ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2005, p. 1229–1231 Vol. 49, No. 3
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.3.1229–1231.2005

Disequilibrium in Distribution of Resistance Mutations among *Mycobacterium tuberculosis* Beijing and Non-Beijing Strains Isolated from Patients in Germany

Doris Hillemann,* Tanja Kubica, Sabine Rüsch-Gerdes, and Stefan Niemann

National Reference Center for Mycobacteria, Forschungszentrum Borstel, Borstel, Germany

Received 25 August 2004/Returned for modification 21 September 2004/Accepted 26 October 2004

Genotypic analysis of 103 multidrug-resistant *Mycobacterium tuberculosis* strains isolated in Germany in 2001 revealed that mutations in codon 531 (75.7%) of the *rpoB* gene and codon 315 (88.4%) of the *katG* gene are most frequent. Beijing genotype strains (60.2% of all isolates) displayed a different distribution of resistance mutations than non-Beijing strains.

The molecular patterns of mutations conferring resistance to rifampin (RMP), mainly in the 81-bp hot spot region of the *rpoB* gene (5, 12, 21), and isoniazid (INH), mainly in *katG*, *inhA*, and *oxyR-ahpC* (9, 13, 16, 20), of *Mycobacterium tuberculosis* strains isolated in Germany in 1994–1995 and 1997 have been explored in previous investigations (3, 5, 17). Since that time, we observed a shift in the population structure of multidrug-resistant (MDR) tuberculosis strains, documented by a rising proportion of the Beijing genotype (7). In order to obtain recent data on drug-resistant strains circulating in Germany, in the present study we investigated resistance mutations of MDR strains isolated in 2001.

Genotypic analysis of RMP and INH resistance of 113 *M. tuberculosis* strains (103 MDR and 10 randomly chosen fully susceptible strains as controls) was carried out by use of real-time PCR and sequencing analysis (19). These samples represent more than 90% of all MDR cases reported in 2001 (7, 18).

In all 103 MDR isolates, mutations in the *rpoB* gene were detected, mostly in the 81-bp hot spot region. However, for one isolate a mutation could be detected only after cultivation on RMP-containing medium and reexamination. Fourteen different mutations in seven codons of the *rpoB* gene were found (Table 1). Codon 531 was most frequently affected in 78 of the 103 strains (75.7%). Other mutations were detected in *rpoB* 526 in 14 strains (13.6%) and in *rpoB* 516 in 3 strains (2.9%), and one was detected in codon 176, outside the hot spot region. One triple mutation, affecting codons 531 and 522 and involving a deletion of codon 519, was found. None of the 10 susceptible control strains carried a mutation in *rpoB*.

Concerning INH resistance, for 96 of the 103 MDR isolates (93.2%) a mutation in the genes analyzed was found (Table 1). Since no mutation was detected for the seven MDR strains (6.8%) after cultivation on INH-containing medium, presumably not heteroresistance but mutations in other regions of *katG* (16, 19) or genes not included in these investigation, such as *kasA* (10) or *ndh* (8), are the explanation for this finding. None of the susceptible strains carried a mutation in the regions investigated. In the majority of MDR isolates (84.5%; 87 of 103), a distinct nucleotide change in *katG* codon 315 from AGC (wild-type sequence) to ACC (S315T) was present. Four isolates (3.9%) carried other exchanges in codon 315 (two AAC and two ACA), three (2.9%) had mutations in the ribosome binding site region of *inhA*, and two (1.9%) had nucleotide exchanges in the regulatory region of the *ahpC* gene. This investigation showed a high prevalence of mutations in *katG* codon 315 (88.4%), which is contrary to results from previous studies performed in low-incidence countries (13) and even a study performed in Germany in 1994–1995 (44% *katG* substitutions) (3). Comparable high frequencies of the *katG* 315 mutations were found in northwestern Russia (93.6%) (11), in Latvia (91.0%) (22), and in Lithuania (85.7%) (2). With the additional information that the majority of patients, although residing in Germany at the time of strain isolation, originated from countries of the former Soviet Union (7), the high frequency of the *katG* mutations can probably be explained by an importation of strains from these regions. This conclusion was further supported when the distribution of resistance-conferring mutations was stratified for Beijing and non-Beijing strains. Of the 103 MDR isolates, 62 (60.2%) have been identified as Beijing genotype strains by IS*6110* restriction fragment length polymorphism and their characteristic spoligotyping pattern (4, 6, 23).

Among the Beijing strains, a high rate of mutations was found in *rpoB* codon 531 (52 of 62 strains; 83.9%), whereas this portion was significantly lower within the 41 non-Beijing strains (26 of 41 strains; 63.4%; $P = 0.02$). In contrast, mutations in codon 526 were more frequent in non-Beijing strains (7 of 41 strains; 17.1%) than in Beijing strains (7 of 62 strains; 11.3%), but this difference was statistically not significant ($P = 0.4$). Concerning the distribution of mutations in the *katG* gene, the prevalence of the S315T mutation was significantly higher in the MDR Beijing group (59 of 62; 95.2%) than in the MDR non-Beijing group (28 of 41; 68.3%; $P < 0.001$). Furthermore, the MDR Beijing strains exhibited a great number of isolates (48 of 62; 77.4%) with an identical pattern of mutations (*rpoB* S531L and *katG* S315T) compared to only 16 of 41 isolates (39.0%) in the non-Beijing group ($P < 0.001$).

In conclusion, comparing MDR Beijing and non-Beijing genotype strains with respect to their mutations conferring RMP

* Corresponding author. Mailing address: National Reference Center for Mycobacteria, Forschungszentrum Borstel, Parkallee 18, 23845 Borstel, Germany. Phone: 49 4537 188761. Fax: 49 4537 188311. E-mail: dhillemann@fz-borstel.de.

TABLE 1. DNA sequencing and real-time PCR data for MDR *M. tuberculosis* strains from Germany, stratified for Beijing and non-Beijing strains[a]

MDR strain group (no. of strains)	Affected *rpoB* codon(s)	Nucleotide/amino acid change(s)	Affected *katG*, *inhA*, or *ahpC* codon	Nucleotide/amino acid change(s)	No. (%) of strains
All (103)	531	TCG→TTG/Ser→Leu	*katG* 315	AGC→ACC/Ser→Thr	64 (62.1)
		TCG→TTG/Ser→Leu	None		3 (2.9)
		TCG→TTG/Ser→Leu	*katG* 315	AGC→ACA/Ser→Thr	2 (1.9)
		TCG→TTG/Ser→Leu	*katG* 315	AGC→AAC/Ser→Asn	2 (1.9)
		TCG→TTG/Ser→Leu	*inhA* 209	C→T	2 (1.9)
		TCG→TTT/Ser→Phe	*katG* 315	AGC→ACC/Ser→Thr	2 (1.9)
		TCG→TGG/Ser→Trp	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
		TCG→TGG/Ser→Trp	*inhA* 209	C→T	1 (1.0)
		TCG→TGG/Ser→Trp	None		1 (1.0)
	526	CAC→AAC/His→Asn	*katG* 315	AGC→ACC/Ser→Thr	4 (3.9)
		CAC→CTC/His→Leu	*katG* 315	AGC→ACC/Ser→Thr	3 (2.9)
		CAC→TAC/His→Tyr	*katG* 315	AGC→ACC/Ser→Thr	2 (1.9)
		CAC→CGC/His→Arg	*katG* 315	AGC→ACC/Ser→Thr	2 (1.9)
		CAC→CGC/His→Arg	*ahpC-oxyR*	C(−52)T	1 (1.0)
		CAC→GAC/His→Asp	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
		CAC→TGC/His→Cys	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
	516	GAC→GTC/Asp→Val	*katG* 315	AGC→ACC/Ser→Thr	2 (1.9)
		GAC→TAC/Asp→Tyr	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
	522	TCG→CAG/Ser→Gln	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
		TCG→TTG/Ser→Leu	None		1 (1.0)
	518	AAC→ATC/Asn→Ile	None		1 (1.0)
	513	CAA→CCA/Gln→Pro	None		1 (1.0)
	517	Del[b]	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
	514–516	Del[b]	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
	176	GTC→TTC/Val→Phe	*ahpC-oxyR*	G(−48)A	1 (1.0)
	519/522/531	Del[b], TCG→TTG/Ser→Leu, and TCG→TTG/Ser→Leu	*katG* 315	AGC→ACC/Ser→Thr	1 (1.0)
Beijing (62)	531	TCG→TTG/Ser→Leu	*katG* 315	AGC→ACC/Ser→Thr	48 (77.4)
		TCG→TTG/Ser→Leu	*inhA* 209	C→T	1 (1.6)
		TCG→TTG/Ser→Leu	None		1 (1.6)
		TCG→TTT/Ser→Phe	*katG* 315	AGC→ACC/Ser→Thr	2 (3.2)
	526	CAC→AAC/His→Asn	*katG* 315	AGC→ACC/Ser→Thr	2 (3.2)
		CAC→CTC/His→Leu	*katG* 315	AGC→ACC/Ser→Thr	2 (3.2)
		CAC→TAC/His→Tyr	*katG* 315	AGC→ACC/Ser→Thr	2 (3.2)
		CAC→CGC/His→Arg	*katG* 315	AGC→ACC/Ser→Thr	1 (1.6)
	518	AAC→ATC/Asn→Ile	None		1 (1.6)
	516	GAC→GTC/Asp→Val	*katG* 315	AGC→ACC/Ser→Thr	1 (1.6)
	519/522/531	Del[b], TCG→TTG/Ser→Leu, and TCG→TTG/Ser→Leu	*katG* 315	AGC→ACC/Ser→Thr	1 (1.6)
Non-Beijing (41)	531	TCG→TTG/Ser→Leu	*katG* 315	AGC→ACC/Ser→Thr	16 (39.0)
		TCG→TTG/Ser→Leu	*katG* 315	AGC→ACA/Ser→Thr	2 (4.9)
		TCG→TTG/Ser→Leu	*katG* 315	AGC→AAC/Ser→Asn	2 (4.9)
		TCG→TTG/Ser→Leu	*inhA* 209	C→T	1 (2.4)
		TCG→TTG/Ser→Leu	None		2 (4.9)
		TCG→TGG/Ser→Trp	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
		TCG→TGG/Ser→Trp	*inhA* 209	C→T	1 (2.4)
		TCG→TGG/Ser→Trp	None		1 (2.4)
	526	CAC→AAC/His→Asn	*katG* 315	AGC→ACC/Ser→Thr	2 (4.9)
		CAC→GAC/His→Asp	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
		CAC→CGC/His→Arg	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
		CAC→CGC/His→Arg	*ahpC-oxyR*	C(−52)T	1 (2.4)
		CAC→TGC/His→Cys	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
		CAC→CTC/His→Leu	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
	522	TCG→CAG/Ser→Gln	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
		TCG→TTG/Ser→Leu	None		1 (2.4)
	516	GAC→GTC/Asp→Val	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
		GAC→TAC/Asp→Tyr	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
	513	CAA→CCA/Gln→Pro	None		1 (2.4)
	517	Del[b]	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
	514–516	Del[b]	*katG* 315	AGC→ACC/Ser→Thr	1 (2.4)
	176	GTC→TTC/Val→Phe	*ahpC-oxyR*	G(−48)A	1 (2.4)

[a] According to reference 21, GenBank accession numbers are as follows: L27989 for the *rpoB* gene, X68081 for the *katG* gene, U66801 for the *inhA* gene, and U16243 for the *ahpC-oxyR* intergenic region.

[b] Del, deletion.

or INH resistance, a marked difference in the distribution of mutations was observed. Comparable differences have also been found for *katG* S315T mutations in a northwestern Russian setting (11). However, no association of specific mutations with a certain spoligotype pattern or genotype could be detected in recently published studies analyzing the prevalence of *rpoB* mutations in southeast Asia (15) or *rpoB* and *katG* mutations in Latvia (22) and England (1).

Since the proportion of Beijing genotype strains among MDR strains from Germany has changed markedly from 19.2% in 1995 to 58.3% in 2001 (7), this has also resulted in a shift of resistance mutations determined in MDR strains. Comparing the data from this study with the distribution of *rpoB* mutations present in RMP-resistant strains isolated in Germany found in previous studies, an increase of the mutations of *rpoB* codon 531 was assessed as follows: 1994-1995, 39% (17); 1997, 65% (5); and 2001, 75.7%. Accordingly, we observed a high rate of *katG* codon 315 mutations compared with the study of Dobner and colleagues (88.4 versus 44%) (3). To the best of our knowledge, this is the first study demonstrating the influence of strain importation on the prevalence of resistance mutations among strains in a given setting. In this context, the fact that the *katG* S315T mutation has no impact on the bacterial fitness (14) is of especial importance. Thus, the presence of particular clones of MDR strains might have a direct impact on transmission dynamics of MDR tuberculosis. As a consequence, the increased rate of strains carrying particular resistance mutations in line with the increasing proportion of Beijing strains may lead to a changed situation concerning transmission of MDR strains in Germany.

This work was supported by a grant from the Bundesministerium für Gesundheit und Soziale Sicherung, Berlin, Germany (325-4539-84/3.2).

We are grateful to Olfert Landt (TIB MOLBIOL Synthese Labor, Berlin, Germany) for designing and providing us with parts of the primers and FRET probes. We thank Kirsten Ott, Ilse Radzio, Merle Fischer, and Frauke Schaefer, Forschungszentrum Borstel, for excellent technical assistance.

REFERENCES

1. **Baker, L., T. Brown, M. C. Maiden, and F. Drobniewski.** 2004. Silent nucleotide polymorphisms and a phylogeny for Mycobacterium tuberculosis. Emerg. Infect. Dis. **10:**1568–1577.
2. **Bakonyte, D., A. Baranauskaite, J. Cicenaite, A. Sosnovskaja, and P. Stakenas.** 2003. Molecular characterization of isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates in Lithuania. Antimicrob. Agents Chemother. **47:**2009–2011.
3. **Dobner, P., S. Rüsch-Gerdes, G. Bretzel, K. Feldmann, M. Rifai, T. Löscher, and H. Rinder.** 1997. Usefulness of *Mycobacterium tuberculosis* genomic mutations in the genes *katG* and *inhA* for the prediction of isoniazid resistance. Int. J. Tuberc. Lung Dis. **4:**365–369.
4. **Glynn, J. R., J. Whiteley, P. J. Bifani, K. Kremer, and D. Van Soolingen.** 2002. Worldwide occurrence of Beijing/W strains of *Mycobacterium tuberculosis*: a systematic review. Emerg. Infect. Dis. **8:**843–849.
5. **Heep, M., B. Brandtstätter, U. Rieger, N. Lehn, E. Richter, S. Rüsch-Gerdes, and S. Niemann.** 2001. Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. J. Clin. Microbiol. **39:**107–110.
6. **Kamerbeek, J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. D. A. van Embden.** 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35:**907–914.
7. **Kubica, T., S. Rüsch-Gerdes, and S. Niemann.** 2004. The Beijing genotype is emerging among multidrug-resistant *Mycobacterium tuberculosis strains* from Germany. Int. J. Tuberc. Lung Dis. **8:**1107–1113.
8. **Lee, A. S. G., A. S. M. Teo, and S. Y. Wong.** 2001. Novel mutations in *ndh* in isoniazid-resistant *Mycobacterium tuberculosis* isolates. Antimicrob. Agents Chemother. **45:**2157–2159.
9. **Mdluli, K., D. R. Sherman, M. J. Hickey, B. N. Kreiswirth, S. Morris, C. K. Stover, and C. E. Barry III.** 1996. Biochemical and genetic data suggest that InhA is not the primary target for activated isoniazid in *Mycobacterium tuberculosis.* J. Infect. Dis. **174:**1085–1090.
10. **Mdluli, K., R. A. Slayden, Y. Zhu, S. Ramaswamy, X. Pan, D. Mead, D. D. Crane, J. M. Musser, and C. E. Barry III.** 1998. Inhibition of a *Mycobacterium tuberculosis* beta-ketoacyl ACP synthase by isoniazid. Science **280:**1607–1610.
11. **Mokrousov, I., O. Narvskaya, T. Otten, E. Limeschenko, L. Steklova, and B. Vyshnevkiy.** 2002. High prevalence of *KatG* Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates from northwestern Russia, 1996 to 2001. Antimicrob. Agents Chemother. **46:**1417–1424.
12. **Musser, J. M.** 1995. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin. Microbiol. Rev. **8:**496–514.
13. **Musser, J. M., V. Kapur, D. L. Williams, B. N. Kreiswirth, D. van Soolingen, and J. D. van Embden.** 1996. Characterization of the catalase-peroxidase gene (*katG*) and *inhA* locus in isoniazid-resistant and -susceptible strains of *Mycobacterium tuberculosis* by automated DNA sequencing: restricted array of mutations associated with drug resistance. J. Infect. Dis. **173:**196–202.
14. **Pym, A. S., B. Saint-Joanis, and S. T. Cole.** 2002. Effect of *katG* mutations on the virulence of *Mycobacterium tuberculosis* and the implication for transmission in humans. Infect. Immun. **70:**4955–4960.
15. **Qian, L., C. Abe, T. P. Lin, M. C. Yu, S. N. Cho, S. Wang, and J. T. Douglas.** 2002. *rpoB* genotypes of *Mycobacterium tuberculosis* Beijing family isolates from East Asian countries. J. Clin. Microbiol. **40:**1091–1094.
16. **Ramaswamy, S., and J. M. Musser.** 1998. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber. Lung Dis. **79:**3–29.
17. **Rinder, H., P. Dobner, K. Feldmann, M. Rifai, G. Bretzel, S. Rüsch-Gerdes, and T. Löscher.** 1997. Disequilibria in the distribution of *rpoB* alleles in rifampicin-resistant M. tuberculosis isolates from Germany and Sierra Leone. Microb. Drug Resist. **3:**195–197.
18. **Robert Koch Institut.** 2003. Bericht zur Epidemiologie der Tuberkulose in Deutschland für 2001. Robert Koch Institut, Berlin, Germany.
19. **Sajduda, A., A. Brzostek, M. Poplawska, E. Augustynowicz-Kopec, Z. Zwolska, S. Niemann, J. Dziadek, and D. Hillemann.** 2004. Molecular characterization of rifampin- and isoniazid-resistant *Mycobacterium tuberculosis* strains isolated in Poland. J. Clin. Microbiol. **42:**2425–2431.
20. **Sreevatsan, S., X. Pan, Y. Zhang, V. Deretic, and J. M. Musser.** 1997. Analysis of the *oxyR-ahpC* region in isoniazid-resistant and -susceptible *Mycobacterium tuberculosis* complex organisms recovered from diseased humans and animals in diverse localities. Antimicrob. Agents Chemother. **41:**600–606.
21. **Telenti, A., N. Honoré, C. Bernasconi, J. March, A. Ortega, H. E. Takiff, and S. T. Cole.** 1997. Genotyping assessment of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*: a blind study at reference laboratory level. J. Clin. Microbiol. **35:**719–723.
22. **Tracevska, T., I. Jansone, L. Broka, O. Marga, and V. Baumanis.** 2002. Mutations in the *rpoB* and *katG* genes leading to drug resistance in *Mycobacterium tuberculosis* in Latvia. J. Clin. Microbiol. **40:**3789–3792.
23. **van Soolingen, D.** 2001. Molecular epidemiology of tuberculosis and other mycobacterial infections: main methodologies and achievements. J. Intern. Med. **249:**1–26.