

Antibiotic resistance genes and integrons as indicators of biotic pollution and resistance load of Arctic ecosystems

Acronym: ATTENTION

According to the World Health Organization (WHO), rapid growth and permanent spread of resistance to antibiotics among bacteria is an indisputable global problem and one of the most serious threats of the 21st century. Currently, drug-resistant diseases cause at least 700,000 deaths globally per year and it is estimated that this number will increase to 10 million deaths globally per year by 2050 if no action is taken. The presence of multi-resistant strains in the environment that do not respond to last resort drugs is a medical problem, and therefore it is crucial and necessary to take integrative international efforts in order to recognize the rate of antibiotic resistance worldwide.

The presence of antibiotic resistance genes (ARGs) and integrons in the environment is considered to be a biotic pollution and an ecological problem. Integrons are DNA platforms, inside which gene cassettes conditioning antibiotic resistance are embedded. Aquatic environments, and in particular wastewater, are the pathways for the spread of antibiotic resistance in the environment. Areas directly related to human activities such as wastewater treatment plants contain a greater variety and number of integrons and ARGs, and therefore they are called "hot spots" where the exchange of ARGs and integrons between bacteria via horizontal gene transfer (HGT) is very common. The diversity and spread of ARGs and integrons in seemingly pristine and rapidly changing polar regions are poorly studied. Even the remote Arctic ecosystems with limited impact of human activities may constitute repositories of previously unknown ARGs and integrons, which originate from wastewater delivered to the fjords, as well as water from melting glaciers and permafrost, which constitutes a global threat to human and animal health.

The main aim of this study is the evaluation of impact that water flowing from melting glaciers, permafrost and wastewater discharged directly into fjords in the Arctic region exert on increasing the genes pool of the global aquatic resistome, being the collection of all ARGs in the environment. The proposed research aims at determining the type and frequency of antibiotic-resistant bacteria (ARB), integrons as a marker of anthropogenic pollution and ARGs in cryospheric resistome and in wastewater in the Svalbard archipelago. Svalbard is located between Europe and the North Pole and serves as an example of remote and a relatively pristine polar area. Samples will be collected from sites such as glacial ice and permafrost (both with no direct human impact). Samples in Ny-Ålesund from a wastewater treatment plant and a stream of wastewater from Longyearbyen as highly human-impacted sites will also be collected.

Research conducted during the project will include the following analyses:

- The determination of frequency and type of ARGs, integrons and variable region of class 1 integrons in bacteria with significant mechanisms of antibiotic resistance isolated from Arctic environments and wastewater.
- Quantifying the abundance of ARGs and integrons in metagenome (total DNA) and plasmidome (collection of all plasmids) from Arctic samples.
- Investigating the diversity of ARGs and gene cassettes carried by class 1 integrons from metagenome and plasmidome of Arctic environments and wastewater.
- Estimating the frequency of ARGs and integrons located on mobile genetic elements.

The idea behind the proposed research is included in the Arctic Monitoring & Assessment Program (AMAP) of the Working Group of the Arctic Council. One of the main goals of AMAP published in a report from 2018 is to trigger global action and develop an effective strategy for monitoring and assessing the unknown pathways of the spread of antibiotic resistance in the Arctic that results from human-induced contamination. All these activities are a part of the global holistic "One Health" approach that is to combat the constantly growing and spreading antibiotic resistance in the environment.

Culture-based methods coupled with metagenomic studies, whose purpose is to investigate the total, environmental DNA, will provide information on the previously unknown diversity and abundance of ARGs in the Arctic. The conducted research will also allow to recognize the pathways of resistance transmission and to assess the impact of melting glaciers, permafrost and an Arctic wastewater treatment plant on the increase of antibiotic resistance genes abundance in the global aquatic environment. Moreover, the research of plasmidome might be helpful in determining the location of the genes and understanding mechanisms of genes exchange which in turn may become vital in controlling the future dissemination of antibiotic resistance.