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Geographic Differences in the Contribution of *ubiA* Mutations to High-Level Ethambutol Resistance in *Mycobacterium tuberculosis*

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Ethambutol (EMB) resistance can evolve through a multistep process, and mutations in the *ubiA* (*Rv3806c*) gene appear to be responsible for high-level EMB resistance in *Mycobacterium tuberculosis*. We evaluated the prevalence of *ubiA* and *embB* (*Rv3795*) mutations in EMB-resistant strains originating from Africa and South Korea. No differences in *embB* mutation frequencies were observed between strains from both origins. However, *ubiA* mutations were present in 45.5% ± 6.5% of the African EMB-resistant isolates but in only 9.5% ± 1.5% of the South Korean EMB-resistant isolates. The *ubiA* mutations associated with EMB resistance were localized to regions encoding the transmembrane domains of the protein, whereas the *embB* mutations were localized to regions encoding the extramembrane domains. Larger studies are needed to investigate the causes of increased *ubiA* mutations as a pathway to high-level EMB resistance in African countries, such as extended EMB usage during tuberculosis treatment.

Ethambutol (EMB), a first-line antituberculosis drug, is often used in combination with other drugs to treat tuberculosis and prevent the emergence of drug resistance (1). Numerous studies have shown that mutations in the *embCAB* operon, particularly the *embB* gene, are a major cause of EMB resistance in *Mycobacterium tuberculosis* (2–8). A second set of mutations, which occur in the *ubiA* gene, has been associated with EMB resistance in clinical *M. tuberculosis* isolates (9–11). Mutations in *ubiA* almost always occur in EMB-resistant strains that also contain *embB* mutations, and *ubiA* appears to have multiplicative effects with *embB* mutations on MICs (9). The evolutionary path leading from low- to high-level EMB resistance has been studied in the laboratory (9). In these studies, high-level EMB resistance appears to develop through the stepwise acquisition of mutations in *embB*, *ubiA*, and *embC*.

In the study described here, we examined the prevalence of *ubiA* mutations in isolates from two different geographic regions. Our results confirm the association between *ubiA* mutations and the presence of *embB* mutations and EMB resistance. We also demonstrate that the prevalence of these mutations varies by geographic location, suggesting that local factors may play a role in the type of mutations which develop as *M. tuberculosis* strains evolve to become EMB resistant.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Fifty-four clinical *M. tuberculosis* strains from different geographic regions were selected from a collection of highly characterized *M. tuberculosis* isolates established by the World Health Organization Special Programme for Research and Training in Tropical Disease (TDR strains; see Table S1 in the supplemental material) (12). A second set of 39 isolates from the National Masan Hospital in Changwon, South Korea and 41 isolates from the National Reference Laboratory in Rwanda was also tested (see Table 3). The last two sets of isolates were part of a drug resistance survey and were cultured from patient sputum after obtaining informed consent from their institutional review boards of the respective institutions (13, 14). In this study, *M. tuberculosis* strains were cultured at 37°C either in Middlebrook 7H9 broth containing 0.05% (wt/vol) Tween 80 or on Middlebrook 7H10 agar supplemented with 0.5% (vol/vol) glycerol, both of which were enriched with 10% oleic acid-albumin-dextrose-catalase (OADC; Becton Dickinson).

DNA isolation, PCR, and DNA sequencing. Genomic DNA was extracted as described previously, with minor modifications (15, 16). To amplify DNA fragments for DNA sequencing, the PCR was performed using a mix containing 1 ng of genomic DNA, 5 pmol of each primer, 200 μM deoxynucleoside triphosphates, 1× PCR buffer, and 1 U of high-fidelity *Pfx Taq* polymerase (Invitrogen). All PCR products were purified using a gel extraction kit (Qiagen). Direct bidirectional Sanger sequencing of the *embB* and *ubiA* genes was performed with a BigDye Terminator kit and analyzed with an ABI 3100 genetic analyzer (Applied Biosystems). All primer sequences used in this study are described in Table 1.

MIC testing and DST. The EMB MICs of the TDR strains were determined in this study using the standard radiometric Bactec 460TB method (Becton Dickinson and Company, Sparks, MD) following the manufacturer's instructions with minor modifications (4). Each strain was tested against serial 2-fold increases in the antibiotic concentration. To confirm the results obtained by the Bactec method, 5×10^3 CFU from the same

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TABLE 1 Primers used for amplification and sequencing

Gene (Rv designation)	Size (bp)	Primer	Sequence	Product location
ubiA (*Rv3806c*)	909	F-ubiA[a]	ACGTTGAGCTTGAGGCTAGC	−116 to +15
		R-ubiA[a]	CGCTGTCGCGAATACTGCT	
		Fin-ubiAseq[b]	GGTAGACGACCATTACCAGC	NA[c]
		Rin-ubiAseq[b]	GGTACTGGGAGTGACATCGC	NA
embB (*Rv3795*)	3297	F1-embB[a]	ATCGGTGGAGCAGTACCA	−117 to 877
		R1-embB[a]	ATGACATGCCAGAGCAGG	
		F2-embB[a]	CACCGTCGTCGCACTGAT	683 to 1675
		R2-embB[a]	CGCAACATGATGAACACCG	
		F3-embB[a]	CGTGGTATACCGAGAACCTG	1549 to +82
		R3-embB[a]	CATACCGAGCAGCATAGGAG	
		Fin3-embBseq[b]	CGCATTCACACTGGGTGTGC	NA
		Rin3-embBseq[b]	GGCAGGATGAGGTAGTAG	NA

[a] Primers used for PCR and sequencing.
[b] Primers used only for sequencing.
[c] NA, not applicable.

inoculum was also spotted onto plates of 7H10 medium containing serial increases in the concentrations of EMB. The plates were incubated at 37°C for 2 to 3 weeks, and the MICs were determined to be the antibiotic concentration that inhibited growth compared to the growth of the 1/100 dilution on antibiotic-free 7H10 medium. The MICs determined by the Bactec method were not significantly different from those determined by the agar method. Drug susceptibility testing (DST) for determination of the EMB susceptibilities of the clinical strains from Rwanda and South Korea was performed previously by local clinical laboratories using the mycobacterial growth indicator tube (MGIT) method with a cutoff of 5 μg/ml, as indicated in the manufacturer's package inserts, and/or by the agar proportion method on Lowenstein-Jensen medium with a cutoff of 2 μg/ml, as described previously (17).

Statistical analysis. For each gene, *embB* and *ubiA*, the associations between mutation status (presence, absence) and both the EMB MIC category and the geographic origin of the strain (Africa, South Korea) were assessed. Either the Pearson chi-square test or Fisher's exact test, if the expected sample counts were small, was used. The association between the frequency of *embB* mutations and *ubiA* mutations among all strains was assessed by McNemar's test for paired samples. A linear trend between mutations in each of the *embB* and *ubiA* genes and the EMB MIC was assessed using the exact Cochran-Armitage test for trend. All statistical tests were performed at a significance level of alpha equal to 0.05. All statistical analyses were performed using SAS (version 9.4) software (SAS Institute, Cary, NC).

Nucleotide sequence accession numbers. In this study, we identified six new mutations in the *ubiA* gene, and the sequences were deposited in the GenBank database under accession numbers KX021867 for UbiA A38V, KX021868 for UbiA V55G, KX021869 for UbiA V148A, KX021870 for UbiA S173A, KX021871 for UbiA V229G, and KX021872 for UbiA S173A/A278V.

RESULTS

Examination of *ubiA* and *embB* genotypes in *M. tuberculosis* TDR strains isolated in South Korea and in African countries. We measured the EMB MICs of 54 *M. tuberculosis* clinical strains isolated in South Korea and in Africa selected from the TDR collection (see Table S1 in the supplemental material) and sequenced the *embB* and *ubiA* genes of each of these strains. We then classified each strain into one of three EMB MIC categories: susceptible (MIC, 2 μg/ml), low-level resistant (MIC, 4 to 16 μg/ml), and high-level resistant (MIC, 32 μg/ml) (Table 2). None of the susceptible strains contained a mutation in either *embB* or *ubiA*. However, *embB* mutations were identified in many of the low-level and high-level EMB-resistant strains. No differences in *embB* mutation frequency were observed between strains of African origin (10/10 [100%] high-level resistant strains and 9/11 [82%] low-level resistant strains) and strains of South Korean origin (5/5 [100%] high-level resistant strains and 17/23 [74%] low-level resistant strains) ($P = 1.00$). Mutations in *ubiA* were also identified in low-level and high-level EMB-resistant strains. Contrary to the findings for the *embB* mutations, *ubiA* mutations occurred more frequently in the strains of African origin (8/10 [80%] high-level resistant strains and 3/11 [27%] low-level resistant strains) than in strains of South Korean origin (2/5 [40%] high-level resistant strains and 1/23 [4%] low-level resistant strains) (Table 2). Combining all EMB-resistant strains (MICs, >2 μg/ml), *ubiA* mutations were significantly more prevalent in strains of African origin (11/21, 52%) than in strains of South Korean origin (3/28, 11%) ($P = 0.001$). Our results also confirmed that mutations in *ubiA* are

TABLE 2 Distribution of *embB* and *ubiA* mutants among TDR strains classified by EMB MIC and geographic origin

	No. of MT[a]/no. of strains tested (%[b])					
	embB			*ubiA*		
EMB MIC (μg/ml)	Africa	South Korea	Total	Africa	South Korea	Total
32	10/10 (100)	5/5 (100)	15/15 (100)	8/10 (80)	2/5 (40)	10/15 (67)
4–16	9/11 (82)	17/23 (74)	26/34 (76)	3/11 (27)	1/23 (4)	4/34 (12)
2	0/3 (0)	0/2 (0)	0/5 (0)	0/3 (0)	0/2 (0)	0/5 (0)

[a] MT, mutant.
[b] Percentage of mutants.

associated with high-level EMB resistance (10/15 [67%] high-level resistant strains versus 4/34 [12%] low-level resistant strains; $P > 0.001$), supporting the findings of a previous study (9). All the *ubiA* mutations occurred in strains that also had *embB* mutations.

***ubiA* genotypes of *M. tuberculosis* EMB-resistant strains isolated in clinical laboratories of hospitals in Rwanda and in South Korea.** The *ubiA* mutation frequencies in African versus South Korean strains were then investigated by use of a second independent set of EMB-resistant strains obtained from Rwanda and South Korea. Because of the similar *embB* mutation frequencies described in African and South Korean EMB-resistant TDR strains, only the *ubiA* gene from this second set of strains was sequenced. Significant differences in *ubiA* mutation frequencies similar to those seen in the first set of strains tested were observed. Mutations in *ubiA* were detected in 16/41 (39%) EMB-resistant Rwandese isolates and 3/39 (8%) EMB-resistant South Korean isolates ($P = 0.001$) (Table 3). Three DNA samples from Rwanda had a mixture of wild-type and mutant *ubiA* sequences, and one DNA sample from South Korea had a double mutation in *ubiA* (Table 3).

Mutations in *ubiA* that are associated with EMB resistance are localized in the region encoding the transmembrane domains of the protein. The *ubiA* mutations from this study and our previously published study provide the largest collection of *ubiA* mutations so far reported in *M. tuberculosis* (9). We previously identified two mutations, R76R and E149D, to be phylogenetic markers associated with ancestral *M. tuberculosis* strains (lineage 1) (9, 18). Indeed, both the R76R and E149D mutations are present in the published genome sequences of *M. bovis* species, *M. africanum*, and *M. canettii* strains in GenBank (ftp://ftp.ncbi.nlm.nih.gov/genomes/all) (see Table S2 in the supplemental material). We examined the location of the *ubiA* mutations in relationship to the predicted secondary structure of the UbiA protein. Computer models have predicted that UbiA and Emb are integral membrane proteins consisting of cytoplasmic loops, transmembrane domains, and extracytoplasmic loops (2, 3, 19). UbiA is predicted to be an α-helical protein with nine transmembrane domains and no large carboxy-terminal region (19). After eliminating the R76R and E149D mutations and other synonymous mutations and mutations found in susceptible clinical isolates, we found that the amino acid changes in the UbiA protein associated with EMB resistance were almost exclusively located in the predicted transmembrane domains of the protein (Fig. 1). Interestingly, the location of the UbiA resistance-associated mutations contrasted markedly with the predicted location of mutations associated with EMB resistance in the EmbB protein. EmbB is predicted to be an α-helical protein with 11 transmembrane domains and a carboxy-terminal region of approximately 375 amino acids (3). In contrast to UbiA, the amino acids of EmbB associated with EMB resistance are predicted to be located in the extracytoplasmic loops of the membrane (3). While the contrasting locations of the UbiA and EmbB mutations associated with EMB resistance are striking, the functional significance of this difference is unclear.

DISCUSSION

This study confirms the previously documented association between *ubiA* mutations and EMB resistance, particularly with high-level EMB resistance (9). We found that *ubiA* mutations are always found together with *embB* mutations in EMB-resistant strains but that *embB* mutations can occur in the absence of *ubiA*

TABLE 3 Distribution of *ubiA* mutations in EMB-resistant *M. tuberculosis* strains isolated in laboratories of hospitals in South Korea and in Rwanda[a]

Origin	Total	No. of strains with the following UbiA codon change:											
		WT	Val148Ala (GTG → GCG) mix	Ser173Ala (TCC → GCC)	Trp175Gly (TGG → GGG)	Ile179Thr (ATC → ACC)	Val229Gly (GTG → GGG)	Ala237Val (GCT → GTT)	Arg240Cys (CGC → TGC)	Ala237Val (GCT → GTT) mix	Arg240Cys (CGC → TGC) mix	Ser173Ala (TCC→GCC), Ala278Val (GCG→GTG) DMT	% MT
South Korea	39	36		1			1					1	8
Rwanda	41	25	1		1	1		7	4	1	1		39

[a] WT, wild type; mix, mixture of wild type and mutant; DMT, double mutant; MT, mutants.

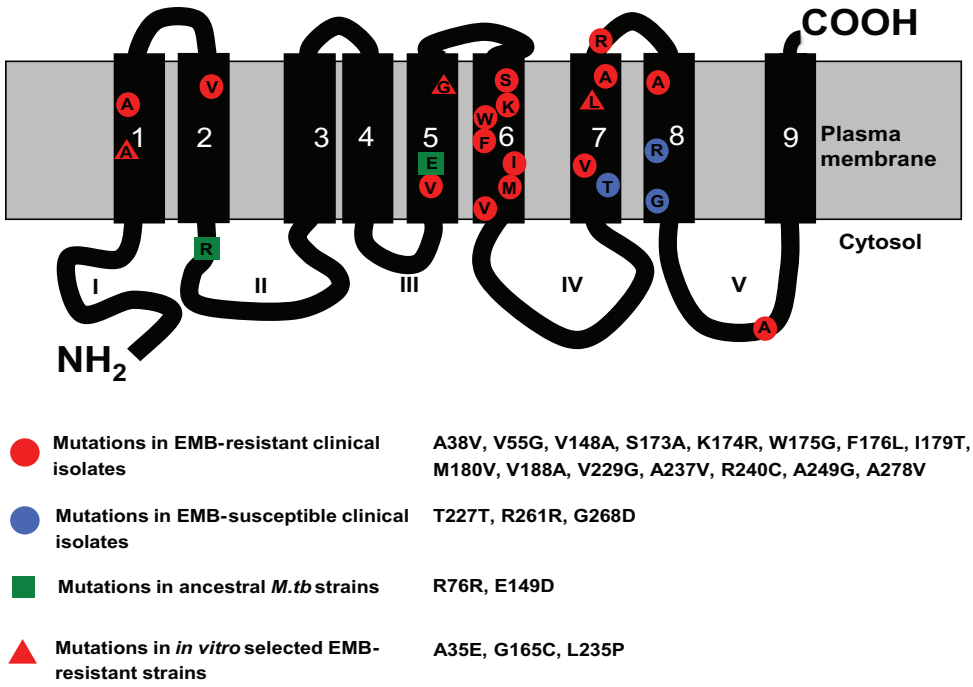

FIG 1 Schematic representation of UbiA amino acid changes associated with EMB resistance. *M.tb*, *M. tuberculosis*. Numbers 1 to 9 indicate transmembrane domains; I, II, III, IV, and V indicate cytoplasmic loops.

mutations. This observation suggests that mutations occur in *embB* as the first step in the evolution of EMB resistance and that *ubiA* mutations occur as secondary mutations, usually in association with high-level EMB resistance.

We have previously identified the association of the R76R and E149D *ubiA* mutations with lineage 1 of *M. tuberculosis* and not with EMB resistance (9). It is well reported that the changes in resistance genes should be evaluated when developing molecular diagnostic assays and managing treatment decisions (20). The V188A, A237V, R240C, and A249G mutations in *ubiA* have been conclusively shown, using allelic exchange studies, to cause EMB resistance (9); and using overexpression studies, K174T, W175C, and F176L mutations have been strongly implicated as a cause of EMB resistance (10). Thus, similar studies are needed to evaluate the contribution of other *ubiA* mutations in EMB resistance, further refine the association of all common *ubiA* mutations and EMB resistance, and improve genotypic drug resistance testing (20).

We found that *ubiA* mutations occurred more frequently in a collection of EMB-resistant isolates of African origin than a collection of EMB-resistant isolates of South Korean origin. This observation was confirmed with a second set of representative EMB-resistant isolates from Rwanda and South Korea. The reasons behind these regional differences in *ubiA* mutations remain unclear. It could be due to regional variations in *M. tuberculosis* strain types. Indeed, virtually all *M. tuberculosis* strains isolated from South Korea are members of the Beijing family of lineage 2 (21, 22), while most strains in Africa are members of lineage 1 or 3, with the T2 family being the most predominant genotype in Rwanda (23–26). Alternatively, variations in treatment practices among geographic regions could induce different resistance mutation profiles among EMB-resistant isolates. Standardized antituberculosis regimens in Rwanda use EMB for both the initial treatment and retreatment of tuberculosis, and drug susceptibility tests (DSTs) were not used to tailor treatments during the sampling period. In the absence of routine DSTs, clinicians may continue to use EMB even in EMB-resistant cases, potentially leading to the development of secondary mutations in *ubiA* and high-level EMB resistance. In contrast, clinicians in South Korea typically adjust their treatment regimens according to the results of DSTs that are performed at the start of treatment. These adjustments may prevent EMB-resistant isolates from acquiring additional EMB resistance mutations, including ones in *ubiA*. Routine DST is thought to improve treatment outcomes and to prevent the acquisition of resistance to new drugs (27). Our results also suggest that the more widespread use of DST could prevent the further evolution of resistance to a drug to which an *M. tuberculosis* strain is already resistant.

Our large panel of mutations permitted us to map UbiA resistance mutations to several transmembrane domains. The active site of the UbiA protein has been shown to be in the N-terminal region located in cytoplasmic loops II and IV (19). Thus, it is not clear how transmembrane mutations could lead to increased decaprenylphosphoryl-β-D-arabinose production and EMB resistance, as previously shown (9). One possibility is that UbiA is involved in a large enzyme complex with Emb proteins and other arabinan biosynthetic pathway components and that mutations in the transmembrane domains of UbiA could affect the stability or efficacy of this complex.

This study suggests that EMB resistance continues to evolve in isolates that are already EMB resistant through the acquisition of additional mutations. Our results also suggest that molecular tests for drug resistance should be implemented in antituberculosis health programs throughout the world to reduce the misuse of antimicrobials and to control the emergence of resistant strains. More clinical studies are needed to determine the contribution of

embCAB, *ubiA*, and *aftA* mutations to the evolution of EMB resistance in different geographic regions. These future studies should help to identify biomarkers for low-level and high-level EMB resistance, which will improve treatment decisions and prevent the emergence of multidrug-resistant organisms.

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REFERENCES

1. **American Thoracic Society, CDC, and Infectious Diseases Society of America.** 2003. Treatment of tuberculosis. MMWR Recommend Rep **52**(RR-11):1–77.
2. **Telenti A, Philipp WJ, Sreevatsan S, Bernasconi C, Stockbauer KE, Wieles B, Musser JM, Jacobs WR.** 1997. The emb operon, a gene cluster of Mycobacterium tuberculosis involved in resistance to ethambutol. Nat Med **3:**567–570. http://dx.doi.org/10.1038/nm0597-567.
3. **Ramaswamy SV, Amin AG, Goksel S, Stager CE, Dou S-J, El Sahly H, Moghazeh SL, Kreiswirth BN, Musser JM.** 2000. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother **44:**326–336. http://dx.doi.org/10.1128/AAC.44.2.326-336.2000.
4. **Safi H, Sayers B, Hazbon MH, Alland D.** 2008. Transfer of *embB* codon 306 mutations into clinical Mycobacterium tuberculosis strains alters susceptibility to ethambutol, isoniazid, and rifampin. Antimicrob Agents Chemother **52:**2027–2034. http://dx.doi.org/10.1128/AAC.01486-07.
5. **Starks AM, Gumusboga A, Plikaytis BB, Shinnick TM, Posey JE.** 2009. Mutations at *embB* codon 306 are an important molecular indicator of ethambutol resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother **53:**1061–1066. http://dx.doi.org/10.1128/AAC.01357-08.
6. **Safi H, Fleischmann RD, Peterson SN, Jones MB, Jarrahi B, Alland D.** 2010. Allelic exchange and mutant selection demonstrate that common clinical *embCAB* gene mutations only modestly increase resistance to ethambutol in Mycobacterium tuberculosis. Antimicrob Agents Chemother **54:**103–108. http://dx.doi.org/10.1128/AAC.01288-09.
7. **Plinke C, Cox HS, Zarkua N, Karimovich HA, Braker K, Diel R, Rüsch-Gerdes S, Feuerriegel S, Niemann S.** 2010. *embCAB* sequence variation among ethambutol-resistant Mycobacterium tuberculosis isolates without embB306 mutation. J Antimicrob Chemother **65:**1359–1367. http://dx.doi.org/10.1093/jac/dkq120.
8. **Cui Z, Li Y, Cheng S, Yang H, Lu J, Hu Z, Ge B.** 2014. Mutations in the *embC-embA* intergenic region contribute to Mycobacterium tuberculosis resistance to ethambutol. Antimicrob Agents Chemother **58:**6837–6843. http://dx.doi.org/10.1128/AAC.03285-14.
9. **Safi H, Lingaraju S, Amin A, Kim S, Jones M, Holmes M, McNeil M, Peterson SN, Chatterjee D, Fleischmann R, Alland D.** 2013. Evolution of high-level ethambutol-resistant tuberculosis through interacting mutations in decaprenylphosphoryl-[beta]-D-arabinose biosynthetic and utilization pathway genes. Nat Genet **45:**1190–1197. http://dx.doi.org/10.1038/ng.2743.
10. **He L, Wang X, Cui P, Jin J, Chen J, Zhang W, Zhang Y.** 2015. *ubiA* (*Rv3806c*) encoding DPPR synthase involved in cell wall synthesis is associated with ethambutol resistance in Mycobacterium tuberculosis. Tuberculosis **95:**149–154. http://dx.doi.org/10.1016/j.tube.2014.12.002.
11. **Xu Y, Jia H, Huang H, Sun Z, Zhang Z.** 2015. Mutations found in *embCAB*, *embR*, and *ubiA* genes of ethambutol-sensitive and -resistant Mycobacterium tuberculosis clinical isolates from China. Biomed Res Int **2015:**951706. http://dx.doi.org/10.1155/2015/951706.
12. **Vincent V, Rigouts L, Nduwamahoro E, Holmes B, Cunningham J, Guillerm M, Nathanson CM, Moussy F, De Jong B, Portaels F, Ramsay A.** 2012. The TDR Tuberculosis Strain Bank: a resource for basic science, tool development and diagnostic services. Int J Tuberc Lung Dis **16:**24–31. http://dx.doi.org/10.5588/ijtld.11.0223.
13. **Umubyeyi AN, Vandebriel G, Gasana M, Basinga P, Zawadi JP, Gatabazi J, Pauwels P, Nzabintwali F, Nyiramasarabwe L, Fissette K, Rigouts L, Struelens MJ, Portaels F.** 2007. Results of a national survey on drug resistance among pulmonary tuberculosis patients in Rwanda. Int J Tuberc Lung Dis **11:**189–194.
14. **Chakravorty S, Lee JS, Cho EJ, Roh SS, Smith LE, Lee J, Kim CT, Via LE, Cho S-N, Barry CE, Alland D.** 2015. Genotypic susceptibility testing of Mycobacterium tuberculosis isolates for amikacin and kanamycin resistance by use of a rapid sloppy molecular beacon-based assay identifies more cases of low-level drug resistance than phenotypic Lowenstein-Jensen testing. J Clin Microbiol **53:**43–51. http://dx.doi.org/10.1128/JCM.02059-14.
15. **van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM.** 1993. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol **31:**406–409.
16. **Safi H, Barnes PF, Lakey DL, Shams H, Samten B, Vankayalapati R, Howard ST.** 2004. IS*6110* functions as a mobile, monocyte-activated promoter in Mycobacterium tuberculosis. Mol Microbiol **52:**999–1012. http://dx.doi.org/10.1111/j.1365-2958.2004.04037.x.
17. **NCCLS.** 2003. Susceptibility testing of Mycobacteria, Nocardiae, and other aerobic Actinomycetes; approved standard. NCCLS, Wayne, PA.
18. **Galagan JE.** 2014. Genomic insights into tuberculosis. Nat Rev Genet **15:**307–320. http://dx.doi.org/10.1038/nrg3664.
19. **Huang H, Berg S, Spencer JS, Vereecke D, D'Haeze W, Holsters M, McNeil MR.** 2008. Identification of amino acids and domains required for catalytic activity of DPPR synthase, a cell wall biosynthetic enzyme of Mycobacterium tuberculosis. Microbiology **154:**736–743. http://dx.doi.org/10.1099/mic.0.2007/013532-0.
20. **Köser CU, Feuerriegel S, Summers DK, Archer JAC, Niemann S.** 2012. Importance of the genetic diversity within the Mycobacterium tuberculosis complex for the development of novel antibiotics and diagnostic tests of drug resistance. Antimicrob Agents Chemother **56:**6080–6087. http://dx.doi.org/10.1128/AAC.01641-12.
21. **Gutacker MM, Mathema B, Soini H, Shashkina E, Kreiswirth BN, Graviss EA, Musser JM.** 2006. Single-nucleotide polymorphism-based population genetic analysis of Mycobacterium tuberculosis strains from 4 geographic sites. J Infect Dis **193:**121–128. http://dx.doi.org/10.1086/498574.
22. **Shamputa IC, Lee J, Allix-Béguec C, Cho E-J, Lee J-I, Rajan V, Lee EG, Min JH, Carroll MW, Goldfeder LC, Kim JH, Kang HS, Hwang S, Eum S-Y, Park SK, Lee H, Supply P, Cho S-N, Via LE, Barry CE.** 2010. Genetic diversity of Mycobacterium tuberculosis isolates from a tertiary care tuberculosis hospital in South Korea. J Clin Microbiol **48:**387–394. http://dx.doi.org/10.1128/JCM.02167-09.
23. **Filliol I, Motiwala AS, Cavatore M, Qi W, Hazbón MH, Bobadilla del Valle M, Fyfe J, García-García L, Rastogi N, Sola C, Zozio T, Guerrero MI, León CI, Crabtree J, Angiuoli S, Eisenach KD, Durmaz R, Joloba ML, Rendón A, Sifuentes-Osornio J, Ponce de León A, Cave MD, Fleischmann R, Whittam TS, Alland D.** 2006. Global phylogeny of Mycobacterium tuberculosis based on single nucleotide polymorphism (SNP) analysis: insights into tuberculosis evolution, phylogenetic accuracy of other DNA fingerprinting systems, and recommendations for a minimal standard SNP set. J Bacteriol **188:**759–772. http://dx.doi.org/10.1128/JB.188.2.759-772.2006.
24. **Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, Nicol M, Niemann S, Kremer K, Gutierrez MC, Hilty M, Hopewell PC, Small PM.** 2006. Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A **103:**2869–2873. http://dx.doi.org/10.1073/pnas.0511240103.
25. **Gagneux S, Small PM.** 2007. Global phylogeography of Mycobacterium tuberculosis and implications for tuberculosis product development. Lancet Infect Dis **7:**328–337. http://dx.doi.org/10.1016/S1473-3099(07)70108-1.
26. **Gafirita J, Umubyeyi A, Asiimwe B.** 2012. A first insight into the genotypic diversity of Mycobacterium tuberculosis from Rwanda. BMC Clin Pathol **12:**20. http://dx.doi.org/10.1186/1472-6890-12-20.
27. **WHO.** 2008. Guidelines for the programmatic management of drug-resistant tuberculosis. Emergency update. WHO, Geneva, Switzerland.