Global Sewage Surveillance Project

– global surveillance of infectious diseases and antimicrobial resistance from sewage

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Protocol for urban sewage collection

1. EXECUTIVE SUMMARY

The Global Sewage Surveillance Project aims to collect urban sewage globally and analyze it by metagenomics to provide continuous surveillance of antimicrobial resistance as well as other infectious disease agents globally.

The Global Sewage Surveillance Project was launched in 2016 and annual point-prevalence samplings have been conducted since then. From 2020, the project aims to conduct sampling twice a year.

2. PROJECT DESCRIPTION

Background

Human and animal populations are increasingly confronted with novel, emerging or re-emerging infectious, zoonotic and communicable diseases including multi-drug resistant microorganisms. The increased exposure can be linked to increased globalization, urbanization, climate change, population growth, and intensive farming.

Surveillance of pathogens and antimicrobial resistance are essential in disease control and prevention strategies. Human disease surveillance is often hampered due to ethical problems with sensitivity of data from clinical samples and healthy individuals. Sewage has been suggested as an alternative for population based surveillance and the anonymous nature of sewage avoids many ethical concerns.

The rapid development in high-throughput sequencing and metagenomic analysis offers the potential to simultaneously determine the presence and prevalence of a large number of DNA and RNA sequences and thus, greatly enhance our ability to rapidly detect emerging pathogens and related antimicrobial resistance genes. If monitoring of pathogens and antimicrobial resistance in sewage can provide timely information on pathogens of concern, the information can be used to assist risk managers with information on appropriate prevention and treatment strategies and needs for environmental remediation.

This joint study between the WHO and the National Food Institute, Technical University of Denmark (DTU FOOD, WHO Collaborating Centre for Antimicrobial Resistance in Food borne Pathogens and

Genomics) funded by the Novo Nordisk Foundation, will serve as proof-of-concept for applying a metagenomic approach to a global surveillance of human infectious diseases from sewage collected in major cities around the world to detect, control, prevent and predict human infectious disease.

Impact

The most important outcome will be a proof-of-concept of "real-time" large-scale population surveillance combining state-of-the-art technology and analytic facilities that provide better and faster detection and control of health risks. In addition, the project is also expected to provide a proof-of-principle regarding the evaluation of WCS (Whole Community Sequencing) performed directly on sewage samples. The project could establish the foundation for the first surveillance of a large, healthy human population and possibly animal populations. Thus, it could reduce morbidity and mortality through rapid disease detection, reduce the development of antimicrobial resistance through proper drug adherence and enable earlier clinical treatment or interventions, and ultimately improve treatment outcome and minimize disease spread. The outcome of this study could lead to a complete paradigm shift in the way infectious disease surveillance of nationwide or disease hot spots are conducted.

Ethical Issues

All analysis will be conducted in accordance with the Danish Act on scientific ethical treatment of health research administrated and confirmed by the Research Ethics Committees of the Capital Region of Denmark (www.regionh.dk), Journal no.: H-14013582. Thus, it will not be possible to trace back samples or data to any individuals, i.e. an ethical approval is not required.

Publication and IP

The samples will not be transferred to third parties and will solely be used for this Global Sewage Surveillance Project. All metagenomic raw data for each sample in combination with minimum meta-data (location and date) will be deposited in the public domain at the time of publication.

An MTA (Material Transfer Agreement) is offered to all participants prior to each sampling round. Please contact XXXXXXXX, if there is a need for other agreements.

The study will result in a number of scientific publications. It is expected that all partners, either country mediator or sample providers, will be co-authors on the first publication (e.g. one per country (preferred)). Persons also actively participating in the analysis of the samples and/or data analysis may become co-authors on multiple publications. All co-authors will have the opportunity to comment on the manuscript prior to submission. Please notice that we kindly ask all of you to report any changes in regard to e-mail address and other contact information. If we cannot reach you at the time of publication, the data for your samples will still be included in the publication (of course only country- or state wise, as always).

3. DETAILED PROCEDURE

Sampling site and sampling procedure

From each location, one sewage sample of 1L is collected from the main sewage flow of the city's main sewage pipelines prior to the inlet to a wastewater treatment plant (WWTP).

For sampling locations where the sewage is not treated (no WWTP) a representative sewage sample is collected from the final outlet of the sewage network, just before the wastewater is discharged into the environment (e.g. to rivers or lakes). If necessary, samples can be obtained following the pre-filtration step (prior to primary treatment), but no further treatment must take place. Sampling directly from river or lake waters cannot be used.

Whenever possible, the collection of the concentrated flow is performed over 24 hours (i.e. continuous sampling). However, if not possible, three crude point samples are collected in a short time interval, i.e. at least 5 minutes between each sample to ensure as much randomness as possible.

SAMPLING INSTRUCTIONS:

1) Use a clean 1000 mL plastic container with no soap or disinfectant residues.
2) Fill a label for the container with country, city, name of the collector, sample name and date. Use a permanent marker and seal the label with tape to avoid smeared text.
3) Fill the container (leave some space to allow expansion during freezing):
    - collect the sample over 24 hours (from the mid-stream of the sewage inlet)
    - *OR* collect the sample in short time intervals with at least 5 minutes between each of three samples of ~ 300 ml and finally pool the samples to a 1L sample.
4) Record the temperature of the sewage flow and the pH of the sample.
5) Take a photo of the sampling site, if possible.
6) Take the GPS coordinates by a mobile phone (or record the coordinates later via Google Maps or Google Earth). We need the format of latitude/longitude (e.g. 55.787058, 12.519933).
7) Keep the sample as cool as possible and bring it to the local lab within 8 hours upon sampling.
8) Clean the surface of the container with alcohol and pack each container in a plastic bag. Store the sample frozen, preferably at -80°C as soon as possible.

Appendix 1 provides a checklist for materials and activities for the sampling.

After sampling, please submit the sample data via a Survey Monkey link that will be send to you by e-mail upon receiving the sample/samples at DTU. Photos of the sampling sites are also uploaded via this survey.

Sample storage and shipment

Store the containers at -80°C for at least 48 hours (preferred) and prepare shipping the samples to the DTU FOOD, Denmark.

The samples should be packed according to *Appendix 2*. Please, do NOT ship the samples with dry ice, as this would complicate shipping and increase the costs. For the same reasons, please arrange the shipping without temperature restrictions. Please send the samples frozen, packed directly from the freezer. *Appendix 2* provides you with an example of the packing procedures. The shipment is not IATA[1] restricted as per SP A197 (see *Appendix 3*) and can therefore be sent without an UN-label.

The international courier services of DHL or FedEx must be used. Letters of authorization are attached as *Appendix 5.* Please use the shipping account numbers provided below:

- **DHL: XXXXXXXXXX**
- **FedEx: XXXXXXXXXX**

The full shipping address is:

National Food Institute, Technical University of Denmark
Post- and goods reception
Henrik Dams Allé, Bygning 205B
2800 Kgs. Lyngby
DENMARK
Att.: XXXXXXXX
Phone: XXXXXXXX
E-mail: XXXXXXXX

Specific Safety Requirements and Responsibilities

This protocol describes how to collect sewage samples containing human and potentially animal faeces. Blood- and airborne pathogen protection (gloves, lab coat, mask, etc.) must be used when handling human and animal clinical samples.

We encourage staff that handle and work with the sewage to take the proper precautions. Biosafety level-2 (BSL-2/RG-2) practices and procedures must be followed when handling sewage or clinical samples suspected to contain pathogenic organisms. Techniques used to enhance and/or culture environmental samples should be conducted at BSL2 or higher biosafety levels in an appropriate containment device, such as a biological safety cabinet or fume hood. It is the responsibility of the collector and local laboratory to comply with the biosafety rules.

[1] International Air Transport Association