Whole genome sequence of *Mycobacterium kansasii* isolates of the genotype 1 from Brazilian patients with pulmonary disease demonstrates considerable heterogeneity

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*Mycobacterium kansasii* is an opportunistic pathogen and one of the most commonly encountered species in individuals with lung disease. We here report the complete genome sequence of 12 clinical isolates of *M. kansasii* from patients with pulmonary disease in Brazil.

Key words: *Mycobacterium kansasii* - genomes - lung disease - genotype I - diversity - drug resistance

Nontuberculous *Mycobacterium* (NTM) species are widespread in the (man-made) environment and some species cause opportunistic infections in humans. *Mycobacterium kansasii*, frequently isolated from tap water, is a slow-growing photochromogenic NTM and a pathogen that is commonly isolated from patients with pre-existing lung disease, similar to other NTM clinical species such as *M. abscessus*, *avium complex*, *malmoense* and *M. xenopi*. In the USA and South America *M. kansasii* is the second most isolated NTM after *M. avium* complex while in Rio de Janeiro, it is the most frequent NTM to cause pulmonary disease. Besides chronic bronchopulmonary disease, *M. kansasii* causes other clinical manifestations such as lymphpadenitis, skin and soft tissue infection, tenosynovitis, arthritis, osteomyelitis and disseminated infection in patients co-infected with human immunodeficiency virus.

Seven major genotypes (I to VII) of *M. kansasii* have been described and while human isolates are mainly of types I and II (with type II mainly HIV-related), environmental isolates are mostly of the other subtypes. Type I has been described as a heterogeneous group, incompletely characterized on the genomic level. Recently, the genome sequence of the ATCC strain 12478 Hauduroy isolated in Kansas in 1955 has been compared with that of the *M. tuberculosis* strain H37Rv, while other *M. kansasii* genomes are available from environmental, human and simian sources.

Here, we report the genome sequence of 12 clinical *M. kansasii* isolates belonging to genotype I, as determined by *hsp65* sequencing. The strains were isolated from human Brazilian patients with pulmonary disease and possibly earlier tuberculosis. Nine isolates were from sputum, two from bronchoalveolar lavage (7287 and 10742) and one of unknown origin (1580). Isolates were from residents of the state of Rio de Janeiro (n = 7), Pernambuco (n = 3), Rio Grande do Sul (n = 1), and Santa Catarina (n = 1). Genomic DNA libraries were constructed using the Nextera XT DNA library kit and whole-genome shotgun sequencing was performed on the Illumina HiSeq2500 platform, generating paired end reads of 2 × 100 bp. Genomes were de novo assembled using SPAdes software version 3.11.1 and, annotated with RAST (Table). No evidence for the presence of plasmids was observed.

To evaluate the overall similarity of the genomes of the isolates from Brazil with that of the isolate ATCC 12478, we also compared the 12 genomes with the ATCC reference genome using the BLAST Ring Image Generator (BRIG) program (Fig. 1).
We observed many deletions spread in the genomes that were either shared by all or part of the isolates. Three isolates (1580, 3657 and 4404) presented a region of difference of about 27 kb causing the loss of five helicases, a restriction endonuclease and two hypothetical proteins. We also observed multiple deletions shared by the isolates 1580, 3657, 4404, 8835, 8837 and 8839 (Fig. 1).

In addition, a reference-based single-nucleotide polymorphism (SNP) calling was performed against the genome of the *M. kansasii* ATCC 12478 strain using both Snippy (version 3.2; https://github.com/tseemann/snippy) and the wgSNP module of BioNumerics v7.6 (Applied Maths, Sint-Martens-Latem, Belgium). Most isolates presented homogeneously distributed SNPs over the entire genome, except for isolates 6849 and 6498 which clearly presented regions of higher SNP frequency (data not shown). As demonstrated by a Neighbour-Joining phylogenetic tree based on 5,607,341 sites and 1000 bootstrap replicates (Fig. 2), three groups were observed when compared to the genome of the ATCC strain, one presenting less than 100 SNP (n = 1) and more than 5000 SNP (n = 1) and more than 10,000 SNPs (n = 1) (lower part), a second group with more than 10,000 SNPs (n = 6) (upper part). We observed an association between SNP-based grouping and phylogeny on basis of large deletions, that led us suggest that the third group has evolved from the common ancestor.

From two patients, we performed whole genome sequencing of two isolates each; the pair of isolates from a patient from Rio de Janeiro (10742 and 10953) had 0 SNPs difference; the second pair from a patient from Recife (8837 and 8839) presented 277 mutations (data not shown).

Additionally, two isolates (6498 and 8835) presented a Minimal Inhibitory Concentration of > 1 µg/mL for rifampicin in vitro and both presented a non-synonymous SNP, localized respectively at position 4267612 (C to G) and 4267613 (A to C), causing an amino acid change in codon 411 of *rpoB* of respectively Gln to Glu and of Gln to Pro. We as such confirm the scarce existing data on the association between resistance against rifampicin and presence of point mutations in *M. kansasii rpoB* gene. Indeed, point mutations in *rpoB* gene are the main reason for resistance against rifampicin and this has been confirmed in several bacteria. Also, molecular determinants of drug resistance in *M. kansasii* has been recently suggested by Bakula et al. (20). Among the present isolates, we observed considerable differences of *in vitro* virulence in macrophages as measured by growth and induction of necrosis and of cytokine production (data not shown).

In conclusion, these are preliminary data on the variability within the *M. kansasii* genotype 1 in Brazil and, even on the basis of this small sample number, we evidenced three genetic groups that are separated by the presence of large number of SNPs throughout the genome and by the pattern of large deletions. One grouping of strains from Rio de Janeiro is highly similar to the ATCC strain Hauduroy (< 100 SNPs) that was isolated more than 70 years ago in the US. A second group of human isolates that was observed by Bakuła et al. (20) is highly similar to the ATCC strain. We also observed an association between genotypes and geographic origin of isolates, separating those from the states of Rio de Janeiro, Pernambuco and Santa Catarina. Our observation of considerable differences of *in vitro* and *in vivo* virulence and their differences on the genome level is under investigation.

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Fig. 1: BLAST ring image of the 12 Brazilian genomes against the ATCC 12478 reference genome.
Fig. 2: Neighbor-Joining SNP-based tree of the 12 Brazilian isolates and the ATCC 12478 reference genome.

AUTHORS’ CONTRIBUTION

PNS, EL, CM, LR, BCJ and MC conceived and designed the experiments; SEGV, LLG, RJ, LDC and VOM performed the experiments; EM, CC, SEGv, LLG, ABM and CM analysed the data; LDC, JPR, PR, CEDC, APSCG, TG, FFM and FCQM were responsible for samples collection, strain isolation and identification; PNS, EM, EL, DS, SEGV, JPR, LR, BCJ and MC contributed for the manuscript writing.

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