JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2005, p. 2026–2027 Vol. 43, No. 4
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.4.2026–2027.2005

High Frequency of Mutations in the *rpoB* Gene in Rifampin-Resistant Clinical Isolates of *Mycobacterium tuberculosis* from Singapore

Rifampin (RIF) is a first-line antituberculosis drug. Resistance to RIF, in the majority of cases, has been associated with mutations within an 81-bp RIF resistance-determining region (RRDR) of the *rpoB* gene, which encodes the β subunit of the RNA polymerase (8). RIF acts by binding to the β subunit of the RNA polymerase, thus interfering with transcription and RNA elongation.

Although targeted molecular analysis of the *rpoB* gene has been shown to be effective for detecting RIF resistance in over 90% of RIF-resistant strains from diverse geographical regions, there is only limited information from Southeast Asia (2, 3, 11, 12). Previous investigations on isoniazid-, pyrazinamide-, and ofloxacin-resistant *Mycobacterium tuberculosis* isolates from Singapore indicated that the mutation frequencies and spectra of the genes studied differed from those of genes from isolates from other geographical locations (4–6). To determine if targeted molecular screening of the *rpoB* gene may be useful in Singapore, we evaluated the frequency of mutations within the RRDR of *rpoB* for 51 RIF-resistant and 4 RIF-sensitive *Mycobacterium tuberculosis* isolates by direct sequencing of the entire RRDR.

Consecutive RIF-resistant isolates were collected from August 1994 to December 1996 as previously described (4). The clinical data of this study population have been detailed elsewhere (1). Drug susceptibility testing was done by using the BACTEC 460 radiometric method (Becton Dickinson, Towson, Md.), and the test drug concentration was 2 μg/ml. DNA extracted from bacterial colonies was used for PCR amplification of a 350-bp fragment of *rpoB*, as previously described (3). The PCR products were purified (Wizard PCR purification kit; Promega, Madison, Wis.) and directly sequenced by using the Applied Biosystems 377 DNA sequencer.

Twelve different missense mutations involving codons 513, 514, 516, 522, 526, and 531 were identified in 49 RIF-resistant strains (Table 1). The most common alterations were a Ser→Leu substitution at codon 531, present in 25 (49.0%) isolates, and a His→Tyr substitution at codon 526 in 7 (13.7%) isolates. Mutations at codon 531 were observed in 28 (55%) of the isolates, at codon 526 in 12 (24%) isolates, and at codon 516 in 6 (12%) isolates. One isolate had mutations in both codons 514 and 516.

A rare mutation at codon 513, resulting in a Gln→Glu substitution, was identified in one isolate. This mutation has only been reported once, in an isolate from Taiwan (7). Similarly, the deletion of codon 518 was observed in one isolate, and this aberration has been observed in only two other studies in Africa and Belgium (9, 10).

No genetic alterations were detected in 2 (3.9%) of the 51 RIF-resistant isolates. Four RIF-sensitive isolates were included as controls, and no mutations were identified in these isolates.

Genetic alterations in the RRDR were present at a high frequency of 96.1% in our Singaporean isolates, and the most commonly mutated residues were Ser-531, His-526, and Asp-516, as has also been demonstrated by the majority of studies worldwide (2, 8, 13). In conclusion, this high rate of mutations within the RRDR of the *rpoB* gene suggests that targeted screening of the RRDR may be feasible for the determination of RIF resistance in clinical isolates of *Mycobacterium tuberculosis* from Singapore.

TABLE 1. Genetic alterations of the *rpoB* gene in 51 rifampin-resistant *Mycobacterium tuberculosis* isolates from Singapore

Codon(s)	Amino acid substitution(s)	Mutation(s)	No. of isolates (%)
513	Gln→Glu	CAA→GAA	1 (2.0)
514; 516	Phe→Leu; Asp→Val	TTC→CTC; GAC→GTC	1 (2.0)[a]
516	Asp→Val	GAC→GTC	4 (7.8)
516	Asp→Tyr	GAC→TAC	1 (2.0)
518	Asn deletion	AAC deletion	1 (2.0)
522	Ser→Leu	TCG→TTG	1 (2.0)
526	His→Tyr	CAC→TAC	7 (13.7)
526	His→Asp	CAC→GAC	3 (5.9)
526	His→Leu	CAC→CTC	1 (2.0)
526	His→Arg	CAC→CGC	1 (2.0)
531	Ser→Leu	TCG→TTG	25 (49.0)
531	Ser→Trp	TCG→TGG	2 (3.9)
531	Ser→Met	TCG→ATG	1 (2.0)
No mutation			2 (3.9)
Total			51 (100)

[a] Mutations at both codons 514 and 516 were observed in one isolate. The corresponding substitutions and mutations are respective to the codons.

We acknowledge the contribution of the Central TB Laboratory, Department of Pathology, Singapore General Hospital, for providing the isolates and the National Medical Research Council of Singapore for funding this project.

REFERENCES

1. **Boudville, I. C., S. Y. Wong, and I. Snodgrass.** 1997. Drug-resistant tuberculosis in Singapore, 1995 to 1996. Ann. Acad. Med. Singap. **26:**549–556.
2. **Hirano, K., C. Abe, and M. Takahashi.** 1999. Mutations in the *rpoB* gene of rifampin-resistant *Mycobacterium tuberculosis* strains isolated mostly in Asian countries and their rapid detection by line probe assay. J. Clin. Microbiol. **37:**2663–2666.
3. **Kapur, V., L. L. Li, S. Iordanescu, M. R. Hamrick, A. Wanger, B. N. Kreiswirth, and J. M. Musser.** 1994. Characterization by automated DNA sequencing of mutations in the gene (*rpoB*) encoding the RNA polymerase beta subunit in rifampin-resistant *Mycobacterium tuberculosis* strains from New York City and Texas. J. Clin. Microbiol. **32:**1095–1098.
4. **Lee, A. S., I. H. Lim, L. L. Tang, A. Telenti, and S. Y. Wong.** 1999. Contribution of *kasA* analysis to detection of isoniazid-resistant *Mycobacterium tuberculosis* in Singapore. Antimicrob. Agents Chemother. **43:**2087–2089.
5. **Lee, A. S., L. L. Tang, I. H. Lim, and S. Y. Wong.** 2002. Characterization of pyrazinamide and ofloxacin resistance among drug resistant Mycobacterium tuberculosis isolates from Singapore. Int. J. Infect. Dis. **6:**48–51.
6. **Lee, A. S., A. S. Teo, and S. Y. Wong.** 2001. Novel mutations in *ndh* in isoniazid-resistant *Mycobacterium tuberculosis* isolates. Antimicrob. Agents Chemother. **45:**2157–2159.
7. **Liu, Y. C., T. S. Huang, and W. K. Huang.** 1999. Line probe assay for rapid detection of mutations in the rpoB gene of Mycobacterium tuberculosis. J. Formos. Med. Assoc. **98:**582–585.
8. **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.
9. **Rossau, R., H. Traore, H. De Beenhouwer, W. Mijs, G. Jannes, P. De Rijk, and F. Portaels.** 1997. Evaluation of the INNO-LiPA Rif. TB assay, a reverse hybridization assay for the simultaneous detection of *Mycobacterium tuber-*

culosis complex and its resistance to rifampin. Antimicrob Agents Chemother. **41:**2093–2098.

10. **Schilke, K., K. Weyer, G. Bretzel, B. Amthor, J. Brandt, V. Sticht-Groh, P. B. Fourie, and W. H. Haas.** 1999. Universal pattern of RpoB gene mutations among multidrug-resistant isolates of Mycobacterium tuberculosis complex from Africa. Int J. Tuberc. Lung Dis. **3:**620–626.
11. **Telenti, A., N. Honore, C. Bernasconi, J. March, A. Ortega, B. Heym, H. E. Takiff, and S. T. Cole.** 1997. Genotypic assessment of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*: a blind study at reference laboratory level. J. Clin. Microbiol. **35:**719–723.12**Valim, A. R., M. L. Rossetti, M. O. Ribeiro, and A. Zaha.** 2000. Mutations in the *rpoB* gene of multidrug-resistant *Mycobacterium tuberculosis* isolates from Brazil. J. Clin. Microbiol. **38:**3119–3122.13**Van Der Zanden, A. G., E. M. Te Koppele-Vije, N. Vijaya Bhanu, D. Van Soolingen, and L. M. Schouls.** 2003. Use of DNA extracts from Ziehl-Neelsen-stained slides for molecular detection of rifampin resistance and spoligotyping of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **41:**1101–1108.

Ann S. G. Lee*
Division of Medical Sciences
National Cancer Centre
Singapore 169610, Singapore

Irene H. K. Lim
Lynn L. H. Tang
Sin Yew Wong
Department of Infectious Diseases
Tan Tock Seng Hospital
Singapore 308433, Singapore

*Phone: 65-6436 8313
Fax: 65-6226 3696
E-mail: dmslsg@nccs.com.sg