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Sequencing of the *pncA* Gene in Members of the *Mycobacterium tuberculosis* Complex Has Important Diagnostic Applications: Identification of a Species-Specific *pncA* Mutation in "*Mycobacterium canettii*" and the Reliable and Rapid Predictor of Pyrazinamide Resistance▽

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Testing for susceptibility to pyrazinamide (PZA) and analysis of the *pncA* gene sequences of 423 *Mycobacterium tuberculosis* complex isolates have revealed a unique silent nucleotide substitution that enables the rapid identification of "*M. canettii*" (proposed name). Moreover, the lack of a defined mutation within the *pncA* gene strongly suggests that an alternative mechanism is responsible for PZA resistance. Our results indicate that DNA sequencing of the *pncA* gene has the potential to shorten the turnaround time and increase the accuracy of PZA susceptibility testing of the *M. tuberculosis* complex.

The *Mycobacterium tuberculosis* complex (MTB complex) consists of the closely related organisms *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. caprae*, *M. microti*, *M. pinnipedii*, the dassie bacillus, and "*M. canettii*" (proposed name) (3, 5, 6, 26). Although the members of the complex may differ in their epidemiologies, host spectra, geographic ranges, pathogenicities, and antituberculosis drug susceptibilities and although they display different phenotypic characteristics by conventional biochemical tests, they show high degrees of genetic homogeneity (10, 25).

Conventional phenotypic methods for the identification of the members of the MTB complex are laborious and time-consuming, and they require a large biomass (13). Thus, molecular methods (such as DNA sequencing of the *oxyR*, *pncA*, *gyrB*, or *hsp65* gene; analysis of spacers between direct repeats in the direct repeat region; and deletion analysis of the regions of difference [RD]) provide a more rapid and accurate approach to the differentiation of the members of the MTB complex (8, 9, 16, 23, 24).

The findings of a recent report that examined seven genes (*katG*, *gyrB*, *gyrA*, *rpoB*, *hsp65*, *sodA*, and the 16S rRNA gene) indicate that *M. canettii* represents the most ancient phylogenetic lineage of the tubercle bacilli (10). Until recently, little attention was paid to *M. canettii*, a rare member of the complex that infects only humans and that shows a geographical restriction to Africa; however, it has been found in patients as they travel to other parts of the world, leading to the need for the identification of this organism outside of the African continent (7, 19, 27).

M. canettii is characterized by eugonic growth, with smooth, white, and glossy colonies on solid medium. Its biochemical characteristics indicate that it is unable to produce niacin but that it is capable of nitrate reduction and has positive urease, Tween 80 hydrolysis, and catalase activities (at 22°C, although not at 68°C). Genetically, it can be distinguished from other members of the MTB complex by an unusual composition of the direct repeat cluster when it is assessed by spoligotyping. Finally, it is naturally resistant to thiophene-2-carboxylic acid hydrazide and pyrazinamide (PZA) (9, 19, 27). PZA (a nicotinamide analog) is a prodrug which is converted to its active form, pyrazinoic acid (POA), by the mycobacterial enzyme pyrazinamidase (PZase) (18, 28). It has been observed that PZA-resistant *M. tuberculosis* isolates usually lose their PZase activity (14). When the gene encoding PZase (*pncA*) from PZA-resistant clinical isolates was sequenced, it was found that 72 to 97% of all isolates tested contained a mutation in the structural gene or in the putative promoter region, both of which were predicted to impair PZase activity (23, 28). In addition, *M. bovis* and *M. bovis* BCG, which are naturally resistant to PZA, encode a single substitution of PZase, His57Asp, which distinguishes them from other members of the MTB complex (22).

The aim of the present study was to determine whether the natural resistance to PZA of *M. canettii* could be correlated with a similar *pncA* mutation and, if so, whether the mutation could be used to distinguish *M. canettii* from other members of the MTB complex. On the basis of the accumulated findings, we also aimed our study to examine the use of *pncA* sequencing for the rapid detection of PZA resistance.

A total of nine *M. canettii* isolates were studied: *M. canettii* CIPT140010059 from the Institut Pasteur, Paris, France; *M. canettii* 910563 from the Institut Pasteur; *M. canettii* 217/94, isolated at the Swiss Reference Laboratory for Mycobacteria,

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TABLE 1. Nucleotide polymorphisms in the *pncA* gene of the 11 *M. canettii* isolates studied

Strains (origin)	Nucleotide (amino acid) changes in *pncA*[a]
M. canettii CIPT140010059 (Institute Pasteur, Paris, France)	**A138G (Ala46Ala)**, T387C (Asp129Asp)
M. canettii 910563 (Institute Pasteur, Paris, France)	**A138G (Ala46Ala)**
M. canettii 217/94 (Swiss Reference Laboratory for Mycobacteria, Zurich, Switzerland)	**A138G (Ala46Ala)**
M. canettii So93 (National Institute of Public Health and Environment, Bilthoven, The Netherlands)	**A138G (Ala46Ala)**
M. canettii So93R (the rough colony type subculture of *M. canettii* So93; National Institute of Public Health and Environment, Bilthoven, The Netherlands)	**A138G (Ala46Ala)**
M. canettii Percy 25 (Percy Military Hospital, Clamart, France)	**A138G (Ala46Ala)**
M. canettii Percy 229 (Percy Military Hospital, Clamart, France)	**A138G (Ala46Ala)**
M. canettii Percy 65 (Percy Military Hospital, Clamart, France)	**A138G (Ala46Ala),** C42T (Cys14Cys), G261C (Thr87Thr), C349T (Leu117Leu), T387C (Asp129Asp), A110C (Glu37Ala), G187A (Asp63Asn), G273C (Glu91Asp), T451A (Leu151Met), T506C, G507A (Val169Ala), G522C (Glu174Asp)
M. canettii AFB0600000720 (Wadsworth Center, Albany, NY)	**A138G (Ala46Ala)**

[a] Boldface indicates an *M. canettii*-specific silent substitution.

Zurich, Switzerland (19); *M. canettii* So93 (smooth colony type), isolated at the National Institute of Public Health and Environment, Bilthoven, The Netherlands (27); *M. canettii* So93R, the rough colony type subculture of a single colony of So93; *M. canettii* Percy 25, Percy 65, and Percy 229, isolated at the Percy Military Hospital, Clamart, France (7); and *M. canettii* AFB 0600720 identified at the Wadsworth Center, Albany, NY. *M. canettii* isolates 217/94 (kindly provided by Gaby Pfyffer); So93 and So93R (kindly provided by Dick van Soolingen); and Percy 25, 65, and 229 (kindly provided by Michel Fabre) were maintained and cultured at the Institut Pasteur de Guadeloupe.

Identification of all strains as *M. canettii* was confirmed by a PCR-based deletion analysis with two panels of RDs (RD1, RD9, and RD10 and RD4, RD12, and TbD1) and by PCR-restriction analysis of the *hsp65* gene, as described earlier (9, 17; L. M. Parsons, J. Dormandy, A. Clobridge, J. R. Driscoll, M. Oxtoby, H. W. Taber, and M. Salfinger, Abstr. National TB Controllers Workshop, 2004). Routine drug susceptibility testing was performed with all strains by use of the radiometric BACTEC 460TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) and by the agar proportion method (12, 13); *M. canettii* AFB 0600720 was tested in the BACTEC 460TB system at the Wadsworth Center. All other *M. canettii* isolates were tested by the agar proportion method at the Unité de la Tuberculose et des Mycobactéries, Institut Pasteur de Guadeloupe, as described earlier (9). *M. canettii* Percy 25, 65, and 229 were also tested with the BACTEC MGIT 960 system (Becton Dickinson Diagnostic Instrument Systems) (M. Fabre, personal communication).

Using two primers, pncA-P1 (5′-GCT-GGT-CAT-GTT-CG C-GAT-CG-3′) and pncA-P6 5′-GCT-TTG-CGG-CGA-GC G-CTC-CA-3′), which flanked the entire *pncA* gene and its upstream promoter, we generated a 700-bp product from each isolate, as described previously (22, 23). The same primers were used for DNA sequencing of both strands with an automated 3700 DNA sequencer (Applied Biosystems, Foster City, CA). The resulting sequences were compared with the wild-type *pncA* sequence from *M. tuberculosis* H37Rv for the detection of mutations associated with PZA resistance. The DNA sequencing was carried out by the Molecular Genetics Core Facility at the Wadsworth Center.

The mutations of the *pncA* gene that were identified in the nine *M. canettii* strains are summarized in Table 1. All strains were resistant to PZA. Analysis of the DNA sequence of *pncA* revealed an A138G (Ala46Ala) silent nucleotide substitution in all nine *M. canettii* strains. In an independent study just published, the same substitution was described as being present in five *M. canettii* strains by Huard et al. (11). Strain CIPT140010059 in our study carried an additional T387C (Asp129Asp) silent substitution, while strain Percy 65 carried an additional 10 nucleotide substitutions, 4 of which were silent and 6 of which introduced amino acid changes in PZase (Table 1).

In a second phase of the study, we analyzed the *pncA* gene by DNA sequencing of a large number (n = 412) of MTB complex isolates (294 *M. tuberculosis*, 45 *M. bovis*, 43 *M. bovis* BCG, 24 *M. africanum*, 4 *M. microti*, and 2 *M. caprae* isolates) to confirm that the A138G substitution identified in the *M. canettii* strains is unique to this member of the MTB complex. Susceptibility testing of these strains was performed with the BACTEC 460TB system. Identification of the strains was determined by PCR-based deletion analysis, as described above. Of the 412 strains, 240 (58.3%) were susceptible and 172 (41.7%) were resistant to 100 μg/ml PZA in the BACTEC 460TB system.

Analysis of the *pncA* gene sequence of this large number of clinical isolates showed that none of the changes in the *M. canettii* strains were present in other members of the MTB complex (Table 2). DNA sequencing confirmed a wild-type *pncA* sequence for 195 (81.2%) of the 240 non-*M. canettii* strains that were susceptible to PZA (Table 2). Three (1.3%) PZA-susceptible *M. tuberculosis* strains carried silent substitutions which differed from those in *M. canettii* (Tables 1 and 2). A further 42 (17.5%) *M. tuberculosis* strains that were susceptible in the BACTEC 460TB system carried the same *pncA* mutation (Thr47Ala). However, it is noteworthy that these *M. tuberculosis* strains, which were resistant to the same drugs and which shared a characteristic spoligotype pattern, all belonged to the Beijing/W family (Table 2). They are therefore derived

TABLE 2. Pyrazinamide susceptibility testing with the BACTEC 460TB system and *pncA* DNA sequencing analysis results for 412 non-*M. canettii* isolates of the *Mycobacterium tuberculosis* complex[a]

No. of strains	Identification	BACTEC 460TB result	*pncA* mutation	Nucleotide change
166	MTB	S	No mutation	NA
42	MTB	S	Thr47Ala	A139G
2	MTB	S	Ser65Ser*	C195T
1	MTB	S	Ala20Ala*	G60A
24	AFR	S	No mutation	NA
4	MICR	S	No mutation	NA
1	CAP	S	No mutation	NA
14	MTB	R	Frameshift mutation	Deletion of nucleotide 70 (G)
13	MTB	R	Leu85Pro	T254C
6	MTB	R	NA	A→−11 upstream
3	MTB	R	Ser104Arg	C312G
3	MTB	R	Leu172Pro	T515C
2	MTB	R	Gln10Pro	A39C
2	MTB	R	Cys14Arg and Ser65Ser*	T40C and C195G
2	MTB	R	His51Gln	C153A
2	MTB	R	Leu116Arg	T347G
2	MTB	R	Trp119Cys	G357T
2	MTB	R	Thr142Pro and Ser65Ser*	A424C and C195T
2	MTB	R	Frameshift mutation	Nucleotide insertion (G) between codons 77 and 78
1	MTB	R	NA	Deletion of start codon and −5 nucleotides upstream
1	MTB	R	Val21Gly	T62G
1	MTB	R	Leu35Arg and His82Asp	T104 and C244G
1	MTB	R	Ala46Val	C138T
1	MTB	R	Frameshift mutation	Deletion of nucleotide 146 (A)
1	MTB	R	His51Arg	A152G
1	MTB	R	Ser67Pro	T199C
1	MTB	R	Trp68Arg	T202C
1	MTB	R	His71Arg	A212G
1	MTB	R	Gly78Cys	G232T
1	MTB	R	Tyr103Asp	T307G
1	MTB	R	Gly108Arg	G322C
1	MTB	R	Frameshift mutation	Nucleotide insertion (G) between codons 104 and 105
1	MTB	R	Frameshift mutation	Deletion of codons 128, 129, 130
1	MTB	R	Val128Gly	T383G
1	MTB	R	Frameshift mutation	Nucleotide insertion (GG) between codons 130 and 131
1	MTB	R	Cys138Tyr	G413A
1	MTB	R	Val139Leu	G415C
1	MTB	R	Val139Ala	T416C
1	MTB	R	Gln141Stop	C421T
1	MTB	R	Thr142Met	C425T
1	MTB	R	Frameshift mutation	Nucleotide insertion (CT) between codons 155 and 156
1	MTB	R	Leu156Pro	T467C
1	MTB	R	Gly162Ser	G485A
1	MTB	R	Gly162Asp	G486A
1	MTB	R	Ser164Pro and Glu173Glu*	T490C and G519A
1	MTB	R	Ala171Val	C512T
1	MTB	R	Frameshift mutation	Nucleotide insertion (CG) at codon 177
1	MTB	R	Val180Phe	G538T
1	MTB	R	Frameshift mutation	Deletion of four nucleotides (542, 543, 544, 545)
45	BOV	R	His57Asp	C169G
43	BCG	R	His57Asp	C169G
1	CAP	R†	No mutation	NA

[a] Abbreviations and symbols: MTB, *M. tuberculosis*; BOV, *M. bovis*; AFR, *M. africanum*; CAP, *M. caprae*; MICR, *M. microti*; S, susceptible; R, resistant; NA, not applicable; *, silent mutation; †, a live culture could not be retrieved to confirm the accuracy of the discrepant result (*M. caprae* strains are naturally PZA susceptible).

from a single clone of a strain that was originally identified during nosocomial outbreaks in New York City in the early 1990s (2). Further analysis of these Beijing/W strains revealed that the mutation observed in *pncA* resulted in elevated PZA MICs compared to those for PZA-susceptible strains but in lower MICs compared to those for PZA-resistant strains, as determined by more rigorous assays (data not shown) (6a).

PZA resistance-associated mutations were detected in 171 (99.94%) of the 172 PZA-resistant strains. The single *M. caprae* strain that exhibited resistance to PZA in the BACTEC 460TB system did not encode any mutations in the sequenced region (Table 2). Unfortunately, a live culture could not be retrieved to confirm the validity of this discrepant result (*M. caprae* strains are naturally PZA susceptible) (1). All of the 45 *M. bovis* isolates and the 43 *M. bovis* BCG isolates encoded the distinguishing His57Asp mutation, consistent with previous results (Table 2) (22).

Testing of the members of the MTB complex for their sus-

ceptibilities to PZA has been complicated by the fact that this drug is active only at acidic pH; a significant number of *M. tuberculosis* isolates would not grow at pH 5.5 in conventional solid media (4, 20). Subsequently, a more accurate, broth-based radiometric test system (the BACTEC 460TB system) with pH 6.0 medium was described, and this has become the current recommended assay (21). However, in a study that examined 428 MTB complex strains, at least 4 (0.8%) grew in the presence of 100 μg/ml PZA in the BACTEC 460TB system and yet were PZase positive, suggesting either that for some strains the radiometric assay is inaccurate or that resistance is mediated by another mechanism (15). Our results indicate that sequence analysis of *pncA* is also an accurate predictor of PZA resistance (99.9%). This capability, combined with the rapid turnaround time of the sequence analysis, makes it an attractive new reference assay for PZA susceptibility testing.

Most importantly, our work with the *M. canettii* isolates suggests that their natural resistance to PZA is not *pncA* based. Rather, another mechanism of PZA resistance must be operative, such as PZA uptake, *pncA* regulation, or POA efflux, a conclusion that is in agreement with the observations of others (15, 28). Further investigations are warranted to determine the mechanism of PZA resistance in *M. canettii*. Moreover, the sequence analysis identified a single nucleotide polymorphism (A138G) as a positive indicator of *M. canettii*, allowing this organism to be easily distinguished from other members of the MTB complex.

The natural reservoir, host range, and mode of transmission of *M. canettii* are unknown. Many clinical laboratories do not differentiate among the members of the MTB complex. Thus, it is very likely that the geographic distribution and prevalence of *M. canettii*, as well as those of other non-*M. tuberculosis* members of the MTB complex, are underestimated. The results of the present study establish that analysis of the *pncA* gene by DNA sequencing can rapidly and accurately identify *M. canettii* and can differentiate it from other members of the MTB complex.

In conclusion, besides the potential to shorten the turnaround time and increase the accuracy of PZA susceptibility testing, the broader use of DNA sequencing analysis of *pncA* will help to accumulate additional valuable information on the epidemiology, transmission, and pathogenesis of *M. bovis*, *M. bovis* BCG, and *M. canettii* infections.

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REFERENCES

1. **Aranaz, A., E. Liebana, E. Gomez-Mampaso, J. C. Galan, D. Cousins, A. Ortega, J. Blazquez, F. Baquero, A. Mateos, G. Suarez, and L. Dominguez.** 1999. *Mycobacterium tuberculosis* subsp. *caprae* subsp. nov.: a taxonomic study of a new member of the *Mycobacterium tuberculosis* complex isolated from goats in Spain. Int. J. Syst. Bacteriol. **49:**1263–1273.
2. **Bifani, P. J., B. B. Plikaytis, V. Kapur, K. Stockbauer, X. Pan, M. L. Lutfey, S. L. Moghazeh, W. Eisner, T. M. Daniel, M. H. Kaplan, J. T. Crawford, J. M. Musser, and B. N. Kreiswirth.** 1996. Origin and interstate spread of a New York City multidrug-resistant *Mycobacterium tuberculosis* clone family. JAMA **275:**452–457.
3. **Brosch, R., S. V. Gordon, M. Marmiesse, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L. M. Parsons, A. S. Pym, S. Samper, D. van Soolingen, and S. T. Cole.** 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc. Natl. Acad. Sci. USA **99:**3684–3689.
4. **Butler, W. R., and J. O. Kilburn.** 1983. Susceptibility of *Mycobacterium tuberculosis* to pyrazinamide and its relationship to pyrazinamidase activity. Antimicrob. Agents Chemother. **24:**600–601.
5. **Cousins, D. V., R. Bastida, A. Cataldi, V. Quse, S. Redrobe, S. Dow, P. Duignan, A. Murray, C. Dupont, N. Ahmed, D. M. Collins, W. R. Butler, D. Dawson, D. Rodriguez, J. Loureiro, M. I. Romano, A. Alito, M. Zumarraga, and A. Bernardelli.** 2003. Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. Int. J. Syst. Evol. Microbiol. **53:**1305–1314.
6. **Cousins, D. V., R. L. Peet, W. T. Gaynor, S. N. Williams, and B. L. Gow.** 1994. Tuberculosis in imported hyrax (*Procavia capensis*) caused by an unusual variant belonging to the *Mycobacterium tuberculosis* complex. Vet. Microbiol. **42:**135–145.

6a. **Dormandy, J., et al.** Chest, in press.

7. **Fabre, M., J. L. Koeck, P. Le Fleche, F. Simon, V. Herve, G. Vergnaud, and C. Pourcel.** 2004. High genetic diversity revealed by variable-number tandem repeat genotyping and analysis of *hsp65* gene polymorphism in a large collection of "*Mycobacterium canettii*" strains indicates that the *M. tuberculosis* complex is a recently emerged clone of "*M. canettii*". J. Clin. Microbiol. **42:**3248–3255.
8. **Goh, K. S., M. Fabre, R. C. Huard, S. Schmid, C. Sola, and N. Rastogi.** 2006. Study of the *gyrB* gene polymorphism as a tool to differentiate among *Mycobacterium tuberculosis* complex subspecies further underlines the older evolutionary age of '*Mycobacterium canettii*.' Mol. Cell. Probes **20:**182–190.
9. **Goh, K. S., E. Legrand, C. Sola, and N. Rastogi.** 2001. Rapid differentiation of "*Mycobacterium canettii*" from other *Mycobacterium tuberculosis* complex organisms by PCR-restriction analysis of the *hsp65* gene. J. Clin. Microbiol. **39:**3705–3708.
10. **Gutierrez, M. C., S. Brisse, R. Brosch, M. Fabre, B. Omais, M. Marmiesse, P. Supply, and V. Vincent.** 2005. Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. PLoS Pathog. **1:**55–61.
11. **Huard, R. C., M. Fabre, P. de Haas, L. Claudio Oliveira Lazzarini, D. van Soolingen, D. Cousins, and J. L. Ho.** 2006. Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. J. Bacteriol. **188:**4271–4287.
12. **Inderlied, C. B., and M. Salfinger.** 1999. Antimycobacterial agents and susceptibility tests, p. 1601–1623. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, DC.
13. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology. A guide for a level III laboratory. Centers for Disease Control, Atlanta, GA.
14. **Konno, K., F. M. Feldman, and W. McDermont.** 1967. Pyrazinamide susceptibility and amidase activity of the tubercle bacilli. Am. Rev. Respir. Dis. **95:**461–469.
15. **Miller, M. A., L. Thibert, F. Desjardins, S. H. Siddiqi, and A. Dascal.** 1995. Testing of susceptibility of *Mycobacterium tuberculosis* to pyrazinamide: comparison of BACTEC method with pyrazinamidase assay. J. Clin. Microbiol. **33:**2468–2470.
16. **Niemann, S., D. Harmsen, S. Rusch-Gerdes, and E. Richter.** 2000. Differentiation of clinical *Mycobacterium tuberculosis* complex isolates by *gyrB* DNA sequence polymorphism analysis. J. Clin. Microbiol. **38:**3231–3234.
17. **Parsons, L. M., R. Brosch, S. T. Cole, A. Somoskovi, A. Loder, G. Bretzel, D. Van Soolingen, Y. M. Hale, and M. Salfinger.** 2002. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. J. Clin. Microbiol. **40:**2339–2345.
18. **Parsons, L. M., A. Somoskovi, R. Urbanczik, and M. Salfinger.** 2004. Laboratory diagnostic aspects of drug resistant tuberculosis. Front. Biosci. **9:**2086–2105.
19. **Pfyffer, G. E., R. Auckenthaler, J. D. van Embden, and D. van Soolingen.** 1998. *Mycobacterium canettii*, the smooth variant of *M. tuberculosis*, isolated from a Swiss patient exposed in Africa. Emerg. Infect. Dis. **4:**631–634.
20. **Salfinger, M., and F. M. Kafader.** 1987. Susceptibility testing of *Mycobacterium tuberculosis* to pyrazinamide. Zentbl. Bakteriol. Parasitenkd. Infektkrankh. Hyg. Abt. 1 Orig. **265:**404–407.
21. **Salfinger, M., L. B. Reller, B. Demchuk, and Z. T. Johnson.** 1989. Rapid radiometric method for pyrazinamide susceptibility testing of Mycobacterium tuberculosis. Res. Microbiol. **140:**301–309.
22. **Scorpio, A., D. Collins, D. Whipple, D. Cave, J. Bates, and Y. Zhang.** 1997. Rapid differentiation of bovine and human tubercle bacilli based on a characteristic mutation in the bovine pyrazinamidase gene. J. Clin. Microbiol. **35:**106–110.
23. **Scorpio, A., and Y. Zhang.** 1996. Mutations in *pncA*, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat. Med. **2:**662–667.
24. **Sreevatsan, S., P. Escalante, X. Pan, D. A. Gillies II, S. Siddiqui, C. N. Khalaf, B. N. Kreiswirth, P. Bifani, L. G. Adams, T. Ficht, V. S. Perumaalla, M. D. Cave, J. D. van Embden, and J. M. Musser.** 1996. Identification of a polymorphic nucleotide in *oxyR* specific for *Mycobacterium bovis*. J. Clin. Microbiol. **34:**2007–2010.

25. **Sreevatsan, S., X. Pan, K. E. Stockbauer, N. D. Connel, B. N. Kreiswirth, T. S. Whittam, and J. M. Musser.** 1997. Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionary recent global dissemination. Proc. Natl. Acad. Sci. USA **94:**9869–9874.
26. **Tsukamura, M., S. Mizuno, and H. Toyama.** 1985. Taxonomic studies on the *Mycobacterium tuberculosis* series. Microbiol. Immunol. **29:**285–299.
27. **van Soolingen, D., T. Hoogenboezem, P. E. de Haas, P. W. Hermans, M. A. Koedam, K. S. Teppema, P. J. Brennan, G. S. Besra, F. Portaels, J. Top, L. M. Schouls, and J. D. van Embden.** 1997. A novel pathogenic taxon of the *Mycobacterium tuberculosis* complex, Canetti: characterization of an exceptional isolate from Africa. Int. J. Syst. Bacteriol. **47:**1236–1245.
28. **Zhang, Y., and A. Telenti.** 2000. Genetics of drug resistance in *Mycobacterium tuberculosis*, p. 235–254. *In* G. F. Hatful and W. Jacobs Jr., R. (ed.), Molecular genetics of mycobacteria. ASM Press, Washington, DC.