genomeA
Journals.ASM.org

Genome Analysis of 17 Extensively Drug-Resistant Strains Reveals New Potential Mutations for Resistance

H. Guio,[a,c] D. Tarazona,[a] M. Galarza,[a] V. Borda,[a] R. Curitomay[b]

Laboratorio de Biotecnología y Biología Molecular, Instituto Nacional de Salud (INS), Lima, Peru[a]; Departamento de Neumología, Hospital Nacional Hipólito Unanue, Lima, Peru[b]; Asociación Latinoamericana de Biotecnología (NGO-ALBIOTEC), Lima, Peru[c]

We report the whole-genome sequence of an extensively drug-resistant (XDR) tuberculosis (TB) strain of Latin American–Mediterranean (LAM) lineage. This strain is phenotypically resistant to aminoglycosides, but carries no related mutations in *rrs*, *tlyA*, and *eis*. Through genome analysis comparison with 16 XDR strains, we found 218 non-synonymous single nucleotide polymorphisms (SNPs) shared that could confer resistance.

Received 7 July 2014 **Accepted** 16 July 2014 **Published** 31 July 2014

Citation Guio H, Tarazona D, Galarza M, Borda V, Curitomay R. 2014. Genome analysis of 17 extensively drug-resistant strains reveals new potential mutations for resistance. Genome Announc. 2(4):e00759-14. doi:10.1128/genomeA.00759-14.

Copyright © 2014 Guio et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to H. Guio, hguio@ins.gob.pe.

For Peru, the incidence rate for tuberculosis (TB) in 2012 was 95/100,000 population, from which 0.3% were new cases of extensively drug-resistant (XDR) TB. 94% of these cases were from Lima (1). XDR-TB strains are resistant to isoniazid (INH), rifampicin (RIF), fluoroquinolones (FLQ), and at least one of the three injectable anti-TB drugs: amikacin (AMK), capreomycin (CAP), or kanamycin (KAN) (2). In 1999, Peru reported one case with this resistant pattern. However, the concept of XDR-TB appears in 2006 with the first outbreak in KwaZulu-Natal (KZN), South Africa (3).

In our quest to identify new possible mutations that can confer resistance in XDR-TB strains, we analyzed the XDR strain, INS-XDR, isolated from a Peruvian patient with active tuberculosis. We determined the strain's lineage to be Latin American–Mediterranean (LAM) using 24 mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) loci (4) and SpolPred (5). The genomic DNA was sequenced on an Illumina HiSeq 2000 with 1,140× coverage. Sequence reads for the isolates were mapped against the corrected reference genome *M. tuberculosis* strain H37Rv The average coverage was 82 for INS-XDR (49,793,402 mapped reads) and assembly with BWA v0.5.9-r16 (6), using H37Rv genome (AL123456.3) (7) as a reference. For annotation process, we used Rapid Annotations using Subsystem Technology (8) and the Prokaryotic Genome Annotation Pipeline. The sequencing data generated has 49,793,402 paired-ends reads. The assembling process resulted in 20 contigs that represents 99.98% of the H37Rv genome. The INS-XDR has a total of 4,391,020 pb with an average G+C content of 65.4%. We used Sanger sequencing to confirm specific mutations. Phenotypically, this strain was resistant to RIF, INH, FLQ, KAN, and CAP. However, sequence analysis of *rrs* and the promoter *eis* do not show mutations for aminoglycosides resistance (9).

To analyze possible mutations causing resistance to CAP and KAN, a polymorphism study was carried out by comparing INS-XDR against KZN605 (a XDR-TB strain of LAM linage) and 16 reported TB-XDR genomes from China, FJ05194, GuangZ0019 (10), XDR1219, XDR1221 (11), GuangZ0008, 293, FJ07070, GuangZ0017, GuangZ0026 (12); Peru, Peru_04_R0292, Peru_03_R0268 (13); Russia, CTRI-4 (14), Russia_03-R1082 (13); Malaysia, UM1072388579 (15), and South Africa, KZN_R506 (16) and KZN_605. Single nucleotide polymorphisms (SNPs) were identified using SNPsFinder (17) and SNP tree (18), and were classified in the Clusters of Orthologous Groups (COG) database (19). These analyses showed evidence of 218 non-synonymous SNPs mainly in four COGs: (1) replication, recombination and repair, L, $n=14$, (2) energy production and conversion, C, $n=14$, (3) amino acid transport and metabolism, E, $n=12$, and (4) secondary metabolites biosynthesis, transport, and catabolism, Q, $n=11$. These 218 non-synonymous SNPs belong to 125 genes. Given that CAP and KAN act by inhibiting protein synthesis and causing production of abnormal proteins, we hypothesize that the 14 non-synonymous SNPs belonging to the C category would be the principal SNPs responsible for CAP and KAN resistance. This provides evidence that it may be worthwhile to explore these new SNPs for diagnosis of XDR-TB.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession JANH00000000. The version described in this paper is version JANH01000000.

ACKNOWLEDGMENTS

We thank Kelly Levano and Harrison Montejo.

This work was supported and approved by the research and ethical committee from the Peruvian National Institute of Health.

REFERENCES

1. **ESNPyCTB.** 2012. Informe de la Estrategia Sanitaria Nacional de Prevención y Control de la Tuberculosis sobre Situación y Control de TB-MDR y TB-XDR en Perú. Estrategia Sanitaria Nacional de Prevención y Control de la Tuberculosis.
2. **Jassal M, Bishai WR.** 2009. Extensively drug-resistant tuberculosis. Lancet Infect. Dis. **9**:19–30. http://dx.doi.org/10.1016/S1473-3099(08)70260-3.

3. **Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G.** 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet **368:**1575–1580. http://dx.doi.org/10.1016/S0140-6736(06)69573-1.
4. **Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, Savine E, de Haas P, van Deutekom H, Roring S, Bifani P, Kurepina N, Kreiswirth B, Sola C, Rastogi N, Vatin V, Gutierrez MC, Fauville M, Niemann S, Skuce R, Kremer K, Locht C, van Soolingen D.** 2006. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **44:**4498–4510. http://dx.doi.org/10.1128/JCM.01392-06.
5. **Coll F, Mallard K, Preston MD, Bentley S, Parkhill J, McNerney R, Martin N, Clark TG.** 2012. SpolPred: Rapid and accurate prediction of *Mycobacterium tuberculosis* spoligotypes from short genomic sequences. Bioinformatics **28:**2991–2993. http://dx.doi.org/10.1093/bioinformatics/bts544.
6. **Li H, Durbin R.** 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **25:**1754–1760. http://dx.doi.org/10.1093/bioinformatics/btp324.
7. **Comas I, Coscolla M, Luo T, Borrell S, Holt KE, Kato-Maeda M, Parkhill J, Malla B, Berg S, Thwaites G, Yeboah-Manu D, Bothamley G, Mei J, Wei L, Bentley S, Harris SR, Niemann S, Diel R, Aseffa A, Gao Q, Young D, Gagneux S.** 2013. Out-of-Africa migration and *Neolithic coexpansion* of *Mycobacterium tuberculosis* with modern humans. Nat. Genet. **45:**1176–1182. http://dx.doi.org/10.1038/ng.2744.
8. **Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R.** 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. **42:**D206–D214. http://dx.doi.org/10.1093/nar/gkt1226.
9. **Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB.** 2009. Tuberculosis drug resistance mutation database. PLOS Med. **6:**e2. http://dx.doi.org/10.1371/journal.pmed.1000002.
10. **Lin N, Liu Z, Zhou J, Wang S, Fleming J.** 22 August 2013. Draft genome sequences of two super-XDR isolates of *Mycobacterium tuberculosis* from China. FEMS Microbiol. Lett. http://dx.doi.org/10.1111/1574-6968.12238.
11. **Wu W, Zheng H, Zhang L, Wen Z, Zhang S, Pei H, Yu G, Zhu Y, Cui Z, Hu Z, Wang H, Li Y.** 2013. *A genome-wide* analysis of multidrug-resistant and extensively drug-resistant strains of *Mycobacterium tuberculosis* Beijing genotype. Mol. Genet. Genomics **288:**425–436. http://dx.doi.org/10.1007/s00438-013-0758-4.
12. **Zhang H, Li D, Zhao L, Fleming J, Lin N, Wang T, Liu Z, Li C, Galwey N, Deng J, Zhou Y, Zhu Y, Gao Y, Wang T, Wang S, Huang Y, Wang M, Zhong Q, Zhou L, Chen T, Zhou J, Yang R, Zhu G, Hang H, Zhang J, Li F, Wan K, Wang J, Zhang X-E, Bi L.** 2013. Genome sequencing of 161 *Mycobacterium tuberculosis* isolates from China identifies genes and intergenic regions associated with drug resistance. Nat. Genet. **45:**1255–1260. http://dx.doi.org/10.1038/ng.2735.
13. **Farhat MR, Shapiro BJ, Kieser KJ, Sultana R, Jacobson KR, Victor TC, Warren RM, Streicher EM, Calver A, Sloutsky A, Kaur D, Posey JE, Plikaytis B, Oggioni MR, Gardy JL, Johnston JC, Rodrigues M, Tang PK, Kato-Maeda M, Borowsky ML, Muddukrishna B, Kreiswirth BN, Kurepina N, Galagan J, Gagneux S, Birren B, Rubin EJ, Lander ES, Sabeti PC, Murray M.** 2013. Genomic analysis identifies targets of convergent positive selection in drug-resistant *Mycobacterium tuberculosis*. Nat. Genet. **45:**1183–1189. http://dx.doi.org/10.1038/ng.2747.
14. **Ilina EN, Shitikov EA, Ikryannikova LN, Alekseev DG, Kamashev DE, Malakhova MV, Parfenova TV, Afanas'ev MV, Ischenko DS, Bazaleev NA, Smirnova TG, Larionova EE, Chernousova LN, Beletsky AV, Mardanov AV, Ravin NV, Skryabin KG, Govorun VM.** 2013. Comparative genomic analysis of *Mycobacterium tuberculosis* drug resistant strains from Russia. PLoS One **8:**e56577. http://dx.doi.org/10.1371/ journal-.pone.0056577.
15. **Ng KP, Yew SM, Chan CL, Chong J, Tang SN, Soo-Hoo TS, Na SL, Hassan H, Ngeow YF, Hoh CC, Lee KW, Yee WY.** 2013. Draft Genome Sequence of the first Isolate of Extensively drug-Resistant (XDR) *Mycobacterium tuberculosis* in Malaysia. Genome Announc. **1**(1):e00056-12. http://dx.doi.org/10.1128/genomeA.00056-12.
16. **Ioerger TR, Koo S, No E-G, Chen X, Larsen MH, Jacobs WR, Jr, Pillay M, Sturm AW, Sacchettini JC.** 2009. Genome analysis of multi- and extensively-drug-resistant tuberculosis from KwaZulu-Natal, South Africa. PLoS One **4:**e7778. http://dx.doi.org/10.1371/journal.pone.0007778.
17. **Song J, Xu Y, White S, Miller KW, Wolinsky M.** 2005. SNPsFinder—a web-based application for genome-wide discovery of single nucleotide polymorphisms in microbial genomes. Bioinformatics **21:**2083–2084. http://dx.doi.org/10.1093/bioinformatics/bti176.
18. **Leekitcharoenphon P, Kaas RS, Thomsen MC, Friis C, Rasmussen S, Aarestrup FM.** 2012. snpTree—a web-server to identify and construct SNP trees from whole genome sequence data. BMC Genomics **13**(Suppl 7):S6. http://dx.doi.org/10.1186/1471-2164-13-S7-S6.
19. **Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, Krylov DM, Mazumder R, Mekhedov SL, Nikolskaya AN, Rao BS, Smirnov S, Sverdlov AV, Vasudevan S, Wolf YI, Yin JJ, Natale DA.** 2003. The COG database: an updated version includes eukaryotes. BMC Bioinformatics **4:**41. http://dx.doi.org/10.1186/1471-2105-4-41.