- Project Title

Evaluation of multidrug-resistant bacteria in the Active soil layer, and Permafrost regions of the Canadian High Arctic

- Lead Researcher's Name and Affiliation

Dr. Krishnan K. P.
Scientist F
Department Name: Arctic Ecology and Biogeochemistry
National Centre for Polar and Ocean Research,
Ministry of Earth Sciences, Govt. of India
Vasco-da-Gama, Goa, India
Desk No : +91-832-2525510
krishnan@ncpor.res.in, kpkrishnan@gmail.com

One of the most noticeable impacts of Arctic warming is the thawing of permafrost – which could potentially reactivate many of the dormant microorganisms that are preserved in the permafrost soils for centuries. Some of these microorganisms may be pathogenic and/or antibiotic-resistant bacteria (ARB) that can lead to the resurgence of zoonotic diseases. Besides, antibiotic resistance genes (ARG) can be transported to the Arctic environment through the migration and movement of humans and birds. Since ARGs are now widespread among microbial communities, whether the extensive diversity of ARGs and ARB in environmental reservoirs is the result of anthropogenic influence is controversial. Thus, in this proposal, we intend to perform a comprehensive survey of the tundra soil, active layer, and permafrost not only to evaluate the presence of potential ARG and ARB using a culture-dependent and independent approach but also to delineate potential sources-anthropogenic and/or ancient origins of ARGs and ARB in the Canadian high Arctic region. Thus, our proposed project will provide baseline data on the ARGs and ARB which could be utilized to demarcate the high-risk permafrost regions for the local population.

In this context, we delineated the following objectives for the proposed project:-

•   Evaluation of Antibiotic-resistant genes (ARGs), virulence-related genes, and antibiotic-resistant bacteria (ARB) in the Canadian high Arctic Soil, and Permafrost regions

•   To evaluate the presence of resistance genes and at analyzing antibiotic resistance patterns, thereby allowing to determine the role of anthropogenic pressure in the apparition of antibiotic resistance.

We are planning for long-term monitoring (2022 to 2026) seasonally. Field methodology includes the collection of samples from the active layer and Permafrost soils using a field core sampler near the CHARS campus. The core samples will be cut aseptically into different layers and stored at -80 °C. These samples will be transported back to India (NCPOR-lab) using a cold shipment. In the NCPOR lab these layers will be used for the isolation, culturing, and

screening of various health-significant bacteria (culture-dependent) and for the extraction of DNA and metagenomics studies (culture-independent).

There are no expected waste or impacts associated with this study. In the unlikely case waste is generated, the proponent shall keep all garbage and debris in bags placed in a covered metal container or equivalent until disposed of at an approved facility. All such wastes shall be kept inaccessible to wildlife at all times.

This study will provide baseline information on the possible reactivation of ARB in the permafrost, the risks associated with the release of potentially drug-resistant pathogens and antibiotic-resistant genes from permafrost, and the implications for policy and international cooperation in light of the Covid-19 pandemic.

Nunavut residents can be involved in the field sampling and the data generated in the project will be stored at data facility center of NCPOR, India and major results of the projects will be shared to Nunavut Research Institute. The data will be shared by the lead Principal Investigator of the Project and coordinator of the Indian Arctic Programme Dr. KP. Krishnan.

Please attach a technical project proposal description including the following information:

a)   Objectives: Provide well-defined short-term and long-term objectives for the overall project

b)   Rationale: Describe the rationale for the project. This should be a detailed section that clearly lays out the scientific basis for the proposed work.

c)   Progress to Date: Describe the results of any work completed to date. This section should also include information on any progress in the areas of capacity building, communications and/or the use of Indigenous knowledge

d)   Methodology: Describe project design, field research methodology, data analysis techniques, where and when the work will be carried out over the lifetime of the project.

e)   Data management: Describe your data management plan, including where and when the data and metadata records will be stored

f)   Research outputs: Describe the major research outputs to be generated through the project (academic theses, publications, presentations, reports, etc.)

Rationale: Climate change is predicted to increase the rate and spread of antibiotic resistance among bacterial pathogens (Cavicchioli et al., 2019; Rodríguez-Verdugo et al., 2020). The evolution and spread of antibiotic resistance among pathogenic bacteria are one of the most important challenges in public health today. Permafrost, the most ancient and extreme environmental ecosystem in which microbial life has been reported, is the potential site to study bacteria dated back to the pre-antibiotic era. Antibiotic resistance genes (ARGs) were functionally diverse before the anthropogenic use of antibiotics began, contributing to the evolution of natural reservoirs of resistance genes. Under the ongoing climatic warming scenario, there is a possibility of horizontal transfer of these natural ARGs among other microbes via mobile genetic elements (MGEs) giving rise to the drug-resistance pathogens. Similar to ARGs, virulence factor genes (VFGs) of pathogenic bacteria can also be transferred to other bacteria by phages and plasmids (Wagner and Waldor, 2002). These transmitted

genes can disrupt the host immune system, allowing bacterial pathogens to invade and cause infectious diseases (Wang et al., 2021). These drug-resistance pathogens may cause infections that are untreatable with currently available antibiotics (Zheng et al., 2021). ARB was identified in permafrost dated 15–290 ka, with species even more abundant in active layers in older and deeper permafrost (Perron et al., 2015). Subsequently, concerns have been raised about the potential for the exchange of genetic material between antibiotic-resistant permafrost microorganisms and contemporary bacteria to create new antibiotic-resistant strains. The local human settlement (the northerners) near the active permafrost region is at risk of being a potential host of these ancient types of ARGs and ARBs. Besides, these indigenous populations could potentially become a vector for the spread of such ARGs and ARBs. Thus, our proposed project will provide baseline data on the ARGs and ARB which could be utilized to demarcate the high-risk permafrost regions for the local population. In this context, we delineated the following objectives for the proposed project:-

Objectives:

- Evaluation of Antibiotic-resistant genes (ARGs), virulence-related genes, and antibiotic-resistant bacteria (ARB) in the Canadian high Arctic Soil, and Permafrost regions
- Genomics of cryopreserved bacteria from permafrost and comparison with their modern counterparts
- To evaluate the presence of resistance genes and analyse antibiotic resistance patterns, thereby allowing to determine the role of anthropogenic pressure in the apparition of antibiotic resistance.

This study will provides baseline information on the possible reactivation of ARB in the permafrost, the risks associated with the release of potentially drug-resistant pathogens and antibiotic-resistant genes from permafrost, and the implications for policy and international cooperation in light of the Covid-19 pandemic.

Progress to Date: Our works on antibiotic resistance in the Svalbard Arctic on migratory birds; Indicated that migratory birds are one of the potential carriers of Antibiotic resistance in the Svalbard Arctic (Prakash et al., 2022). Our studies Antibiotic-resistant gene and bacteria from the Tundra, Marine and permafrost samples from the Svalbard Arctic are going on.

Prakash, E.A., Hromádková, T., Jabir, T., Vipindas, P.V., Krishnan, K.P., Hatha, A.M. and Briedis, M., 2022. Dissemination of multidrug-resistant bacteria to the polar environment-Role of the longest migratory bird Arctic tern (Sterna paradisaea). Science of The Total Environment, 815, p.152727.

Methodology: Field methodology includes the collection of samples from the active layer and Permafrost soils using a field core sampler near the CHARS campus. The core samples will be cut aseptically into different the layers and stored at -80°C. These samples will be transported back to the India (NCPOR-lab) using a cold shipment. In the NCPOR lab these layers will be used for the isolation, culturing, and screening of various health significant bacteria (culture-dependent) and for the extraction of DNA and metagenomics studies (culture-independent).

Data management: Describe your data management plan, including where and when the data and metadata records will be stored

All data will be made available for use by the wider community, but with embargo period (from date of collection) to allow publication of the results beforehand. On publication, the data will be publically

available. Data will be stored at NCPOR, India data centre and will be shared on request before publication also.

Research outputs: Describe the major research outputs to be generated through the project (academic theses, publications, presentations, reports, etc.)

Major Research output expected from the projects were publications and reports.
   Publications include
- Diversity of multidrug-resistant bacteria and antibiotic-resistant genes from Canadian High Arctic.
- Reports on antibiotic-resistant genes and antibiotic-resistant bacteria from Canadian High Arctic.