Rifampicin-resistant *Mycobacterium bovis* BCG strain isolated from an infant with NEMO mutation

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Objectives

It is well known that disseminated *Mycobacterium bovis* BCG infection is developed after BCG vaccination in infants with congenital cellular immune deficiencies such as mutations in genes along the interleukin (IL)-12,/interferon (IFN)-γ pathway and mutations in "nuclear factor-kB essential modulator" (NEMO). In this report, rifampicin (RIF)-resistant M. *bovis* BCG strain isolated from an infant with NEMO defect was presented.

Case report

Mendelian susceptibility to mycobacterial disease (MSMD) is a rare disorder resulting in genetic predisposition to infections by poorly pathogenic mycobacterial species or *Mycobacterium tuberculosis* in otherwise healthy children. Different mutations in genes along the interleukin (IL)-12/interferon (IFN)-γ pathway were found in these patients highlighting the crucial role of this axis in human immunity to mycobacteria. Autosomal mutations in *IFNGR1, IFNGR2, STAT1, IL12B, IL12RB1, ISG15* and *IRF8* and X-linked mutations in *NEMO* and *CYBB* have been known to cause MSMD. Mycobacterial infections were reported in about 40% of NEMO mutated patients.

An 8-month-old male infant with NEMO defect admitted with fever, generalized lymphadenopathy and hepatosplenomegaly. Microscopic examination of lymph node and liver biopsy specimens obtained from patient were acid-fast bacilli (AFB) 3+. The real-time PCR-GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA) detected RIF-susceptible *Mycobacterium tuberculosis* complex in the samples. A mycobacterial growing was determined the MGIT960 (Becton Dickinson Microbiology System, Sparks, NV, USA) culture of the samples on the 20th day. Isolate was identified as the *Mycobacterium bovis* BCG by GenoType MTBC Kit (Hain Lifescience, Nehren, Germany) and defined as *M. bovis* BCG [SIT 482 (BOV_1)] by spoligotyping. The MGIT 960 method was used to test resistance to the primary anti-tuberculosis drugs. Isolate was susceptible to RIF, isoniazid (INH), streptomycin and ethambutol. Following, the anti-tuberculosis treatment was started to patient.

He admitted again with hepatosplenomegaly when he was 2 years old. Smear of spontaneously draining abscess material obtained from subcutaneous nodules were AFB 3+. GeneXpert detected RIF-resistant *M. tuberculosis* complex in the specimen (Figure 1a). A mycobacterial growing was determined the culture of the samples on the 13th day and isolate was identified as *Mycobacterium bovis* BCG (Figure 1b). Isolate was susceptible to INH, streptomycin and ethambutol but resistant to RIF. A mutation in the *rpoB* gene (codon 531, S531L) associated with RIF resistance was detected by using the partial sequencing of the *rpoB* gene (Figure 2). Patient died due to disseminated bovis BCG infection and multiple organ failure.

Figure 1. A) Rifampicin-resistant *Mycobacterium tuberculosis* complex result by GeneXpert MTB/RIF **B)** Positive probes (4, 7, 9, 10, and 13) specific for *M.bovis* BCG by Genotype Mycobacterium CM assay

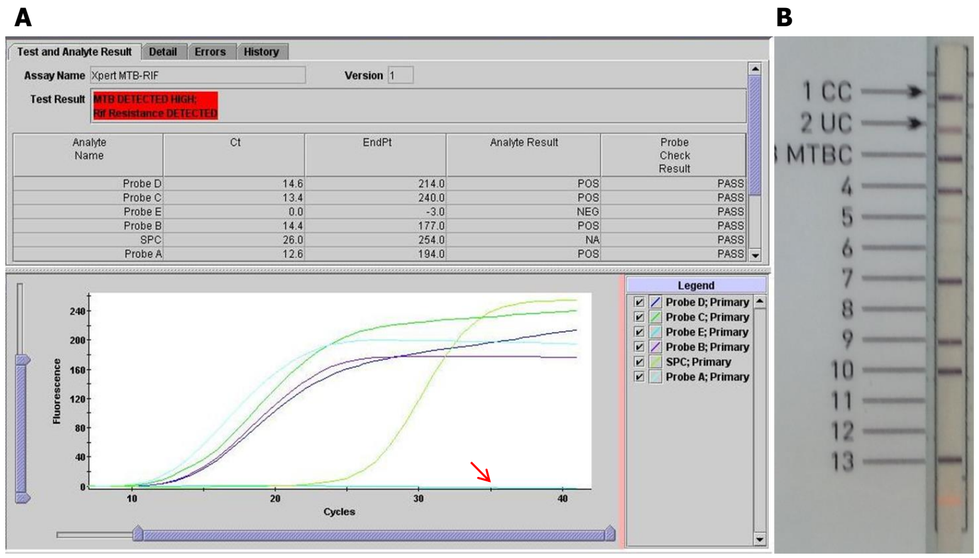
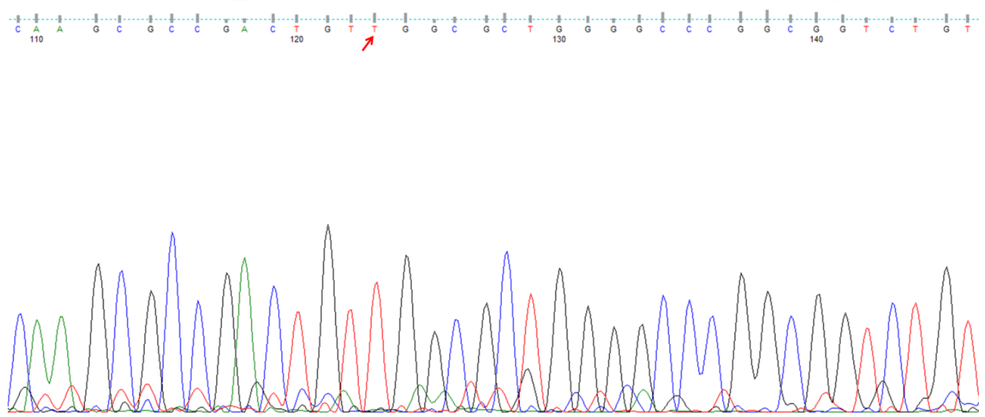


Figure 2. TCG 531TTG (S531L) mutation at codon 531by DNA sequencing



Conclusions

To our knowledge, there are only six RIF-resistant *M. bovis* BCG strains isolated from patients in the literature. However, this is the first *M. bovis* BCG strain isolated from a NEMO deficient patient. Disseminated BCG infection is almost always seen in patients with severe immunodeficiency. During the antituberculosis therapy bacilli can not be completely eliminated in such patients and in this case can lead on the selection of a small number of drug resistant-mutant bacilli in the population. Therefore, to prevent development of drug resistance it is necessary to treat the underlying disease together with bovis BCG infection.