About the Author

Dr. Getahun works at the national reference laboratory for Ethiopia. Her main areas of work include conducting research on priority public health problems, providing technical assistance on TB research, and providing supportive supervision for surveillance and program evaluation.

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Address for correspondence: Muluwork Getahun, Ethiopian Public Health Institute, National TB Reference Laboratory, Addis Ababa, 1242, Ethiopia; email: mimishaget@yahoo.com

Metagenomics of Imported Multidrug-Resistant Mycobacterium leprae, Saudi Arabia, 2017

Qingtian Guan, Talal S. Almutairi, Tahani Alhalouli, Arnab Pain,1 Faisal Alasmari

Author affiliations: King Abdullah University of Science and Technology, Thuwal-Jeddah, Saudi Arabia (Q. Guan, A. Pain); King Saud University Medical City, Riyadh, Saudi Arabia (T.S. Almutairi); King Fahad Medical City, Riyadh (T.S. Almutairi, T. Alhalouli, F. Alasmari)

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Using shotgun metagenomics, we identified an imported case of multidrug-resistant Mycobacterium leprae in a Filipino resident of Saudi Arabia in 2017. We determined the phylogenomic lineage (3K1) and identified mutations in rpoB and rrs corresponding to the multidrug-resistance phenotype clinically observed. Metagenomics sequencing can be used to identify multidrug-resistant M. leprae.

Leprosy is a chronic dermatologic and neurologic disease caused by the infectious agent Mycobacterium leprae and can lead to severe disabilities; >200,000 new cases are reported annually worldwide, according to the World Health Organization. A total of 242 leprosy cases were reported in Saudi Arabia during 2003–2012; however, little is known about the subtypes and prevalence of drug resistance among these M. leprae cases (1).

In May 2017, a 30-year-old woman from the Philippines sought treatment at the dermatology clinic of King Fahad Medical City (KFMC) Hospital in Riyadh, Saudi Arabia, for painful systemic skin nodules and joint pain without joint swelling. She had no medical history of leprosy. The initial clinical diagnosis of this patient was inconclusive, but her initial signs and symptoms were suggestive of a connective tissue disease such as systemic lupus erythematosus, and initial clinical improvement was recorded after a short course of empiric steroids and hydroxychloroquine treatment. Other suspected diagnoses included lepromatous leprosy with type 2 erythema nodosum leprosum reaction or other nontuberculosis mycobacterial infection.

We performed a punch skin biopsy of the extensor surface of the forearm and performed Ziehl-Neelsen staining; we observed a florid histiocytic

1These senior authors contributed equally to this article.
proliferation containing numerous *Mycobacterium* bacilli without an obvious granuloma (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/26/3/19-0661-App1.pdf). We referred the patient to the infectious disease clinic, which performed QuantiFERON-TB Gold (QIAGEN, https://www.quantiferon.com) and took a biopsy for bacterial and fungal culture, and all test results were negative. Mycobacterial culture showed >9 acid-fast bacilli/high-power field on smears, and no growth was observed on Lowenstein-Jensen slants after 8 weeks of incubation.

Her treatment started with a daily regimen of clofazimine (50 mg), dapsone (100 mg), and rifampin (600 mg). Treatment with moxifloxacin (400 mg/d) and macrolides was briefly added (clarithromycin and azithromycin were both stopped because of gastrointestinal side effects) in case of possible nontuberculosis mycobacterial infection. The patient had multiple relapses during 12 months of follow-up and became steroid dependent (i.e., her skin lesions reappeared shortly after steroid treatment ended).

Because initial test reports were inconclusive and the etiologic agent was unconfirmed, we attempted to confirm the etiology by subjecting the patient’s skin biopsy sample to metagenomic sequencing; a DNA sequencing protocol without target DNA-enrichment steps (2) was needed to unambiguously identify the etiologic agent. From the metagenomics datasets, we reconstructed the near-complete genome of the *M. leprae* species (which we named KFMC-1) at 99.2% completeness when compared with *M. leprae* TN, a strain commonly used for reference (3). We assembled the 3.24-Mb genome of *M. leprae* KFMC-1 in 19 DNA segments, and average coverage was 20.02× (Appendix Table 1, Figure 2, panel A). A single-nucleotide polymorphism comparison of *M. leprae* KFMC-1 with a globally representative set of *M. leprae* revealed KFMC-1 was most closely related to 3K1 Ryukyu-2 (Appendix Figure 2, panel B), which was originally isolated in Japan (4).

We identified 158 polymorphic sites in the genome (Appendix Table 2), which corresponded to 136 single-nucleotide polymorphisms and 22 insertion/deletions. In total, 53 of the 158 changes were new, and 63 appeared within gene-coding regions, a couple of which helped us predict the multidrug-resistance profile. We identified a G→T nucleotide change, which leads to a nonsynonymous change (Q438H) in the *rpoB* gene (Appendix Figure 2, panel C). This substitution results in rifampin resistance (5), matching our clinical records. The C1414A mutation in the *rrs* locus is predicted to confer capreomycin resistance, as observed previously in *M. tuberculosis* (6).

After we confirmed the clinical diagnosis as an *M. leprae* infection, we halted moxifloxacin treatment and kept the patient on 3 standard antimicrobial drugs (clofazimine, dapsone, and rifampin). Afterward, the patient left Saudi Arabia and continued her antimicrobial drug course in her country of origin.

The predominant genotypes of *M. leprae* strains in the Middle East are subtypes 2 and 3 (7). Most 3K cases are found in countries of East Asia, such as China (8), Japan (9), Korea (2), and the Philippines (8). In addition, >37% of the lespro cases in Saudi Arabia occur in persons from other countries (1). Our results suggest that this case of leprosy was imported from the patient’s country of origin. Saudi Arabia hosts a massive number of expatriates from all over the world, including persons from *M. leprae*-endemic countries, and also hosts one of the largest recurring religious gatherings in the world. Therefore, genomics-guided infection control efforts are needed to monitor the potential importation and prevent the spread of *M. leprae* infections in the region.

**About the Author**

Mr. Guan is a graduate student in the Pathogen Genomics Laboratory of King Abdullah University of Science and Technology. His research focuses on the applications of bioinformatics tools in understanding of pathogens and infectious diseases.

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6. Suzuki Y, Katsukawa C, Tamaru A, Abe C, Makino M, Mizuguchi Y, et al. Detection of kanamycin-resistant
Three New Cases of Melioidosis, Guadeloupe, French West Indies

Bénédicte Melot, Sylvaine Bastian, Nathalie Doumon, Eric Valade, Olivier Gorgé, Anne Le Fleche, Charlotte Idier, Mireille Vernier, Elisabeth Fernandes, Bruno Hoen, Sébastien Breurec, Michel Carles

Author affiliations: University Hospital of Guadeloupe, Pointe-à-Pitre, France (B. Melot, S. Bastian, N. Doumon, C. Idier, B. Hoen, S. Breurec, M. Carles); Ecole du Val-de-Grâce, Paris, France (E. Valade); Aix Marseille University, Marseille, France (E. Valade, O. Gorgé); Institut Pasteur, Paris (A. Le Fleche); Hospital of Basse-Terre, Basse-Terre, France (M. Vernier, E. Fernandes); Pasteur Institute of Guadeloupe, Pointe-à-Pitre (S. Breurec); University of the French West Indies and French Guiana, Pointe-à-Pitre (B. Hoen, S. Breurec, M. Carles)

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Melioidosis has been detected in the Caribbean, and an increasing number of cases has been reported in the past few decades, but only 2 cases were reported in Guadeloupe during the past 20 years. We describe 3 more cases that occurred during 2016–2017 and examine arguments for increasing endemicity.

Address for correspondence: Arnab Pain, King Abdullah University of Science and Technology, BESE Division, Thuwal-Jeddah, 239556900, Saudi Arabia; email: arnab.pain@kaust.edu.sa
Metagenomics of Imported Multidrug-Resistant *Mycobacterium leprae*, Saudi Arabia, 2017

Appendix

Materials and Methods

The research protocol was approved by the Institutional Review Board of King Fahad Medical City (Riyadh, Saudi Arabia; #16–345) and Institutional Biosafety and Bioethics Committee of King Abdullah University of Science and Technology (Jeddah, Saudi Arabia; #18IBEC23).

The DNA from the patient sample was extracted using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, Freiburg, Germany). NuGEN Ovation Ultralow Library System V2 (NuGen, Manchester, UK) was used for library preparation. A total of 3.51 billion 150 bp pair-end Illumina reads were obtained from Illumina Hiseq4000 instrument. We performed the reference-guided assembly using *M. leprae* TN strain as the reference. Single-nucleotide polymorphisms (SNPs) were called for two iterations and filtered with Genome Analysis Toolkit (1) (GATK) which had a read depth of 3 or higher; an alignment quality score (MQ) ≥40; SNPs
subtyping of *M. leprae* KFMC-1 was done based on SNP subtypes (A–P) defined by surveying the informative SNPs described by Marc Monot et al. in 2009 (2). Phylogenies were generated by aligning all SNPs from representative genomes from various lineages of leprosy genomes (3) using maximum likelihood.

The SNP in the *rpoB* gene was examined by nested PCR of 10 ng of genomic DNA with the method described (4) and the C1414A variant in *rrs* was verified with semi-nested PCR with 98°C for 2 min followed by 35 cycles of denaturation (98°C for 10 sec), primer annealing (62°C, 30 sec for first-round PCR, forward 5′-CGCGTTGTTCGTAAATCT-3, reverse 5′-ATGCTCGCAACCACCTATCCA-3; or 60°C 30 sec for second-round PCR, 1492R GGCTACCTTGTTACGACTT) and extension (72°C, 30 sec), and final extension at 72°C for 2 min. A PCR premix (Q5 Hot Start High-Fidelity 2X Master Mix, New England Biolabs).

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\begin{table}
\centering
\begin{tabular}{lcc}
\hline
Category & \textit{M. leprae} KFMC & \textit{M. leprae} TN \\
\hline
Sequence size & 3243015 & 3268203 \\
Number of contigs & 19 & 1 \\
GC content (%) & 57.8 & 57.8 \\
ccontigs (\geq 50000 bp) & 14 & 1 \\
Median sequence size & 94825 & 3268203 \\
Mean sequence size & 141002.1 & 3268203 \\
Longest contig size & 497079 & 3268203 \\
N50 value & 318796 & NA \\
L50 value & 5 & 1 \\
\hline
\end{tabular}
\caption{Comparison of \textit{M. leprae} KFMC-1 and \textit{M. leprae} TN assemblies*}
\end{table}

*KFMC, King Fahad Medical City; NA, not applicable.
| POS | Type | REF (TN) | ALT (KFMC) | Novelty | Annotation | ML code |
|-----|------|----------|------------|---------|------------|---------|
| 73  | SNP  | T        | G          | No      | missense_variant | ML0001 |
| 12484 | Indel | A         | AAACCACAGCTAGAC | No | intergenic_region | MLP000002-ML0008c |
| 14226 | Indel | C         | CATAT      | Yes     | intergenic_region | ML0009-ML0010c |
| 15439 | SNP  | G         | A          | No      | pseudogene | ML0010c |
| 17425 | SNP  | G         | A          | Yes     | intergenic_region | ML0013c-ML0014 |
| 26545 | SNP  | G         | A          | No      | synonymous_variant | ML0020c |
| 40852 | SNP  | G         | T          | Yes     | pseudogene | ML0034 |
| 52851 | SNP  | T         | C          | No      | missense_variant | ML0042 |
| 57633 | SNP  | T         | G          | No      | pseudogene | ML0046c |
| 61425 | SNP  | A         | G          | No      | missense_variant | ML0049c |
| 62545 | SNP  | G         | T          | No      | missense_variant | ML0051c |
| 73073 | Indel | CGATCAA, GCCAGGA, ATCAAGT, TGATCAA, GCCAGGA, ATCAAGTT | C | Yes | pseudogene | ML0058c |
| 77864 | SNP  | T         | C          | No      | synonymous_variant | ML0061 |
| 86658 | SNP  | G         | A          | Yes     | intergenic_region | ML0064c-ML0065 |
| 100574 | SNP  | A         | G          | No      | pseudogene | ML0080c |
| 132150 | SNP  | G         | A          | Yes     | synonymous_variant | ML0102 |
| 157960 | SNP  | C         | T          | No      | intergenic_region | ML0116c-ML0117 |
| 160627 | SNP  | G         | T          | Yes     | synonymous_variant | ML0119c |
| 175636 | SNP  | C         | T          | Yes     | missense_variant | ML0131 |
| 286105 | SNP  | C         | T          | Yes     | synonymous_variant | ML0214 |
| 313361 | SNP  | A         | G          | No      | missense_variant | ML0238c |
| 328634 | SNP  | G         | C          | No      | missense_variant | ML0252 |
| 330125 | SNP  | G         | A          | No      | missense_variant | ML0252 |
| POS  | Type  | REF (TN) | ALT (KFMC) | Novelty | Annotation            | ML code       |
|------|-------|----------|------------|---------|-----------------------|---------------|
| 365435 | SNP   | G        | A          | No      | missense_variant      | ML0283        |
| 383599 | SNP   | C        | G          | No      | pseudogene            | ML0301c       |
| 441420 | Indel | CAT      | C          | Yes     | intergenic_region     | ML0349c-ML0350c |
| 459887 | SNP   | C        | T          | No      | pseudogene            | ML0368        |
| 481476 | SNP   | A        | G          | No      | synonymous_variant    | ML0387        |
| 494674 | SNP   | T        | G          | No      | missense_variant      | ML0397c       |
| 504437 | SNP   | G        | C          | Yes     | intergenic_region     | ML0405-ML0406 |
| 508481 | SNP   | T        | C          | No      | missense_variant      | ML0410        |
| 509325 | SNP   | C        | G          | No      | missense_variant      | ML0411        |
| 517971 | SNP   | C        | T          | Yes     | intergenic_region     | ML0841-ML0842 |
| 528451 | SNP   | C        | T          | No      | pseudogene            | ML0428        |
| 533403 | SNP   | A        | G          | No      | pseudogene            | ML0433        |
| 561823 | Indel | G        | GGT        | Yes     | pseudogene            | ML0463c       |
| 686240 | SNP   | A        | C          | No      | pseudogene            | ML0567        |
| 694090 | SNP   | T        | C          | No      | missense_variant      | ML0569c       |
| 711197 | SNP   | T        | C          | No      | pseudogene            | ML0585c       |
| 714396 | SNP   | C        | T          | No      | synonymous_variant    | ML0589c       |
| 736703 | SNP   | G        | T          | Yes     | missense_variant      | ML0605        |
| 790218 | SNP   | C        | T          | Yes     | pseudogene            | ML0652        |
| 831215 | SNP   | G        | T          | No      | pseudogene            | ML0693        |
| 832152 | SNP   | T        | C          | No      | pseudogene            | ML0694c       |
| 890453 | SNP   | A        | G          | No      | missense_variant      | ML0747c       |
| 904824 | SNP   | G        | C          | No      | synonymous_variant    | ML0767c       |
| 938372 | SNP   | C        | T          | No      | pseudogene            | ML0794c       |
| 944191 | Indel | C        | CA         | Yes     | pseudogene            | ML0797c       |
| 958228 | Indel | A        | AC         | Yes     | pseudogene            | ML0809        |
| 972005 | SNP   | T        | G          | No      | pseudogene            | ML0821        |
| 1000186 | SNP  | C        | T          | Yes     | intergenic_region     | ML0841-ML0842 |
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| 1076949 | SNP | G    | C     | No       | missense_variant    | ML0909     |
| 1087397 | SNP | T    | C     | No       | synonymous_variant  | ML0917     |
| 1104232 | SNP | C    | G     | No       | pseudogene          | ML0934     |
| 1133492 | SNP | T    | G     | No       | intergenic_region   | ML0964-ML0965c |
| 1133721 | Indel | C   | CG    | Yes      | intergenic_region   | ML0964-ML0965C |
| 1143423 | SNP | T    | C     | No       | pseudogene          | ML0975c    |
| 1144840 | SNP | T    | G     | Yes      | synonymous_variant  | ML0977     |
| 1155582 | SNP | T    | G     | No       | synonymous_variant  | ML0988     |
| 1227051 | SNP | G    | T     | No       | intergenic_region   | ML1061-ML1062 |
| 1257185 | SNP | T    | C     | No       | intergenic_region   | ML1092c-ML1093 |
| 1265267 | SNP | T    | G     | No       | pseudogene          | ML1097     |
| 1295192 | SNP | A    | G     | No       | missense_variant    | ML1119     |
| 1324009 | SNP | C    | G     | No       | missense_variant    | ML1132     |
| 1339813 | SNP | T    | C     | No       | synonymous_variant  | ML1150     |
| 1342557 | SNP | C    | A     | Yes      | non_coding_transcript_exon_variant | MLP000016 |
| 1348426 | SNP | T    | C     | No       | intergenic_region   | ML1152c-ML1153 |
| 1351149 | SNP | C    | G     | No       | intergenic_region   | ML1154c-ML1155 |
| 1529088 | SNP | A    | G     | No       | pseudogene          | ML1284c    |
| 1532258 | SNP | G    | A     | No       | missense_variant    | ML1286     |
| 1533315 | Indel | C | CG    | Yes      | pseudogene          | ML1287     |
| 1587912 | SNP | G    | T     | Yes      | missense_variant    | ML1334     |
| 1605956 | SNP | G    | A     | No       | intergenic_region   | ML1345-ML1346 |
| 1607562 | SNP | A    | G     | Yes      | missense_variant    | ML1346     |
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| 1643162 | SNP | T    | C     | No       | pseudogene          | ML1378     |
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| 1700105 | SNP  | T        | C         | Yes       | missense_variant     | ML1417           |
| 1701590 | SNP  | G        | C         | No        | intergenic_region    | ML1418c-ML1419c  |
| 1725797 | SNP  | C        | G         | No        | pseudogene           | ML1436c          |
| 1741830 | Indel| G        | GCAACAACGTC | Yes       | pseudogene           | ML1449c          |
| 1813428 | Indel| A        | AGT       | No        | pseudogene           | ML1502c          |
| 1839270 | SNP  | A        | G         | No        | pseudogene           | ML1524c          |
| 1841279 | Indel| C        | CG        | Yes       | pseudogene           | ML1527c          |
| 1843283 | SNP  | C        | A         | No        | pseudogene           | ML1528           |
| 1868993 | SNP  | G        | T         | No        | pseudogene           | ML1545           |
| 1876289 | SNP  | G        | A         | Yes       | pseudogene           | ML1552           |
| 1926696 | SNP  | T        | C         | No        | intergenic_region    | ML1600c-ML1601c  |
| 1955004 | SNP  | C        | A         | Yes       | synonymous_variant   | ML1629           |
| 1971193 | SNP  | G        | A         | No        | pseudogene           | ML1636           |
| 2011747 | SNP  | T        | G         | No        | pseudogene           | ML1668c          |
| 2011783 | SNP  | G        | A         | Yes       | pseudogene           | ML1668c          |
| 2030803 | SNP  | G        | A         | No        | missense_variant     | ML1685c          |
| 2040883 | SNP  | G        | A         | No        | pseudogene           | ML1693           |
| 2043287 | SNP  | A        | G         | No        | synonymous_variant   | ML1694c          |
| 2066936 | SNP  | G        | T         | Yes       | missense_variant     | ML1713           |
| 2100523 | SNP  | C        | G         | No        | missense_variant     | ML1740c          |
| 2104127 | SNP  | T        | C         | No        | intergenic_region    | ML1743c-ML1744c  |
| 2142011 | Indel| CT       | C         | No        | pseudogene           | ML1767c          |
| 2148809 | SNP  | G        | A         | No        | pseudogene           | ML1773c          |
| 2155013 | SNP  | T        | G         | No        | pseudogene           | ML1778c          |
| 2174865 | SNP  | G        | C         | No        | intergenic_region    | ML1795-ML1796    |
| 2205779 | Indel| TA       | T         | No        | pseudogene           | ML1822           |
| 2211034 | Indel| TAC      | T         | Yes       | intergenic_region    | ML1825c-ML1826c  |
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| 2275492 | SNP    | C        | A          | Yes     | missense_variant     | ML1891c          |
| 2278551 | SNP    | C        | A          | No      | pseudogene           | ML1893c          |
| 2312059 | SNP    | C        | G          | No      | start_lost           | ML1926c          |
| 2317249 | SNP    | C        | T          | Yes     | pseudogene           | ML1933           |
| 2344787 | SNP    | G        | A          | No      | synonymous_variant   | ML1957c          |
| 2441339 | SNP    | C        | T          | No      | missense_variant     | ML2053c          |
| 2459766 | SNP    | A        | G          | No      | missense_variant     | ML2069           |
| 2468130 | SNP    | C        | A          | No      | missense_variant     | ML2075c          |
| 2514637 | SNP    | C        | T          | Yes     | intergenic_region    | ML2111c-MI2115c  |
| 2515916 | SNP    | G        | T          | Yes     | pseudogene           | ML2115c          |
| 2526543 | SNP    | G        | T          | Yes     | pseudogene           | ML2125c          |
| 2547925 | SNP    | C        | G          | Yes     | pseudogene           | ML2145           |
| 2553176 | SNP    | T        | G          | No      | pseudogene           | ML2149           |
| 2567248 | Indel  | AGTG     | A          | No      | intergenic           | ML2159c-ML2160   |
| 2631245 | SNP    | G        | A          | Yes     | synonymous_variant   | ML2213c          |
| 2651703 | SNP    | C        | A          | Yes     | intergenic_region    | ML2233-MI2234    |
| 2691666 | SNP    | G        | A          | Yes     | pseudogene           | ML2267           |
| 2706236 | SNP    | T        | G          | No      | pseudogene           | ML2281c          |
| 2711942 | SNP    | T        | G          | No      | pseudogene           | ML2287c          |
| 2747358 | SNP    | T        | C          | Yes     | missense_variant     | ML2320c          |
| 2751783 | SNP    | A        | G          | No      | synonymous_variant   | ML2322c          |
| 2757405 | Indel  | GC       | G          | No      | pseudogene           | ML2325c          |
| 2804726 | SNP    | C        | A          | No      | synonymous_variant   | ML2354c          |
| 2818521 | SNP    | T        | C          | No      | synonymous_variant   | ML2357c          |
| 2835913 | Indel  | CGTGT    | C          | Yes     | pseudogene           | ML2367           |
| 2844969 | Indel  | CAT      | C          | Yes     | intergenic_region    | ML2375c-ML2376c  |
| 2887094 | Indel  | C        | CATA       | Yes     | intergenic_region    | ML2415-MI2416c   |
| 2935685 | SNP    | A        | C          | No      | pseudogene           | ML2462c          |
| POS     | Type | REF (TN) | ALT (KFMC) | Novelty     | Annotation         | ML code          |
|---------|------|----------|------------|-------------|--------------------|------------------|
| 2964999 | SNP  | T        | C          | No          | synonymous_variant | ML2490c          |
| 2981212 | SNP  | G        | A          | No          | synonymous_variant | ML2501           |
| 3016175 | SNP  | T        | C          | No          | synonymous_variant | ML2534c          |
| 3057114 | SNP  | G        | C          | No          | intergenic_region  | ML2563-ML2564c   |
| 3063817 | SNP  | C        | A          | Yes         | missense_variant   | ML2568c          |
| 3076050 | SNP  | G        | C          | No          | intergenic_region  | ML2574c-ML2575c  |
| 3102778 | SNP  | A        | C          | No          | missense_variant   | ML2597           |
| 3132639 | SNP  | G        | A          | No          | synonymous_variant | ML2622c          |
| 3152586 | SNP  | C        | T          | No          | synonymous_variant | ML2634c          |
| 3175296 | SNP  | A        | C          | No          | intergenic_region  | ML2652-ML2653    |
| 3202695 | SNP  | A        | G          | No          | intergenic_region  | ML2670c-ML2671   |
| 3221210 | SNP  | G        | A          | Yes         | pseudogene         | ML2676c          |
| 3221615 | Indel| AATAT    | A          | Yes         | intergenic_region  | ML2676c-ML2677   |
| 3236317 | SNP  | G        | A          | No          | missense_variant   | ML2687c          |
| 3243731 | SNP  | A        | G          | No          | pseudogene         | ML2694           |
| 3254050 | SNP  | C        | T          | Yes         | synonymous_variant | ML2700           |
| 3256572 | SNP  | C        | T          | No          | synonymous_variant | ML2700           |
| 3257047 | Indel| GCCCA    | G          | Yes         | pseudogene         | ML2701           |
| 3268175 | SNP  | G        | T          | No          | intergenic_region  | ML2713c-ML0001   |

*ALT, alternative; KFMC, King Fahad Medical City; POS, position; REF, reference; SNP, single-nucleotide polymorphism.
Appendix Figure 1. A) Photographs of forearm skin lesions. B) Histopathology of the skin biopsy using Ziehl-Neelsen staining (100X). Arrows showing *M. leprae* bacilli.
Appendix Figure 2. A) *M. leprae* KFMC-1 genome comparison against the *M. leprae* TN as the reference genome. BLASTn matches above 90% nucleotide identity are colored in gray. The vertical bars represent the polymorphic sites in *M. leprae* KFMC-1 when compared to the *M. leprae* TN strain. The SNP positions in the genome are shown in red while the nucleotide insertions and deletions (Indels) shown in blue; The gray outer circle shows *M. leprae* KFMC-1 shared identity (according to BLASTn) with *M. leprae* TN genome as the reference. B) Phylogenetic lineages of *M. leprae* KFMC-1 with the representatives from *M. leprae* isolates based on SNP sites using a maximum likelihood approach using the Tamura-Nei model. Bootstrap percentages from 1,000 replicates are shown next to the branches. The scale indicates the
number of substitutions per site. C) Chromatograms of *M. leprae* KFMC-1 showing the Q438H mutation in *rpoB* and C1414A mutation in *rrs*. KFMC, King Fahad Medical City; SNP, single-nucleotide polymorphism.