lates. Mutations in \textit{rrl} and \textit{rrs} genes were not detected. AST for 4 MAM isolates confirmed that inducible resistance is not present even with \textit{erm(41)} T28 mutation. AST for 3 MAA with \textit{erm(41)} C28 polymorphism showed MIC values below 2 mg/L which is interpreted by CLSI guidelines as sensitive strain. AST showed that MIC values for amikacin are between 8—16 mg/L interpreted as sensitive and concordant with molecular analysis.

In Slovenia, for macrolide and aminoglycoside resistance, phenotypic and genotypic results of \textit{Mycobacterium abscessus} complex are concordant. Prevalent subspecies is MAA where high percentage of strains have inducible resistance which is important for treatment of CF, where clarithromycin is first drug of choice.

\section*{6.54 MOLECULAR FEATURES OF \textit{MYCOBACTERIUM TUBERCULOSIS} ISOLATES FROM PATIENTS LIVING IN CLOSED CITY IN THE URAL REGION, RUSSIA}

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Novouralsk is a closed town in the Sverdlovsk region, Middle Ural area in Russia, with a total population of 81 500 and travel and residency restrictions. We aimed to identify the molecular-epidemiological features of \textit{M. tuberculosis} circulating in Novouralsk under these specific conditions.

A total of 87 \textit{M. tuberculosis} clinical isolates obtained between 2013 and 2016 from TB patients living in Novouralsk town were analyzed. According to clinical data, 34 (39.1\%) of TB patients were HIV-infected. 53 (60.9\%) of TB cases were newly diagnosed. Using real-time PCR we divided \textit{M. tuberculosis} clinical isolates into Beijing/non-Beijing genetic groups. Beijing genotype variant B0/W148 was detected by multiplex PCR assay. VNTR loci MIRU26 and QUB26 were used for subtyping Beijing strains. Spoligotyping was used for further subtyping non-Beijing isolates. Drug susceptibility testing for first and second line drugs was performed by absolute concentration method.

Genotyping identified the predominance of the Beijing genotype isolates (75.5\%), among new TB cases, that is almost 20\% higher than the average for the Ural region (p < 0.05). 52.8\% isolates belonged to variant Beijing B0/W148. The majority of Beijing isolates — 35 (40.2\%) had seven copies in MIRU26 and QUB-26 loci. Nine (10.3\%) Beijing B0/W148 isolates had 2 copies in QUB26 locus that was unusual for this genetic cluster; six patients from this group had TB/HIV co-infection. Seven (8.0\%) of non-Beijing isolates belonged to SIT35 spoligotype (Ural family). 20.7\% of patient had prison history and 72.2\% of them were infected with B0/W148 genotype. The MDR prevalence rate was higher than in Sverdlovsk region (66\% vs 43.9\%, p < 0.05) and MDR status was associated with the Beijing B0/W148 genotype (94\% and 6\% of its isolates were MDR and polyresistant, respectively).

Epidemiological situation with TB in Novouralsk is characterized by high level of TB/HIV co-infection, predominance of Beijing B0/W148 isolates, which is an underlying reason of high level of MDR-TB.
an important role in the modern epidemiological investigations of tuberculosis in animals at the regional and international level. Bovine tuberculosis represents a significant economic burden to the agriculture of the affected countries. From 2000 to 2015 the disease shows cyclicity in private farms in different regions of Bulgaria. This study is a first molecular investigation of animal tuberculosis in the veterinary medicine in country. The macroscopic and microscopic observation of 35 diagnostic materials from slaughtered cattle, received in the National Reference Laboratory of animal tuberculosis were studied with the three molecular methods: RD4-PCR, spoligotyping and MIRU-VNTR. In 27 of the examined lymph nodes we found specific lesions for bovine tuberculosis. The findings were confirmed bacteriologically and by conventional PCR. To differentiate M. bovis from other M. tuberculosis complex subtypes, we used primers flanking specific deletion (RD4) in the genome of M. bovis and obtained the 446 bp DNA product. The amplified copies were further investigated by molecular investigations of M. bovis strains need to characterize the genetic diversity and population structure of M. bovis strains isolated from cattle in Bulgaria. New information will be added to the global database in the field of molecular epidemiology of the prevalence of M. bovis strains in the cattle population in Bulgaria, which will allow comparative analysis with data from the Balkan region and Europe.

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INTERNATIONAL VALIDATION OF ANALYSIS PIPELINES FOR WHOLE GENOME SEQUENCING DATA OF MYCOBACTERIUM TUBERCULOSIS ISOLATES

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The aim of this multicenter study was to validate and compare different pipelines used for analysis of the Whole Genome Sequencing (WGS) data of Mycobacterium tuberculosis isolates.

All 535 M. tuberculosis isolates of culture positive cases in the Netherlands in 2016, were subjected to WGS, in addition to the routine application of VNTR typing. Transmission suggested on basis of identical VNTR profile of cases in 2016 was further investigated by molecular and epidemiological laboratories pipelines, facilitating the comparability of results.

Several differences were observed among the pipelines with regard to the version of reference genome used, software used for mapping and SNP calling, (repetitive) regions excluded in the analysis, the minimum number of reads to support SNPs, and the minimum allele frequencies. The RIVM pipeline was adapted in the light of these results to function more in line with other international laboratories pipelines, facilitating the comparability of results.

Multidrug resistant tuberculosis (MDR-TB) is one of the major WHO health concerns. One of the challenges that hampers the effective response to MDR-TB is the long turnaround time of phenotypic Drug Susceptibility Testing (DST) of Mycobacterium tuberculosis. To counter this, new fast and sensitive DNA-based methods were successfully introduced over the last years. However, these (a) are based on the knowledge on resistance mutations, (b) do not distinguish living from dead cells, (c) ignore all intrinsic resistance mechanisms, and (d) ignore the influence of compensatory mutations.

We introduce a next-generation diagnostic test based on quantification of drug-specific RNA biomarkers. The basic principle is that a brief antibiotic exposure triggers specific transcriptional responses in susceptible, but not in resistant, microbes within a few hours. This has the advantage that long culture-dependent steps are avoided, yet the resistance phenotype is detected independent of the specific cause of resistance.

First, the global transcriptional response of two M. tuberculosis strains to 10 anti-TB drugs was determined using RNAseq-Seq. A set of highly responsive genes was selected for each drug and RNA-targeting probes were designed.

Next, the RNA-based DST was developed in 96 well format. In short, 200 μl of a positively flagged MGIT™ culture is spiked with a drug, while a replicate is incubated in absence of the drug. Multiplex mRNA quantification is performed directly on crude cell lysates using a combination of the bead-based MagPix™ (Luminex) and Quantigene™ Plex (Thermo Fisher) technology.