



Sébastien Gagneux in the biosafety laboratory airlock at the Swiss Tropical and Public Health Institute.

Systems biology of drug-resistant tuberculosis

## The stealthy superbug

Tuberculosis is transmitted via tiny, coughed-up droplets. In Switzerland, this disease was almost forgotten, but is now back again. Over the last sixty years, multi-drug-resistant strains have developed in Central Asia and Eastern Europe and are now spreading. The scientists working on the TbX project intend to forestall this development.

Each year, tuberculosis, caused by the *Mycobacterium tuberculosis* bacterium, leads to the death of 1.5 million people worldwide. Nine million new cases are registered each year. However, the main problem lies elsewhere. Three percent of all new infections are triggered by bacterial strains which have developed multi-drug resistance, meaning they can no longer be eliminated by a number of antibiotics. “Cases caused by such strains are very difficult to treat”, explains Sébastien Gagneux, head of the tuberculosis research unit at the Swiss Tropical and Public Health Institute in Basel.

Around the world, approximately half a million patients develop multi-drug-resistant tuberculosis each year. This is a matter of serious concern to global health authorities as well as Gagneux, which is why he and his TbX RTD Project team are attempting to identify the biological processes underlying resistance development.

### Prisons – breeding grounds for superbugs

To this end, they are collaborating with the Georgian Health Ministry. In Georgia, multi-drug-resistant tuberculosis is relatively common and represents 20 to 30 percent of all cases. The reasons for this are in part historical, says Gagneux. “Georgia was part of the USSR. During this time, the prisons were full and sanitary and medical conditions were poor.” Not only could tuberculosis easily spread in these surroundings, but it was also able to develop resistance against standard antibiotics.

For treatment to be effective, a patient must be treated with four different types of antibiotic over six months. Only then can one be sure that all bacteria have been killed. In Georgian prisons, antibiotics were often in short supply and the treatment duration was insufficient. Under these conditions, weak strains were killed whereas more robust ones survived and were thereafter immune to the drug. “These are the so-called superbugs”, Gagneux warns.

After the collapse of the Soviet Union, many prisoners were released, and this led to the spread of the resistant tuberculosis strains among the population at large. Poor healthcare after the fall of the USSR then promoted their proliferation.

### Arms race in a flask

Gagneux has the Georgian strains sent to his lab in Basel where he examines their genetic differences. “We compare the strains to determine which ones are more successful and which ones less so.”

In practice, this means bringing two strains together so they can compete against each other. To this end, he adds them to a growth medium in a flask and observes which one has the upper hand after one month. In a second step, he compares them on the molecular level, using tandem mass spectrometry and a high-performance computer to analyze the protein composition of the bacteria. This procedure was developed by the Aebersold group at the ETH Zurich.

Using this data, the scientists can draw conclusions regarding the mode of action of various genes, as these directly affect protein production. “By doing so, we can establish a model that takes into account a bacterium’s genes as well as its molecular phenotype”, explains Gagneux. This could lead to novel therapeutic options in the future, such as the switching off of specific genes of a tuberculosis strain, thereby cancelling its resistance to an antibiotic. Gagneux is collaborating with an industrial partner, BioVersys AG in Basel, to realize this goal (see article, page 12).

### Sluggish evolution

From an evolutionary point of view, tuberculosis is somewhat lazy. Cell division in tuberculosis-causing bacteria occurs only once every 24 hours, whereas other bacteria divide every hour. Besides being slow, *M. tuberculosis* has a further disadvantage. The bacterium lacks so-called plasmids, circular DNA molecules which allow the bacteria to exchange genetic information between themselves. Plasmids notably code for resistance to antibiotics.

When plasmids are not available, each bacterial strain is forced to develop the resistance on its own through numerous divisions and random mutations. Due to these unfavorable preconditions, the tuberculosis bacteria need more time to develop resistance. Even so, hundreds of multi-resistant tuberculosis strains now exist. “These are the strains of interest to us, as they raise many important questions”, ponders Gagneux.

One of them concerns fitness costs. A bacterial strain must pay for resistance development, not in cash but in terms of its own fitness, which decreases. “Resistant strains are usually less infectious in an initial phase”, concedes Gagneux. The bacterium pays for immunity against an antibiotic by becoming less virulent. However, its evolution does not stand still. Many strains offset this dis-

advantage after a few generations as a result of further mutations in their genetic material. Gagneux points out that “the strains are subsequently just as virulent as before”.

### Models help design appropriate therapy

In order to better understand the mechanisms behind these processes, the data collected during mass spectrometry is fed into a metabolic model. Gagneux summarizes the aims of his team: “We hope to be able to predict whether or not a given strain of *M. tuberculosis* can proliferate.” Depending on the result, a patient may or may not have to be isolated. This has a significant impact on the quality of life of the patient and also has an influence on medical costs. “This allows us to design individual treatment strategies”, explains Gagneux. The data and models might also prove useful in the development of novel antibiotics.

The scientists have also developed a model for the geographic distribution of tuberculosis. “We will use this model to predict where a given strain will appear next”, says Gagneux. This is also relevant for Switzerland, where approximately 500 new cases are registered each year. Three quarters of these patients are immigrants. The others are long-time residents, for instance grandparents who contracted the disease 50 years ago. According to Gagneux, “the bacteria can remain inactive in the body for decades”. If, at some point, the immune system is weakened, an outbreak can occur.

To date, few cases of multi-resistant tuberculosis have been registered in Switzerland. “But with the eastward expansion of the EU and the accompanied free movement of its citizens, it will be all the easier for these germs to be introduced here too. It is therefore only a matter of time until further cases appear in Switzerland as well.”

### TbX at a glance

**Principal investigator:** Prof. Sébastien Gagneux

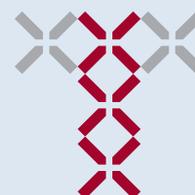
**Research groups:**

- Prof. Sébastien Gagneux, Swiss Tropical and Public Health Institute, University of Basel – Infection and evolutionary biology, molecular epidemiology
- Dr. Xueli Guan, Swiss Tropical and Public Health Institute – Lipidomics
- Prof. Ruedi Aebersold, Institute of Molecular Systems Biology, ETH Zurich – Proteomics
- Prof. Uwe Sauer, Institute of Molecular Systems Biology, ETH Zurich – Metabolomics
- Dr. Christian Beisel, Department of Biosystems Science and Engineering (D-BSSE), ETH Zurich – Genomics
- Prof. Tanja Stadler, Department of Biosystems Science and Engineering (D-BSSE), ETH Zurich – Phylodynamic modeling
- Jörg Stelling, Department of Biosystems Science and Engineering (D-BSSE), ETH Zurich – Metabolic modeling

**Industrial partner:** BioVersys AG, Basel

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**TbX**  
Systems Biology of  
Drug-resistant Tuberculosis  
in the Field