Assessment of Mycobacterial Interspersed Repetitive Unit–QUB Markers to Further Discriminate
the Beijing Genotype in a Population–Based Study of the Genetic Diversity of Mycobacterium
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Abstract
The present investigation focused on genetic diversity and drug–resistance of 101 Mycobacterium
tuberculosis strains isolated between July 2003 to February 2005 in the Okinawa prefecture,
Ryukyu Islands, Japan. A high rate of clustering (87%, eight clusters, 2 to 69 strains/cluster) was
observed upon spoligotyping; most of it was due to the lower discriminatory power of this method
for the Beijing lineage (n=72; 71.3% of the isolates). The remaining diversity was limited to seven
clusters (two to five isolates/cluster), with the following distribution of major lineages: ill–defined T
(n=13; 12.8%), ancestral East African–Indian (n=6; 5.9%), Haarlem (n=4; 4%), Latin
American–Mediterranean (n=2; 2%), X1 (n=1; 1%), and a total absence of the central–Asian clade.
Three remaining strains could not be classified on the basis of their spoligotype pattern and were
labeled “unknown”. Subtyping with mycobacterial interspersed repetitive units (MIRUs) in
association with additional QUB minisatellites was performed to discriminate among the Beijing
strains. Based on an “in–house” spoligotyping/MIRU database (n=694 Beijing strains), eight highly
discriminative MIRU loci for Beijing strains were selected (loci numbered 10, 16, 23, 26, 27, 31, 39,
and 40). The highest discriminatory power (h) observed in our sample (n=72; M–26, 0.385; M–10,
0.38; M–31, 0.255; M–16, 0.238) was too low, and 73.6% of the Beijing strains from Okinawa
remained clustered. Typing of Beijing strains with additional QUB loci (with the exception
of “one–copy” QUB–1451) resulted in higher discriminatory powers: QUB–11b, 0.68; QUB–11a,
0.656; QUB–26, 0.644; QUB–18, 0.553; QUB–4156, 0.5; QUB–1895, 0.453. A definitive algorithm on
the use of QUB markers to subtype Beijing isolates in expanded studies would shed light on their
hypervariability, which may sometimes blur recognition between epidemiologically linked Beijing
isolates. The total absence of multiple drug resistance among Beijing isolates from Okinawa, as
well as the relatively older age of the patients (majority above 60 years), shows that tuberculosis
(TB) is a declining disease in Okinawa, and an adequate TB control program has successfully
avoided both the emergence and the spread of multi–drug–resistant TB in this insular setting.