GLOBAL EXPRESSION RESPONSE OF CLINICAL *MYCOBACTERIUM TUBERCULOSIS* ISOLATES TO ANTITUBERCULOUS DRUGS

Claudia Plinke[1], Andrea Hueser[2], Jörn Kalinowski[2], Sabine Rüsch-Gerdes[1], Stefan Niemann[1]

[1]National Reference Center for Mycobacteria, Research Center Borstel, Borstel, Germany,

[2]Institute for Genome Research, University Bielefeld, Bielefeld, Germany

Background. The emergence of drug resistant and multidrug resistant (MDR) strains of the *Mycobacterium tuberculosis* complex (MTBC) represents a serious public health threat. Interestingly, the occurrence of resistance is not equally distributed when different MTBC genotypes were considered pointing to individual strain capacities to acquire and spread resistance. The availability of MTBC whole genome microarrays has now enabled novel approaches to further investigate this phenomenon by analysing the global expression response of clinical isolates to antituberculous drugs. Therefore, we have developed a medium throughput challenge system for clinical MTBC isolates with special attention to enhanced safety requirements for the work with MDR strains.

Methods. Strains where grown in 7H9 to early log phase (OD 0.2-0.4) and challenged with isoniazid (INH) and ethambutol (EMB), respectively. RNA was isolated at different time points with a modified GTC (guanidiumthisisocyanate)/Trizol method. Bacterial cells were opened using the Fast Prep® System (Qbiogene). Analysis of gene expression was carried out applying spotted whole genome microarrays (oligo set AROS Version 1.1, Operon Biotechnologies) and real time PCR experiments.

Results. In the challenge system the action of both drugs could be clearly shown as susceptible strains stop growing after adding INH or EMB while the growth of resistant strains was not affected. Prior starting with array experiments, we have tested the killing efficacy of the different steps involved in RNA isolation. While sole incubation with GTC buffer has no killing effect, 15 min trizol incubation has 100% killing efficacy in 20 randomly chosen clinical isolates. A initial INH challenge experiment of two clinical isolates revealed first insights in drug-induced genes. These include genes reported before in reference publications as well as some other genes that have not been recognized previously.

Conclusions. The standardized MTBC challenge system developed here provides the basis for studying the pharmacogenomics of clinical isolates.