Double Infection with a Resistant and a Multidrug-Resistant Strain of *Mycobacterium tuberculosis*

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An immunocompetent patient was dually infected with a resistant and a multidrug-resistant strain of *Mycobacterium tuberculosis* (TB). The multidrug-resistant strain, which belongs to the W-strain/Beijing family, was first isolated after 3 months of therapy. Inappropriate treatment led to further drug resistance and unsuccessful therapy. Thus, additional infections with resistant *M. tuberculosis* strains should be considered when tuberculosis therapy fails.

During the last decade, drug-resistant *Mycobacterium tuberculosis* (TB) strains have emerged, posing a major threat to global TB control efforts. The incidence of drug-resistant TB has increased in many parts of the world, not only in developing countries but also in industrialized countries, where the prevalence of drug-resistant TB had been low (1). The emergence of drug resistance during antituberculosis therapy results mainly from inadequate therapy, i.e., improper prescription of treatment regimens, addition of single drugs to failing treatment regimens, and patient noncompliance. However, inconsistent drug-susceptibility patterns or delayed responses to TB therapy may also indicate exogenous reinfection with a strain resistant to multiple drugs or mixed infection with a sensitive and a multidrug-resistant TB strain. Such infections occur in immunocompromised and immunocompetent persons (2-7) and may be more common in areas with high prevalence of resistant TB.

We report the case of an immunocompetent patient initially infected with an isoniazid- and streptomycin-resistant TB strain, who after the first 3 months of TB therapy was found to be infected with a second multidrug-resistant TB strain, resulting in treatment failure. In all, the patient's cultures were resistant to nine anti-TB drugs.

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The Study

A 24-year-old man from Kazakhstan was admitted to hospital A in February 1996, 2 weeks after his arrival in Germany for further diagnosis of a cavernous lesion in the upper lobe of his left lung. The patient reported coughing, but no weight loss, night sweats, or hemoptysis. An elevated erythrocyte sedimentation rate was the only abnormal laboratory finding. He was seronegative for HIV. A tuberculin skin test was positive (12 mm), acid-fast bacilli were detected in gastric aspirates, and two sputum cultures were positive for TB. Antituberculosis therapy was started with isoniazid, rifampin, ethambutol, and pyrazinamide. The patient was then transferred to hospital B, where therapy was continued with three drugs only (ethambutol was discontinued). After 2 months of therapy, the patient showed clinical improvement, resulting in a sputum smear- and culture-negative phase of approximately 4 weeks (Table). When susceptibility testing of the first culture obtained 2 months previously revealed resistance to isoniazid and streptomycin, ethambutol was readded to the treatment regimen. Isolates were identified as *M. tuberculosis* complex by using gene probes (ACCUProbe, GenProbe, San Diego, California, USA). Drug susceptibility was determined by the proportion method on Löwenstein-Jensen medium, the modified proportion method in BACTEC 460TB, or both.

Suspicion of nonadherence to therapy during the first 3 months led to transfer of the patient to hospital C and 3 weeks later to hospital D, where

Table. Treatment regimens and characteristics of *Mycobacterium tuberculosis* isolates[a]

Therapy (months)	Hospital	Treatment regimen	Culture	Susceptibility testing: Culture obtained	Susceptibility testing: Current resistance pattern	Susceptibility testing: Time delay (months)[b]	Spoligotype
0	A/B	H, R, E,[c] Z	pos.	02/28/96	H, S	2	type I
1	B	H, R, Z	pos.	03/17/96	H, S	2	type I
2	B	H, R, E, Z	neg.	-	-	-	-
3	B	H, R, E, Z	neg./ pos.	-	n.d.	-	n.d.
4	C	H, E, Pt	pos.	06/06/96	H, S, R, (E), (Rb)	2	type II
5	D	R, E, Z	pos.	07/12/96	H, S, R, (E), (Rb)	2	type II
6	D	E, Z, Rb, Of	pos.	-	n.d.	-	n.d.
7-12	D	E, Z, Rb, Pt	pos.	-	n.d.	-	n.d.
13	D	E, Z, Rb, Pt	pos.	03/15/97	H, S, R, E, Rb, Pt	1	type II
14-18	D	Z, Pt, Ci	pos.	04/25/97	H, S, R, E, Rb, Pt, Z	5	type II
19	D	Z, Pt, Rb, Ci	pos.	-	n.d.	-	n.d.
20-21	D	Rb, Ci, Am, Pa	pos.	-	n.d.	-	n.d.
22-24	D	Rb, Ci, Am, Pa, Cl	pos.	-	n.d.	-	n.d.
25-28	D	Rb, Ci, Am, Pa, Cl	pos./ neg.	02/18/98	H, S, R, E, Rb, Pt Z, Pa	4	type II
29-32	D	Rb, Ci, Am, Pa, Cl	pos.	06/23/98	H, S, R, E, Rb, Pt, Z, Pa, Am	4	type II
33	D	Rb, Ci, Cl, Ca	pos.	-	n.d.	-	n.d.

[a]Abbreviations and symbols: H, isoniazid; S, streptomycin; R, rifampin; E, ethambutol; Rb, rifabutin; Pt, protionamide; Z, pyrazinamide; Pa, *p*=aminosalicylic acid; Am, amikacin; Of, ofloxacin, Ci, ciprofloxacin; Cl, clofazimine; Ca, capreomycin; borderline results are shown in parentheses; pos., positive culture result; neg., negative culture result; n.d., not determined.
[b]Time between the date on which the specimen was obtained and the date on which the drug-susceptibility pattern was available for the clinician (drug-susceptibility tests were not always performed directly after the cultures were grown but were done retrospectively).
[c]E was given in the first 4 days of therapy only.

treatment was administered on a closed ward (Table). At that time, cultures tested positive again, and the chest X-ray showed slight deterioration. On August l, 1996, the latest susceptibility tests showed resistance to isoniazid, rifampin, and streptomycin, and an intermediate result for rifabutin and ethambutol. Therapy was switched to ethambutol, pyrazinamide, rifabutin, and ofloxacin. Subsequently, treatment regimens were changed several times, but cultures continued to be positive for mycobacteria. In early 1997, atelectasis of the left lower lobe and thickening of the wall of the cavernous lesion in the left upper lobe became apparent. Lung resection was suggested, but thoracic surgeons declined to operate because of the extensive lung involvement. By year-end, susceptibility tests showed resistance to seven drugs: isoniazid, rifampin, ethambutol, pyrazinamide, protionamide, rifabutin, and streptomycin. A regimen with rifabutin, ciprofloxacin, amikacin, para-aminosalicylic acid, and clofazimine led to a short phase of negative sputum cultures, but a chest X-ray showed no improvement. Later, additional resistance to para-aminosalicylic acid and amikacin was documented.

To elucidate the reasons for therapy failure, we compared the current susceptibility patterns with the treatment regimens applied earlier. We found that during several phases of treatment, this patient was treated with only one effective drug. During extended periods, he was culture positive, but no susceptibility tests were performed, even after relapse. Additionally, treatment regimens were changed, and single drugs were added several times without determination of the actual resistance pattern. Treatment regimens, drug-susceptibility patterns, and detailed information on the history of the case are summarized in the Table.

All isolates obtained were submitted to spoligotyping and IS*6110* fingerprinting (5,8). Spoligotypes (Figure), as well as the IS*6110* restriction fragment length polymorphism (RFLP) patterns (data not shown) of the first two isoniazid- and streptomycin-resistant cultures, were identical (type I) but differed clearly (IS*6110* identity of less than 30%) from those of

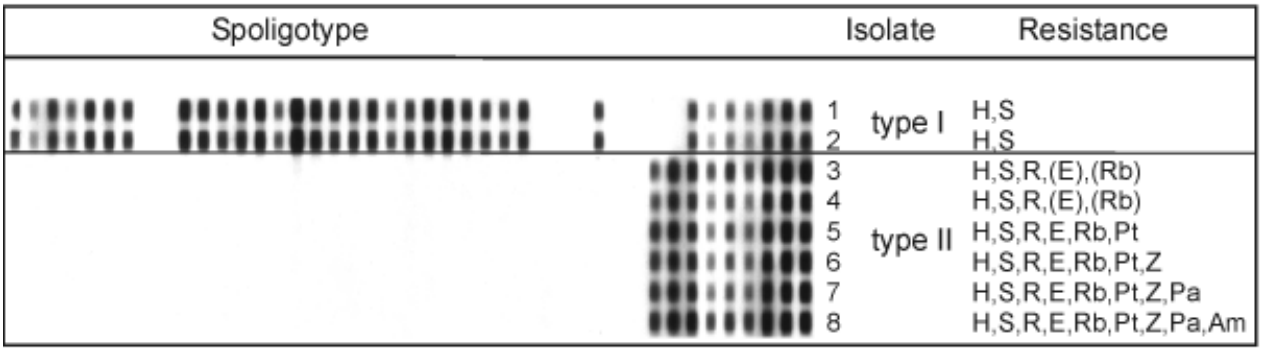

Figure. Spoligotypes and drug-resistance patterns of isolates of patient's first (1 and 2, type I) and second culture-positive phase (3-8, type II). The spoligotypes of the first two cultures differ clearly from those of the latter ones. The eight isolates were obtained on the following dates: 1, February 28, 1996; 2, March 17, 1996; 3, June 6, 1996; 4, July 12, 1996; 5, March 15, 1997; 6, April 25, 1997; 7, February 18, 1998; and 8, June 23, 1998. H, isoniazid; S, streptomycin; R, rifampin; E, ethambutol; Rb, rifabutin; Pt, protionamide; Z, pyrazinamide; Pa, *p*=aminosalicylic acid; Am, amikacin. Borderline results are displayed in parentheses.

later multidrug-resistant isolates (second phase of sputum culture positivity, type II; see Table). These results indicate that the patient was infected with a second TB strain, which showed initial resistance to isoniazid, streptomycin, and rifampin, and borderline resistance to ethambutol and rifabutin. IS*6110* RFLP patterns of multidrug-resistant isolates from the patient were compared with those from other patients who had been treated in hospitals B and C during the same period and with IS*6110* RFLP patterns from resistant TB strains isolated from unrelated patients living in other areas of Germany. Gelcompar software was used for this analysis (Windows 95, version 4.0; Applied Maths, Kortrijk, Belgium) (5). No isolate showing an identical IS*6110* RFLP pattern was identified (data not shown). Spoligotype and the IS*6110* RFLP patterns of the patient's multidrug-resistant strain were similar to those of the W-strain or Beijing family, which have been found in New York, USA, and Beijing, China (9,10).

Conclusions

We report an immunocompetent patient with pulmonary TB who had double infection with a resistant and a multidrug-resistant TB strain, leading to therapy failure. After 2 years of treatment, resistance to eight antituberculosis drugs—including the most potent first- and second-line treatments—occurred, despite clinically supervised hospital therapy. Four months later, resistance to a ninth drug occurred. Progressive disease caused by a second multidrug-resistant TB strain, as demonstrated by molecular strain typing methods, was the initial cause for this occurrence. A possible variation of the initial strain has been excluded since the spoligotype patterns of the multidrug-resistant isolates completely differed from the two isolates of the first TB period (spoligotype patterns have been shown to be highly stable among serial patient isolates [11]).

In this case, TB therapy was often based on drug-resistance data not representing the current drug-resistance pattern, resulting in improper treatment and many periods in which the patient received only one effective drug; the second TB strain could have acquired further resistance during these many periods of monotherapy. Earlier identification of the second infection might have led to treatment with a more appropriate drug regimen, resulting in a more successful outcome. The second multidrug-resistant TB strain could have been acquired by mixed-strain infection or exogenous reinfection (2-7). However, our investigation did not identify a possible index patient. Moreover, the fact that the patient was an immigrant from Kazakhstan, a country with high rates of resistant TB (1), suggests that he may have been infected with the second multidrug-resistant strain in his homeland.

This patient was seronegative for HIV and had no clinical measurements indicative of immunosuppression, suggesting that additional

infection with multidrug-resistant TB during treatment can be largely independent of a host's immune status. These mixed-strain infections with at least one resistant strain may lead to unsuccessful TB therapy. Although few have been reported (6, 7), such cases may become more frequent in areas with high rates of drug-resistant TB. Standard TB treatment apparently is not sufficient to protect patients from infection with a second multidrug-resistant TB strain.

Clinicians should consider the possibility of additional infection with multidrug-resistant TB in cases when TB therapy fails. In such cases, inappropriate treatment regimens and delayed follow-up of susceptibility tests can permit additional resistance to develop, which can dramatically complicate TB therapy. However, regardless of the cause, when a clinical course is abnormal, adding single drugs to failing treatment regimens should be avoided, and retreatment programs should not be initiated before culture sensitivity results are available.

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Dr. Niemann is working in a postdoctoral position at the German National Reference Center for Mycobacteria, Research Center Borstel, Borstel, Germany. He is responsible for molecular characterization and typing of mycobacteria. Research interests include the characterization of the MTB complex by molecular techniques and the epidemiology of TB by DNA fingerprinting.

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