Non-tuberculous mycobacteria (NTM) is a term which refers to those *Mycobacterium* species that are not members of the *Mycobacterium tuberculosis* complex. The NTM are for the most part environmental organisms but may under certain conditions (i.e. in patients with significantly impaired immunity, as a result of for example malignancy, immunosuppressive therapy, or HIV/AIDS) cause a disease known as mycobacteriosis. The major clinical manifestations attributable to the NTM include pulmonary disease and much less frequently lymphadenitis, skin and soft-tissue infections, and disseminated disease. The proportion of NTM infections is steadily increasing in the world. This, in turn, explains the growing interest of researchers in this group of bacteria.

*Mycobacterium kansasii* is the sixth most commonly isolated NTM species across the world. In Poland, the isolation rate of this pathogen is particularly high, exceeding 35% of total NTM isolations, compared with respective rates of 5% in Europe and 4% globally. The heterogeneity within the *M. kansasii* species is evidenced by the presence of seven distinct genotypes (I-VII). The results of the so far performed investigations have suggested that *M. kansasii* type I is the most prevalent type among clinical isolates worldwide, rarely recovered from the environmental sites.

The key purpose of this doctoral thesis was a multi-faceted analysis of *M. kansasii* isolates, recovered from Polish pulmonary patients. The comprehensive species characteristics included recognition of its genetic polymorphism, susceptibility profiles, and insight into genetic determinants of drug resistance. The PhD project also aimed to describe sociodemographic, clinical and radiological features of *M. kansasii* disease. Among key goals of the study was also a proposal and implementation of some new methodological genotyping and intraspecies differentiation tools to improve molecular diagnostics of *M. kansasii*.

The study comprised of 105 patients treated in the Department of Internal Medicine, Pulmonary Diseases and Allergy of the Medical University of Warsaw between 2000 and
2015 (63 females, 42 males, mean age 64.6 ± 17.8 years). Of these, based on American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA) criteria, 86 (81.9%) were diagnosed as having *M. kansasii* disease. The remaining 19 (18.1%) patients were identified as isolation cases. There were no statistically significant differences between *M. kansasii* disease and isolation cases in terms of clinical symptoms or comorbidities.

Patients with *M. kansasii* disease presented most commonly (43/86, 50%) with fibro-cavitary disease upon radiology. Lesion distribution usually showed bilateral upper lobe involvement.

Among the 191 isolates genotyped, all were identified as *M. kansasii* type I.

Drug susceptibility profiles of *M. kansasii* isolates were determined using two different methodologies, i.e. broth microdilution and diffusion (E-test) assay, with 13 and 8 antimycobacterial agents, respectively. All isolates tested were susceptible to rifampicin (RIF), amikacin (AMK), co-trimoxazole (SXT), rifabutin (RFB), moxifloxacin (MXF), and linezolid (LZD), when using microdilution method. Resistance to ethambutol (EMB), ciprofloxacin (CIP), and clarithromycin (CLR) was found in 83 (97.7%), 17 (20%), and 1 (1.2%) isolate, respectively. The calculated concordance between the E-test and dilution method was 22.6% for AMK, 4.8% for streptomycin (STR), 3.2% for CLR, 1.6% for RIF and 0% for EMB, INH, and SXT.

Screening for mutations associated with resistance was performed on isolates which, upon microdilution method, were declared resistant, based on the published breakpoints, or showed, for a given drug, the highest five MIC values, if no breakpoints were available. Nine genetic loci (eight structural genes and one regulatory region) associated with drug resistance in *M. tuberculosis* were analyzed, upon PCR sequencing. The only mutations disclosed were A2266C transversion at *rrl* gene (CLR-resistant strain) and A128G transition at *rpsL* gene (strain with STR MIC > 64 mg/L).

To design a new, high-resolution fingerprinting method, complete genome sequence of the *M. kansasii* ATCC12478 reference strain was searched for satellite-like repetitive DNA elements. A total of 1447 possible Variable Number Tandem Repeat (VNTR) motifs were found. A 17-loci panel was used to study polymorphism among 67 isolates. The results of VNTR typing were compared with those of pulsed-field gel electrophoresis (PFGE). A combined analysis of VNTR typing and PFGE revealed a total of 45 distinct patterns, including 11 clusters with 33 isolates and 34 unique patterns. The Hunter-Gaston’s discriminatory index was 0.95 and 0.66 for PFGE and VNTR typing respectively, and 0.97 for the two methods combined. Use of only six VNTRs (VNTR 1, 2, 8, 14, 20 and 23) had the same discriminatory power as use of the whole 17-loci panel.

For representatives of the *M. kansasii* subtypes I-VI, the nucleotide sequences of the partial *tuf* gene, coding for the elongation factor EF-Tu, were determined. Based on the obtained data, Mval, the only one enzyme which produced distinct patterns for each *M. kansasii* subtype was selected for PCR-REA (PCR restriction enzyme analysis) assay. The designed here approach was then tested on *M. kansasii* clinical isolates from Poland (n = 80) and representatives of six *M. kansasii* genotypes (I-VI, n = 15). The PCR-REA proposed here produce identical results as with the former typing variants (PCR-REA of *tuf, hsp65* and ITS sequence analysis) but has benefits over the latter, as it involves one digestion reaction with one restriction enzyme instead of multiple digestions with different enzymes.
Within the doctoral study, a new *M. kansasii* identification/genotyping system based on matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) was developed. A total of 32 strains, representing genotypes I-VI of *M. kansasii* were used to establish a spectra reference database. Evaluation was performed on 100 *M. kansasii* clinical isolates. Twenty-seven spectra in total per strain were generated to create a unique main spectra library for each genotype. The spectra were matched against the commercial BDAL database (ver. 6.0). The augmented database resulted in an overall identification success rate of 97% *M. kansasii* isolates used for evaluation purposes.

In conclusion, the doctoral thesis presented here provides a comprehensive description of sociodemographic, clinical and radiological features of patients with *M. kansasii* disease. A detailed characteristic of the species included the genetic structure of *M. kansasii* isolates, susceptibility profiles and genetic determinants of drug resistance. One of the expected outcomes was formulation of a new genotyping and intraspecies differentiation methods for *M. kansasii* to improve molecular and epidemiological diagnostics of the species.