MAJOR ARTICLE

# The Effect of Initial Drug Resistance on Treatment Response and Acquired Drug Resistance during Standardized Short-Course Chemotherapy for Tuberculosis

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***Background.*** In Tomsk Oblast, Russian Federation, during the period of 1996–2000, most previously untreated patients with tuberculosis received standardized short-course chemotherapy, irrespective of drug-susceptibility testing results. A retrospective analysis was done to determine the effect of initial drug resistance on treatment outcome and acquired drug resistance in new patients receiving standardized short-course chemotherapy.

***Methods.*** During the period of 1 November 1996 through 31 December 2000, a total of 2194 patients received a category 1 treatment regimen. Drug susceptibility test results for 1681 patients were available for analysis. Drug resistance patterns before and during treatment were compared for 73 patients whose culture results were persistently positive during treatment. Acquired resistance was defined as new drug resistance (during or at the end of treatment) that was not present at the beginning of treatment.

***Results.*** Pretreatment drug resistance was strongly associated with treatment failure. In patients who had strains with pretreatment resistance patterns that included isoniazid or rifampin resistance, but not resistance to both, 17 (70.8%) of 24 cases involving treatment failures acquired new multidrug resistance. In patients with pretreatment pan-susceptible or streptomycin-monoresistant strains, 13 (41.9%) of 31 cases involving treatment failures acquired new multidrug resistance.

***Conclusions.*** Early diagnosis of drug-resistant tuberculosis and judicious use of second-line drugs is recommended to decrease transmission of drug-resistant strains and to prevent the creation of multidrug-resistant strains. Finally, if drug susceptibility tests are not available or results are delayed, physicians should recognize that patients who do not respond to directly observed empirical short-course chemotherapy are at high risk of having multidrug-resistant tuberculosis and should be treated accordingly.

Drug-resistant tuberculosis has been found in every geographic area surveyed [1]. Although drug-resistant tuberculosis is observed in previously treated patients because of past incorrect or irregular treatment, it is also observed in new, previously untreated patients because of transmission of drug-resistant strains. The treatment of drug-resistant tuberculosis often requires second-line drugs and should be guided by drug susceptibility test (DST) results [2]. Unfortunately, in many countries that have high burdens of drug-resistant tuberculosis, new patients with drug-resistant and even multidrug-resistant tuberculosis (MDR-TB) are often treated empirically with first-line drugs. The reasons for this include a lack of access to second-line drugs, a lack of laboratory facilities capable of performing DSTs, and a lengthy turnaround time for DST results for many commonly used techniques [3–6].

The DOTS (directly observed therapy, short course) strategy is recommended by the World Health Organization for the control of tuberculosis. In DOTS, standardized short-course chemotherapy is recommended for patients with previously untreated tuberculosis [7]. Standardized short-course chemotherapy has been shown to be less effective against drug-resistant tuber-

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culosis than against drug-susceptible tuberculosis [8]. In addition, concern has been raised over the possibility of "amplification" of resistance—adding to the resistance of a previously resistant strain through an inadequate DOTS treatment regimen [9].

Tomsk Oblast, Siberia, Russian Federation, has a population of ~1,048,000. In 1999, the incidence of smear-positive tuberculosis was estimated to be 47.7 cases per 100,000 persons, and the prevalence of drug-resistant tuberculosis among previously untreated patients was estimated to be 29% [1]. DOTS was instituted in Tomsk in 1994 in an effort to improve tuberculosis control [10, 11]. During the period of 1996–2000, there was a secure supply of first-line drugs but a limited supply of second-line drugs. Although DSTs were performed for all patients with culture-positive tuberculosis, most previously untreated patients with drug-resistant tuberculosis received standardized short-course chemotherapy. Therefore, the Tomsk experience provides a unique opportunity to examine the effect of initial drug resistance in new patients receiving standardized short-course chemotherapy. Here, we present a retrospective analysis of program and laboratory data with the objective of determining the risk of treatment failure and acquired resistance in patients with varying levels of drug resistance.

## METHODS

***Cohort selection.*** Tomsk Oblast Tuberculosis Services (TOTBS) coordinates the DOTS program in the civilian sector. All patients listed in the Tomsk regional reference laboratory as having started a category 1 regimen during the period of 1 November 1996 through 31 December 2000 were included for analysis. The Tomsk regional reference laboratory routinely performs cultures and DSTs for all TOTBS patients, except for a small number (estimated to be <2.5%) of all new patients with tuberculosis who live in remote rural areas. Prisoners with tuberculosis are not TOTBS patients, because their treatment is coordinated by the penitentiary system, which has a separate clinic and laboratory network. Therefore, this study has no data from prisoners, although some civilian patients may have been ex-prisoners. The study protocol was approved by the Siberian State Medical University Ethics Committee (Tomsk Oblast).

***Treatment protocol.*** The diagnosis of tuberculosis was made by sputum smear, culture, and chest radiography. Patients without a previous history of tuberculosis treatment generally received a category 1 regimen (i.e., daily doses of isoniazid, rifampin, pyrazinamide, and ethambutol or streptomycin during the intensive phase and daily doses of isoniazid and rifampin during the continuation phase). The intensive phase generally lasted 2 months, and the continuation phase generally lasted 4 months, but both could be extended depending on radiological and bacteriological data and the clinical judgment of the treating physician. There were 212 new patients who did not receive a category 1 regimen for various reasons. In the Tomsk laboratory database, they were not recorded as having received a category 1 regimen and therefore were not represented in this cohort.

All drugs were free to patients. Most patients received fixed-dose combinations procured and quality assured by the International Dispensary Association. During the period of this study, there were no stock-outs of first-line drugs. In 1999, a total of 63% of all patients received their intensive phase treatment in the regional tuberculosis hospital, and the remainder received it as outpatients. The majority of patients received the continuation phase treatment as outpatients. Outpatient services included a day hospital, medical care given at home, outreach nurse visits, and polyclinic treatment. DOT was practiced in all settings.

***Laboratory testing.*** Smear and culture were routinely done for all patients at the beginning of treatment and then were repeated at months 2 and 5 of treatment and when requested by the treating physician. Two to 3 smears and 2 cultures were performed each time. Sputum samples were decontaminated with trisodium phosphate, centrifuged, then inoculated on 2 types of media (Lowenstein-Jensen and Finn 2). Finn 2 is a modification of Lowenstein-Jensen medium used in Russia [12].

DSTs were routinely performed at the beginning of treatment and were then repeated approximately every 2 months for patients whose sputum test results were persistently positive. Susceptibility testing was performed for isoniazid, rifampin, ethambutol, and streptomycin with the absolute concentration method on Lowenstein-Jensen medium. This method has generally been replaced by the proportion method in the United States, but it is one of the oldest techniques for DSTs and is used commonly in Russia [12, 13]. Susceptibility was determined on the basis of the following drugs and concentrations, with appropriate controls: MIC of isoniazid, 1 $\mu$g/mL; MIC of rifampin, 40 $\mu$g/mL; MIC of ethambutol, 5 $\mu$g/mL; and MIC of streptomycin, 10 $\mu$g/mL. Pyrazinamide susceptibility was not tested. External quality control was done in cooperation with the Massachusetts State Laboratory Institute (Boston), a supranational reference laboratory. This largely consisted of evaluation of strains recovered from patients with chronic tuberculosis for treatment with second-line drugs. In 2000, the Tomsk regional reference laboratory correctly identified 154 (98.7%) of 156 strains with isoniazid resistance, 160 (94.7%) of 169 strains with rifampin resistance, 162 (98.8%) of 164 strains with streptomycin resistance, and 85 (59.4%) of 143 strains with ethambutol resistance.

***Data collection and analysis.*** The Tomsk regional reference laboratory records DST results in several ways. Daily DST results are immediately written into a system of laboratory notebooks. In addition, DST results are recorded in a paper-

based database of individual patient cards that is designed to keep all laboratory results for each patient in one place. There is also a computerized database containing basic clinical information about each patient, including name; smear/culture positivity; starting date of treatment; smear and culture results at the second, fifth, sixth, and eighth month of treatment; DST dates and results; treatment outcome; duration of treatment; and place of treatment. This computerized database was used for the analysis in this study. Three data-entry staff persons are responsible for maintaining all databases.

TOTBS definitions were used to designate the outcomes of treatment, as follows: cure was defined as a negative culture result at the end of treatment; failure was defined as a positive culture result at the fifth month of treatment or at the end of treatment; default was defined as an interruption in treatment of >2 months; transfer was defined as transfer out of TOTBS; and death was defined as death due to any cause during treatment. Acquired resistance was defined as new drug resistance found during or at the end of treatment that was not present at the beginning of treatment. If several DSTs were performed during the treatment course, the last result was considered to indicate the posttreatment resistance pattern. Resistance patterns were grouped according to the probability of treatment failure with DOTS: pan-susceptible or other resistance not including isoniazid or rifampin (group 1), resistance to isoniazid or rifampin but not multidrug resistance (group 2), and multidrug resistance (group 3). Statistical analysis was performed using SAS software, version 8.02 (SAS Institute).

## RESULTS

***Pretreatment drug resistance.*** During the period of 1 November 1996 through 31 December 2000, a total of 2194 patients received category 1 treatment regimen in the Tomsk Oblast civilian DOTS program. In 302 of these patients, the results of both sputum smears and sputum cultures were negative; tuberculosis was diagnosed on the basis of clinical and radiographic criteria. In 191 patients, sputum smear results were positive, but sputum culture results were negative. Twenty patients had culture-positive sputum, but DSTs failed for technical reasons. DST results for the remaining 1681 patients are shown in table 1. A total of 1212 strains (72.1%) were pan-susceptible, 169 (10.1%) were monoresistant to streptomycin, 180 (10.7%) had resistance patterns that included resistance to isoniazid but not to rifampin, and 33 (2.0%) had resistance patterns that included resistance to rifampin but not to isoniazid. Seventy-one strains (4.2%) were resistant to both isoniazid and rifampin.

***Treatment outcomes.*** Table 2 shows treatment outcomes in patients who had isolates with similar patterns of resistance. Eighty-one percent of patients in group 1 were cured, but only 69% of patients in group 2 and 36.6% of those in group 3 were cured. The relative proportion of cured and noncured patients in each group was significantly different ($P < .0001$, by $\chi^2$ test of trend). Likewise, treatment failed for 3.2% of patients in group 1, but it failed for 11.3% of those in group 2 and 31% of those in group 3. The relative proportion of patients who experienced treatment failure and of those who did not in each group was significantly different ($P < .0001$, by $\chi^2$ test of trend).

**Table 1. Initial drug resistance patterns in patients with new cases of tuberculosis receiving short-course chemotherapy in Tomsk, Russian Federation.**

| Resistance pattern | No. (%) of patients |
|---|---|
| Pan-susceptible | 1212 (72.1) |
| Ethambutol | 9 (0.5) |
| Streptomycin | 169 (10.1) |
| Streptomycin and ethambutol | 7 (0.4) |
| Rifampin | 13 (0.8) |
| Rifampin and ethambutol | 2 (0.1) |
| Rifampin and streptomycin | 14 (0.8) |
| Rifampin, streptomycin, and ethambutol | 4 (0.2) |
| Isoniazid | 34 (2.0) |
| Isoniazid and ethambutol | 3 (0.2) |
| Isoniazid and streptomycin | 118 (7.0) |
| Isoniazid, streptomycin, and ethambutol | 25 (1.5) |
| Isoniazid and rifampin | 3 (0.2) |
| Isoniazid, rifampin, and streptomycin | 36 (2.1) |
| Isoniazid, rifampin, streptomycin, and ethambutol | 32 (1.9) |
| Total | 1681 (100.0) |

**NOTE.** Resistance profile indicates resistance to the drugs listed.

***Acquired drug resistance in strains from patients who experienced treatment failure.*** Ninety-nine patients were designated by TOTBS as having category 1 treatment failure. Nine of these patients had positive smear results and negative culture results before they started treatment, so pretreatment DSTs could not be performed. For 14 patients, DSTs were never repeated. One patient was excluded because the DST was performed 2.5 months after treatment had been suspended. Two more patients were excluded because they had genitourinary tuberculosis without evidence of pulmonary tuberculosis. This left 73 patients available for analysis.

In 31 patients, pretreatment DST revealed pan-susceptible or streptomycin monoresistant strains (table 3). Acquired resistance, defined as new drug resistance that was not present before treatment, was documented in isolates from 18 patients (58.1%). In 13 patients (41.9%), multidrug resistance was acquired during treatment. In patients 22, 27, and 28, testing performed during treatment initially indicated no change, but strains with acquired drug resistance were observed in subsequent tests. In patients 29, 30, and 31, drug resistance among isolates was acquired in a step-wise process.

Twenty-four patients had isolates with pretreatment resis-

**Table 2. Treatment outcomes for patients with new cases of tuberculosis receiving short-course chemotherapy in Tomsk, Russian Federation.**

| | Patient group, by susceptibility profile(s) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Group 1 | | | Group 2 | | | Group 3 |
| Outcome | Pan-susceptible[a] | E | S and SE[a] | H and HE | HS and HSE | R, RE, RS, and RSE | HR, HRS, and HRSE |
| Cure | 981 (81.0) | 8 (88.9) | 141 (80.6) | 30 (81.1) | 96 (67.1) | 21 (63.6) | 26 (36.6) |
| Treatment failure | 36 (3.0) | … | 8 (4.6) | 3 (8.1) | 18 (12.6) | 3 (9.1) | 22 (31.0) |
| Default | 72 (5.9) | … | 8 (4.6) | 2 (5.4) | 9 (6.3) | 4 (12.1) | 6 (8.5) |
| Transfer | 39 (3.2) | 1 (11.1) | 8 (4.6) | … | 5 (3.5) | 1 (3.0) | 8 (11.3) |
| Death | 83 (6.9) | … | 10 (5.7) | 2 (5.4) | 15 (10.5) | 4 (12.1) | 9 (12.7) |
| Total | 1211 | 9 | 175 | 37 | 143 | 33 | 71 |

**NOTE.** For the susceptibility profile, *Mycobacterium tuberculosis* was resistant to the agents listed. Groups are defined in Methods. E, ethambutol; H, isoniazid; R, rifampin; S, streptomycin.

[a] Missing outcome for 1 patient.

tance patterns that included isoniazid or rifampin resistance, but not both (table 4). Two of these patients (patients 35 and 37) had inconsistent results during treatment; the subsequent clinical response was considered in deciding which result was likely to be erroneous. Acquired resistance was documented in isolates from 19 patients (79.2%). In 17 patients (70.8%), multidrug resistance was acquired during treatment.

Eighteen patients had pretreatment MDR-TB. One patient's strain was resistant to isoniazid and rifampin; 13 patients' strains were resistant to isoniazid, rifampin, and streptomycin; and 4 patients' strains were resistant to isoniazid, rifampin, streptomycin, and ethambutol. All additional DSTs performed during treatment showed MDR-TB. Of the 14 patients whose strains did not have resistance to all 4 drugs tested before treatment, acquired resistance was documented in isolates from 5 patients.

***Amplification matrix.*** The changes in resistance group before and during treatment are summarized in table 5. Each cell in this $3 \times 3$ matrix contains the number of patients whose pretreatment resistance pattern is indicated by the row number and whose posttreatment resistance pattern is indicated by the column number. Patient 35 was the only patient in whom the resistance group decreased during treatment. In this patient, DSTs were performed twice during treatment and produced discrepant results. Streptomycin monoresistance was thought to be more likely than pan-resistance, because this patient was subsequently cured with a category 2 regimen. The change in resistance group in this cohort of patients who experienced treatment failure was highly statistically significant ($P < .0001$, by the sign test).

## DISCUSSION

Resource and technical limitations in many countries mean that new patients with drug-resistant tuberculosis are often treated empirically with short-course chemotherapy. The effect of such an intervention will not be obvious unless DSTs are routinely performed at the beginning of treatment. In Tomsk, DSTs are routinely performed for all new patients, but because of shortages of second-line drugs, most new patients with initial drug resistance between 1996 and 2000 received empirical short-course chemotherapy anyway. Our retrospective study examined the effect of initial drug resistance on treatment outcomes and acquired drug resistance during short-course chemotherapy administered under DOTS program conditions.

As expected, there was a strong association between initial drug resistance and treatment outcome. The TOTBS definition of cure—negative culture results at the end of treatment—probably overestimates the true cure rate in patients with drug-resistant tuberculosis, because many of these "cured" patients will have relapse shortly after finishing treatment [14]. Nevertheless, there was a strong association between the proportion of cured patients and pretreatment drug resistance that was consistent with previous studies [8].

All patients who did not respond to standardized short-course chemotherapy were at risk of acquiring MDR-TB, but the highest risk was in patients who began treatment with isolates that were initially drug resistant. A total of 70.8% of patients with pretreatment isoniazid- or rifampin-resistant strains, but without MDR-TB, were found to have acquired MDR-TB after treatment had failed. The total number of patients with acquired drug resistance was probably underestimated for a number of reasons. DSTs for pyrazinamide were not performed, so acquired resistance to pyrazinamide could not be estimated, even though it is administered to all new patients with tuberculosis. This is particularly relevant for patients with initial multidrug resistance; because many of these patients had strains that were resistant to 3 or 4 drugs resistance to begin with, an accurate estimate of acquired resistance among isolates in this group is not possible without accurate testing of susceptibility to ethambutol and pyrazinamide. The mortality rate of 9.9%

**Table 3. Acquired drug resistance in new patients initially infected with pan-susceptible or streptomycin-monoresistant strains of *Mycobacterium tuberculosis*.**

| Patient | Duration of treatment, months | Pretreatment resistance pattern | Resistance pattern (month)[a] | Acquired resistance |
|---|---|---|---|---|
| 1 | 5.0 | S | S (2) | No |
| 2 | 5.8 | S | HS (5) | Yes |
| 3 | 6.2 | S | HRS (6) | Yes |
| 4 | 9.3 | S, pan-susceptible | HRSE (7) | Yes |
| 5 | 6.8 | S | HRSE (2, 3) | Yes |
| 6 | 6.3 | S | HRSE (2) | Yes |
| 7 | 9.5 | S | HRSE (2, 8) | Yes |
| 8 | 6.2 | Pan-susceptible | Pan-susceptible (6) | No |
| 9 | 5.1 | Pan-susceptible | Pan-susceptible (2) | No |
| 10 | 5.6 | Pan-susceptible | Pan-susceptible (2) | No |
| 11 | 8.1 | Pan-susceptible | Pan-susceptible (5) | No |
| 12 | 6.1 | Pan-susceptible | Pan-susceptible (6) | No |
| 13 | 3.9 | Pan-susceptible, pan-susceptible | Pan-susceptible (2) | No |
| 14 | 6.0 | Pan-susceptible | Pan-susceptible (1) | No |
| 15 | 4.2 | Pan-susceptible, pan-susceptible | Pan-susceptible (4, 4) | No |
| 16 | 5.6 | Pan-susceptible | Pan-susceptible (2, 5) | No |
| 17 | 5.3 | Pan-susceptible, pan-susceptible | Pan-susceptible (2) | No |
| 18 | 5.9 | Pan-susceptible | Pan-susceptible (2) | No |
| 19 | 5.1 | Pan-susceptible | Pan-susceptible (2) | No |
| 20 | 5.1 | Pan-susceptible | S (1, 5) | Yes |
| 21 | 7.0 | Pan-susceptible | HS (4, 4, 7) | Yes |
| 22 | 12.4 | Pan-susceptible | Pan-susceptible (2), HSE (10) | Yes |
| 23 | 6.1 | Pan-susceptible | R (1, 3), HS (6, 6) | Yes |
| 24 | 8.1 | Pan-susceptible | HRSE (8) | Yes |
| 25 | 11.4 | Pan-susceptible | HRSE (7,10) | Yes |
| 26 | 9.8 | Pan-susceptible | HRSE (5) | Yes |
| 27 | 5.2 | Pan-susceptible | Pan-susceptible (3), HRS (4) | Yes |
| 28 | 6.9 | Pan-susceptible | Pan-susceptible (2), HRSE (6) | Yes |
| 29 | 8.2 | Pan-susceptible | RE (4), HRSE 6) | Yes |
| 30 | 4.6 | Pan-susceptible | S (2), HRS (6) | Yes |
| 31 | 6.2 | Pan-susceptible | RS (4), HRSE (6) | Yes |

**NOTE.** For some patients, 2 pretreatment drug susceptibility test results were in the laboratory database. E, ethambutol; H, isoniazid; R, rifampin; S, streptomycin.

[a] For drug susceptibility tests performed during treatment, the pattern of resistance is followed in parentheses by the month of treatment in which the drug susceptibility test was performed.

among patients with drug-resistant tuberculosis and of 12.7% among those with MDR-TB is significant, but only for a small proportion of patients were DSTs repeated. Acquired drug resistance is a very likely possibility in these patients, and this is significant from the point of view of infection control, because highly resistant strains could have been transmitted before death to contacts.

Finally, there were a number of new patients who did not receive category 1 regimens and were therefore excluded from the study. Although the exact reasons that they did not receive category 1 treatment cannot be determined without a detailed chart review, it was often because of drug resistance. Approximately 80% of these patients had drug-resistant disease; ~35% had MDR-TB, 25% had tuberculosis that was resistant to isoniazid and streptomycin, and 8% had tuberculosis that was resistant to isoniazid, streptomycin, and ethambutol. The absolute number of patients who experienced treatment failure and of patients who had strains with acquired drug resistance certainly would have been greater if second-line drugs had been completely unavailable in Tomsk.

**Table 4. Acquired drug resistance in new patients initially infected with isoniazid- or rifampin-resistant strains but with not multidrug-resistant strains of *Mycobacterium tuberculosis*.**

| Patient | Duration of treatment received, months | Pretreatment resistance pattern | Resistance pattern (month)[a] | Acquired resistance |
|---|---|---|---|---|
| 32 | 5.1 | HS | HS (3) | No |
| 33 | 6.6 | HS | HS (3) | No |
| 34 | 5.8 | HS | HS (3, 5) | No |
| 35 | 5.2 | HS | HRS (3), S (5) | Probably no[b] |
| 36 | 9.0 | RS | HS (5), RS (9) | Probably no |
| 37 | 11.4 | H | Pan-susceptible (5), HS (11) | Probably yes[c] |
| 38 | 9.8 | HS | HSE (2, 6) | Yes |
| 39 | 9.0 | HS | HS (7), HRS (9, 9) | Yes |
| 40 | 7.4 | HE | HRE (7) | Yes |
| 41 | 9.4 | HS | Pan-susceptible (2), HRSE (9) | Yes |
| 42 | 13.6 | HS | HRS (4, 8), HRSE (13), HR (13) | Yes |
| 43 | 4.0 | HS | HRS (4) | Yes |
| 44 | 8.9 | HSE | HRSE (6), HRS (8) | Yes |
| 45 | 6.3 | HSE | HRSE (6) | Yes |
| 46 | 5.3 | H | HRSE (5) | Yes |
| 47 | 5.4 | HS, S | HRS (3), HRSE (5) | Yes |
| 48 | 6.3 | HS | HRSE (6) | Yes |
| 49 | 4.0 | HS | HRS (4) | Yes |
| 50 | 5.1 | HSE | HRS (2) | Yes |
| 51 | 2.7 | HSE | HRSE (1) | Yes |
| 52 | 5.7 | HS | HS (2), HRS (4) | Yes |
| 53 | 4.3 | HS | HRS (2, 4) | Yes |
| 54 | 7.3 | HS | HRS (6) | Yes |
| 55 | 13.4 | RS | HRS (13) | Yes |

**NOTE.** E, ethambutol; H, isoniazid; R, rifampin; S, streptomycin.

[a] For drug susceptibility tests performed during treatment, the pattern of resistance is followed in parentheses by the month of treatment in which the drug susceptibility test was performed.

[b] Patient was subsequently cured with category 2 treatment.

[c] Subsequent treatment with second-line drugs failed and patient died of tuberculosis.

One limitation of this study is that, because DNA fingerprinting of strains was not performed, reinfection or multiple infection cannot be ruled out. Unfortunately, all cultures were discarded from the laboratory soon after susceptibility testing was performed. Exogenous reinfection has been documented in patients with tuberculosis and is thought to be more common in HIV-positive patients in areas where there is a high incidence of tuberculosis [15]. The majority of the patients in this study were treated in hospitals, day care hospitals, or psychiatric hospitals during the intensive phase of treatment. Such institutions likely have a higher incidence of tuberculosis than the community. In contrast to previous reports of reinfection, however, these patients did not have a past history of tuberculosis followed by a disease-free period during which reinfection ostensibly occurred. In many patients, reinfection would have had to occur within a few months after starting effective treatment for tuberculosis. In a few patients (patients 29, 30, 31, and 47), sequential reinfection with 2 different strains would have been necessary to produce the resistance patterns demonstrated during treatment. In addition, none of these patients were HIV positive. Nevertheless, reinfection with a more highly resistant strain cannot be ruled out as a possible explanation for some of the cases presented here. Additional studies of the incidence of reinfection in hospital settings of Tomsk are needed.

Another possibility is that some of the acquired drug resistance was the result of laboratory errors in which resistant pretreatment strains were incorrectly identified as susceptible. A concordance of <60% with ethambutol-resistant strains was found in external quality assurance with a supranational reference laboratory. This discrepancy may be the result of laboratory error, the inherent technical difficulties of ethambutol susceptibility testing [16], or differences in the method and media used. At any rate, in only 5 patients was there emergence

**Table 5. Amplification matrix relating resistance group before and after treatment failure with short-course chemotherapy in patients with new cases of tuberculosis.**

| Resistance group at start of treatment | Resistance group after treatment failure | | | Total |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Pan-susceptible and streptomycin monoresistance | 13 | 5 | 13 | 31 |
| Isoniazid or rifampin resistance, but not multidrug resistance | 1 | 6 | 17 | 24 |
| Multidrug-resistant tuberculosis | … | … | 18 | 18 |
| Total | … | … | … | 73 |

**NOTE.** Groups are defined in Methods.

of ethambutol resistance alone, and 4 of those cases were in the highly plausible scenario involving pretreatment resistance to isoniazid, rifampin, and streptomycin. Laboratory contamination with a susceptible strain also cannot be ruled out, because DSTs were normally performed on only 1 culture before the patient started treatment. However, contamination cannot explain the DST results for patients 22, 27, 28, 39, and 52, for whom the first DST result during treatment was the same as the result from before treatment was started.

One of the more striking findings of this study is that initially pan-susceptible strains can acquire multiple-drug resistance while the patient is receiving failed treatment with standardized short-course chemotherapy. Of 24 patients with initially pan-susceptible strains, 12 patients showed evidence of strains with acquired resistance in subsequent DSTs; in most cases, the strains had resistance to multiple drugs. The drug resistance patterns demonstrated in patients 29, 30, and 31 suggest that resistance was acquired in a step-wise fashion; performance of DSTs at more frequent intervals may have demonstrated more clearly the step-wise acquisition of resistance mutations. The laboratory evidence of acquired drug resistance is consistent with the clinical history of treatment failure. In any case of bacteriological treatment failure under DOT, *Mycobacterium tuberculosis* is actively replicating in the presence of antituberculosis drugs, and drug resistance must be considered as a possible explanation.

In Tomsk, the length of the intensive phase depends on radiological, bacteriological, and clinical improvement of the patient. In this approach, the physician waits to reduce the number of drugs in the treatment regimen until there is evidence that the patient is improving. This approach might improve cure rates in new patients with isoniazid-monoresistant strains [17]. On the other hand, it is unclear how much this approach to treatment creates environmental pressure that selects for resistant mutants. In a study of the influence of initial drug resistance on treatment outcomes in the clinical trials of the British Medical Research Council, the emergence of rifampin resistance was rare when it was given for 2 months but frequent when it was given for >4 months [18]. There are many other important clinical and radiological factors that could have affected the rate of acquired drug resistance, such as the severity of disease, malnutrition, malabsorption, and alcoholism. Detailed clinical and radiographic information was unfortunately unavailable for this analysis, but it should be the subject of further studies.

The findings of this study have important ramifications wherever patients with drug-resistant tuberculosis are treated with short-course chemotherapy. Early diagnosis of drug-resistant tuberculosis and the judicious use of second-line drugs is recommended to decrease transmission of drug-resistant strains and to prevent the creation of multidrug-resistant strains. Finally, if DSTs are not available or results are delayed, physicians should recognize that patients who are not responding to directly observed empirical short-course chemotherapy are at high risk of having MDR-TB and should be treated accordingly.

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