tries across 4 continents. For PCR restriction-enzyme analysis (PCR-REA) subtyping, protocols described by Telenti et al. (2003) (*hsp65* gene) and Bakuła et. al. (2016) (*tuf* gene) were used. The patients were categorized as having *M. kansasii* disease following the American Thoracic Society 2007 diagnostic criteria.

The vast majority of isolates (392; 82.5%) presented patterns characteristic for subtype I. Forty-three (9%) isolates exhibited subtype II pattern. There were 19 (4%), 2 (0.4%), 2 (0.4%) and 13 (2.7%) isolates representing subtypes III, IV, V, and VI, respectively. Four (0.8%) isolates gave inconsistent results (mixed subtype — I/II). The subtype I–VI isolates were obtained from both disease-associated and non-disease associated cases. Of two subtype IV isolates, one was obtained from a non-disease associated case, and the other one from a patient with unknown status. For two subtype V isolates, data concerning *M. kansasii* disease were unavailable.

The highest frequency of *M. kansasii* subtype I isolations was observed for Poland (140/142; 98.6%), and the lowest for Estonia (2/7; 28.6%).

This study demonstrated that subtype I represented the vast majority of *M. kansasii* clinical isolates worldwide. Since all *M. kansasii* subtypes detected (I-VI) were isolated from both disease-related and non-related cases, subtyping of the species does not permit differentiation between disease and non-disease states that did and did not cause definite disease. Furthermore, the genetic diversity of the *M. kansasii* population showed important regional variations.

The study was performed within the framework of the Fight Against Tuberculosis in Central & Eastern Europe consortium (https://fate-consortium.org).

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GENETIC DIVERSITY OF MULTIDRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* ISOLATES IN PAKISTAN

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Tuberculosis (TB) remains an inglorious leader among infectious diseases in mortality, with its annual toll of 1.7 million lives worldwide. Pakistan ranks 5th among the world's highest TB burden countries and the 6th among countries with the highest burden of drug-resistant TB, including multi-drug resistant (MDR)-TB. However, very limited data are available on the genetic structure of *M. tuberculosis* strains circulating in this country.

The objective of this study was to explore the genetic diversity of multidrug-resistant *M. tuberculosis* isolates from Pakistan with two different methodologies, i.e. spoligotyping and 24-loci MIRU-VNTR typing.

The study included 130 MDR-TB isolates, recovered from as many patients from Pakistan, between January 2013 and June 2015. Conventional drug susceptibility testing was performed using the standard 1% proportion method on the Löwenstein-Jensen medium, as described elsewhere. Spoligotyping was performed with a commercially available kit (Mapmygenome India Ltd., Madhapur, India) according to the manufacturer's protocol. MIRU-VNTR analysis was carried out at 24 loci, as described earlier. Phylogenetic clades of *M. tuberculosis* were assigned according to signatures provided in the SITVIT database (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE).

Spoligotypes were obtained for 127 (97.8%) isolates. Based on a SIT number in the SITVIT database, all isolates presented 53 different profiles split into 14 clusters (n = 88, 69.3%, 2–30 isolates per cluster) and 39 (30.7%) unique patterns. MIRU-VNTR typing identified 128 unique types (98.5%) and one cluster (n = 2, 1.5%). When spoligotyping and MIRU-VNTR typing was used in combination, only two, out of 130 isolates, clustered both in both methods, resulting in a clustering rate of 1.5%.

Upon phylogenetic analysis, 101 (77.7%) isolates were classified into 12 clades, with the most prevalent being CAS1_DELHI (n = 53, 41.7%) followed by T1 (n = 14, 11%) and BEIJING (n = 10, 7.8%). The remaining 9 families (CAS, MANU2, EAI5, T2, LAM10_CAM, H1, X1, H4 and CAS2) involved 24 (18.9%) isolates. Twenty-six (20.5%) isolates could not be assigned to any specific lineage.

This study provides a snapshot of the genetic diversity of *M. tuberculosis* strains circulating in Pakistan. The compactness of the drug resistant *M. tuberculosis* population structure was apparent, as three major lineages, i.e. CAS1_DELHI, T1, and BEIJING comprised more than half (60.6%) of the isolates studied. Furthermore, the exceptionally low clustering rate

Suggest that recent transmission does not play an important role in the incidence of MDR-TB in Pakistan.

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UPDATE ON VIRULENCE FACTORS IN MYCOBACTERIA

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Although the majority of mycobacteria represent harmless environmental bacteria, a few mycobacterial species have evolved into major human pathogens. *Mycobacterium tuberculosis*, the etiological agent of human tuberculosis, is the most dominant mycobacterial pathogen in terms of global patient numbers and gravity of disease.

The molecular mechanisms by which *M. tuberculosis* induces disease are complex and result from a long-lasting host-pathogen co-evolution that might have started already by its *Mycobacterium canettii*-like progenitors. Recent research has revealed numerous factors implicated in the pathogenesis of *M. tuberculosis*, although the pathogen still holds many secrets of its successful strategy to circumvent host defences and persist in the host. As many pathogenicity factors relate to the exchange and secretion of biomolecules by *M. tuberculosis*, special emphasis is given to secretion pathways that enable *M. tuberculosis* to circumvent immune defence mechanisms mounted by the host. These factors might represent new, alternative targets for development of combination therapies that would enhance the efficacy of the immune system in controlling *M. tuberculosis* infections. Similarly, selected secretion systems may also represent important virulence factors in selected non-tuberculous mycobacteria. Here, recent insights into evolution of selected factors of *M. tuberculosis* and selected other mycobacteria that are involved in host-pathogen interaction will be discussed.