal* (http://www.journals.uchicago.edu/JID/journal/). However, all values of the SR were \(<1\), suggesting that, in our study population, drug-resistant TB was less likely to lead to a secondary case of TB than was drug-susceptible TB and that the SR was not strongly influenced by the largest clusters. When we removed the TB cases that involved only extrapulmonary TB, the SR estimate was 0.45 (95% CI, 0.324–0.627).

**DISCUSSION**

We used data from a 9-year, prospective, population-based, molecular epidemiology study to quantify the differential capacity of drug-resistant and drug-susceptible strains of *M. tuberculosis* to generate secondary cases of TB. The number of drug-resistant secondary cases generated varied considerably by drug-resistance pattern and place of birth. We found that, in the context of an effective TB control program, drug-resistant strains of *M. tuberculosis* were approximately half as likely as

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**Figure 2.** Examples of 3 prototypical tuberculosis clusters. ○, Drug-susceptible cases; ●, drug-resistant cases. In cluster type A, a drug-susceptible *Mycobacterium tuberculosis* strain was transmitted from 1 patient to another. There were 122 such clusters. In cluster type B, at least 1 patient had a strain resistant to \( \geq 1 \) first-line drug, but there was no documented secondary case with the drug-resistant strain. There were 30 such clusters. In cluster type C, there were both drug-resistant and drug-susceptible isolates and \( \geq 1 \) secondary cases of drug-resistant tuberculosis. In these 19 clusters, there were only 42 secondary cases from a drug-resistant strain.
The pathogenesis of TB involves 3 phases; transmission/acquisition of infection, containment/latency, and recrudescence of latent infection. Different host-pathogen interactions are operable during each of these phases, although they are not well elucidated. Because molecular epidemiologic assessments require the development of active TB (i.e., progression through each of these phases), we cannot determine whether drug resistance influences only 1 or 2 or all 3 of these processes. However, a case-control study by Snider et al. [9] demonstrating that contacts of patients with drug-resistant and drug-susceptible incident cases of TB had an equal prevalence of positive tuberculin skin test results suggests that infectivity was not diminished by drug resistance. Moreover, our finding of a reduced SR for isoniazid-resistant strains in a human population is consistent with the observed decreased pathogenicity of isoniazid-resistant strains in animal models [10–12]. For example, mutations or deletions within the katG gene of isoniazid-resistant strains of M. tuberculosis have been associated with a decrease in pathogenicity in animal models [25, 26]. Our present findings are most consistent with a decrease in pathogenicity associated with isoniazid resistance, although both environmental and host factors could also be playing a role. For example, prolonged and close contact with a highly infectious patient could overcome the decreased pathogenicity of the organism and result in transmission and progression to TB. Relevant to this issue, it is important to note that, in our cohort, the difference in relative SR was detected despite the counterbalancing bias that patients with drug-resistant strains of TB were more likely to be AFB smear positive than were patients with drug-susceptible strains.

By tracking the circulating drug-resistant and drug-susceptible strains of M. tuberculosis in specific populations, we were able to infer the contribution of host factors to the development of disease. For example, we found that the relative SR of drug-resistant strains, although still <1, was higher among US-born patients than among foreign-born patients. US-born patients are known to generate more secondary cases in our setting [17]. We also found that the overall SR of drug-resistant strains among patients infected and not infected with HIV was essentially equivalent. Please see table 1A in the Appendix in the electronic edition of the Journal (http://www.journals.uchicago.edu/JID/journal/). However, a large proportion of patients with rifampin-resistant strains were both US born and infected with HIV. Resistance to rifampin alone has been reported nearly exclusively among patients with advanced HIV disease, although the reasons for this association have not been determined [27].

In our study population, we did not observe a single instance of transmission resulting in a secondary case of MDR TB, despite having 31 case patients with MDR disease. Although we cannot calculate an SR value for MDR strains, the lack of secondary cases is striking, and the mechanism is probably the same as that for isoniazid-resistant strains: the same mutations that lead to isoniazid resistance also occur in the development of multidrug resistance [25, 26].

In San Francisco, the same levels of intensive TB control measures are applied to capture TB cases and interrupt transmission, regardless of place of birth and drug-susceptibility test results [16]. In addition, drug-susceptibility test results are generally not available during the first 4 weeks of therapy, the time during which patients could be more infectious. Most of our patients with MDR TB received treatment as outpatients, and some remained infectious for prolonged durations, up to a year in some

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Table 1. Secondary case rate ratio (SR) of drug-resistant (DR) strains, by human immunodeficiency virus (HIV) serostatus and place of birth.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Secondary cases from DR case</th>
<th>Secondary cases from DS case</th>
<th>SR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant to &gt;1 drug</td>
<td>42</td>
<td>424</td>
<td>0.51 (0.37–0.69)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HIV serostatus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>146</td>
<td>0.49 (0.30–0.80)</td>
<td>.003</td>
</tr>
<tr>
<td>Negative</td>
<td>23</td>
<td>278</td>
<td>0.45 (0.30–0.69)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Place of birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>29</td>
<td>268</td>
<td>0.66 (0.43–1.02)</td>
<td>.055</td>
</tr>
<tr>
<td>Foreign</td>
<td>13</td>
<td>156</td>
<td>0.41 (0.24–0.70)</td>
<td>.004</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; DS, drug susceptible. For the definition of SR, see the Patients and Methods section.
cases. Both of these factors would favor a greater SR for MDR strains. Furthermore, in the present study, the median number of contacts for case patients with either drug-resistant or drug-susceptible TB was not statistically different (data not shown).

All molecular epidemiological studies that seek to address pathogenicity suffer from methodological issues that can bias the results [28]. Differences in sampling, misclassification of source cases, treatment practices, prior exposure to TB, previous vaccination with bacille Calmette-Guérin, and behavioral heterogeneity among patients could also explain the differences we observed. However, we do not believe that these factors can explain all the differences described in the present study. By using a population-based approach over the course of many years, we were unlikely to have preferentially missed drug-resistant, compared with drug-susceptible, cases. Misclassification of source cases is possible. We assumed that the first patient in the cluster was the initial source of transmission. It is possible that a different source case in the chain of transmission could alter the number of drug-susceptible or drug-resistant secondary cases generated. However, this assumption was made for both drug-resistant and drug-susceptible source cases. Furthermore, as noted in a previous study from San Francisco describing transmission of TB from smear-negative source cases, misclassification was unlikely to be a significant factor [24].

Other possible sources of bias in our study are host and environmental factors that we could not measure and that could have influenced the difference in the secondary-case rates observed. For example, variables such as previous vaccination with bacille Calmette-Guérin and prior TB infection are more common among patients who are foreign born, and these factors could have decreased their risk of developing TB. To address this possible bias, we calculated the SR for US-born and foreign-born case patients. As noted above, US-born patients with drug-resistant TB were more likely to produce a secondary case than were foreign-born patients with drug-resistant TB, but they were still less likely than patients with drug-susceptible TB to produce a secondary case.

Our findings cannot be extrapolated to all settings. There are areas where drug resistance is neither detected nor treated effectively and where the longer duration of infectiousness for patients with drug-resistant strains treated with standard regimens might offset the bacterium’s diminished capacity to cause secondary cases. In areas with high prevalence rates of HIV, the increased host susceptibility, even to strains with diminished pathogenicity, may offset bacterial differences. Thus, because poor TB control and underlying HIV infection are common in many areas, drug resistance may flourish locally, despite the diminished propensity of drug-resistant strains to cause disease.

In conclusion, our data suggest that, in the context of an effective TB control program, M. tuberculosis strains that are resistant to at least isoniazid result in fewer secondary cases. Consequently, this apparent reduced propensity to cause disease diminished the epidemiologic effect of isoniazid-resistant and MDR strains in San Francisco. In contrast, rifampin-resistant strains were more likely to result in a secondary case of TB, because most of the secondary cases caused by rifampin-resistant strains occurred in HIV-infected individuals. It will be important to identify the molecular basis of these observations and to determine whether similar findings are documented in different populations and under different epidemiological conditions.

**Acknowledgments**

We thank Antonio Paz, Masae Kawamura, and the staff at the Tuberculosis Clinic, San Francisco Department of Public Health, who maintained an excellent standard of care for tuberculosis patients; Melvin Javonillo for assistance with IS6110 and polymorphic guanine-cytosine–rich sequence typing; and Dennis Osmond for his thoughtful comments.

**References**


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**Table 2. Secondary-case rate ratio (SR), by initial drug-resistance phenotype.**

<table>
<thead>
<tr>
<th>Drug-resistance phenotype</th>
<th>Secondary cases from DR case</th>
<th>Secondary cases from DS case</th>
<th>SR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>9</td>
<td>424</td>
<td>0.29 (0.15–0.57)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>19</td>
<td>424</td>
<td>0.88 (0.53–1.47)</td>
<td>.629</td>
</tr>
<tr>
<td>Rifampin</td>
<td>11</td>
<td>424</td>
<td>2.33 (1.04–5.25)</td>
<td>.035</td>
</tr>
<tr>
<td>Isoniazid and streptomycin</td>
<td>2</td>
<td>424</td>
<td>0.10 (0.02–0.42)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MDR tuberculosis</td>
<td>0</td>
<td>424</td>
<td>0.00 (0.0–undefined)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**NOTE.** For the definition of SR, see the Patients and Methods section. CI, confidence interval; DR, drug resistant; DS, drug susceptible; MDR, multidrug resistant.

* One-sided, 97.5% CI.