



Antibiotic Resistance in Wastewater

Transmission Risks for Employees and Residents
around Wastewater Treatment Plants

NEWSLETTER

Volume 3 | December 2020

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MESSAGE FROM THE COORDINATION

With the COVID-19 pandemic raging across the world the AWARE research team was also affected. How you can read on the next pages of this last Newsletter. The virus reached Germany the 28th of January, the Netherlands at the 27th of February and had affected all EU/EEA countries by the 25th of March. Lockdowns and other intervention measures were enacted in all AWARE partner countries. When I first learned that the novel coronavirus SARS-CoV-2 was excreted in feces at the end of January we studied whether people working with sewage would be at risk. And we learned that when the current practice in personal protective measures for people working with sewage to protect them from pathogens are also effective against coronaviruses. To date the virus has not been cultured from wastewater and no one has been reported contracting the disease from sewage supporting that sewage is not a transmission route for the virus. And within the AWARE team we will continue research to investigate whether employees of wastewater treatment plants have a higher risk of carrying resistant bacteria. We will keep you posted



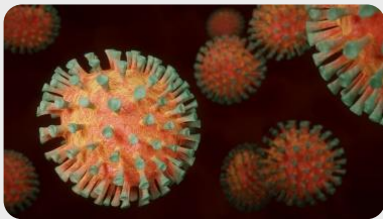
**Prof. Dr. Ana Maria de Roda
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Study Coordination
AWARE



MORE INFO

- Covid-19:
 - Infectious disease caused by coronavirus SARS-CoV-2
 - Worldwide pandemic
 - Most common symptoms: fever, dry cough, tiredness
 - Virus detectable in wastewater



<https://www.pexels.com>

How Covid-19 has impacted our study centres

After recruiting participants in three Bavarian cities the German study team planned to include another town into the study to increase the data available for analyses. Unfortunately, the Covid-19 pandemic has thwarted our plans and we needed to decide whether or not to continue with our work. The Dutch and Romanian study teams were also confronted with the same problem. Since we are aware of our responsibility towards our participants and our study teams the field phase of AWARE had to be closed in all countries in April 2020. During air and water sampling our teams would have been in close contact to workers of the wastewater treatment plants and the risk of spreading the virus in either direction would have been too high. To avoid exposing the participants to any danger while handing in their stool samples, we decided to end the field phase earlier than planned and to start analysing our already collected data.

Another reason for termination was that because of changes in daily human activities (i.e. decreased travel, social distancing, increased hand hygiene) the transmission of the investigated target resistant bacteria and resistance genes was likely to have changed as well, which would have obliterated comparability of pre- and post-Covid-19 participants. Further, considering the fact that current studies assume that the virus is detectable in wastewater, ending the field phase was the right decision. Samples taken after the outbreak of the pandemic would not have been comparable to the ones taken before.

REWARDS

Rewards for participants

We thank everybody who supported this study by providing us with filled-in questionnaires and stool samples. Without the help of hundreds of participants this study would not have been possible. In order to acknowledge the participation in our study and to appreciate the effort involved, each study centre has come up with a small reward for its participants.



<https://www.pexels.com>

Results of the analyses

After analysing all received stool samples regarding antibiotic resistant bacteria in our labs, all study centres sent their participants their personal results via post. The Romanian labs isolated the DNA from the stool samples collected in Romania and the Netherlands and used antibiograms and PCR to analyse resistance genes. Besides the stool samples, the bacterial strains from the water samples taken from the wastewater treatment plants of all included countries have already been analysed by the Romanian team. Further analyses of the stool isolates and the air samples taken in the plants are planned for the future.

PUBLICATIONS

Progress of our publications

Currently, we are working on the publications by analysing and interpreting data as well as by researching important information on the topic of antibiotic resistant bacteria in wastewater. A methods paper describing the conduct of our study will be available soon.

A few weeks ago, the Swedish team published a paper with a topic that will be examined with data of the AWARE study as well.

The research team has discovered more than a hundred previously undiscovered genes predicted to provide resistance to the commonly used antibiotics tetracycline. By using probabilistic models specifically designed to identify new tetracycline resistance genes, the team has analyzed large amounts of publically available genomic and metagenomic data. The data came from various environments such as the human gut, polluted water and wastewater treatment plants. Seventeen of the predicted genes were selected for experimental verification with seven of them resulting in a resistance phenotype when expressed in an *Escherichia coli* host. The results from this study show that the tetracycline resistome is far greater than previously known and describe resistance genes that may be mobilized and transferred into pathogens in the future.

INFO

Analysed bacteria:

- ESBL-producing *Escherichia coli* (*E. coli*)
- Carbapenemase-producing Enterobacteria (CPE) analysed by Chrom ID OXA and Chrom ID CARBA

Antibiogram:

- A collection of data summarizing the antimicrobial resistance and susceptibility of individual bacterial pathogens

PCR (= polymerase chain reaction):

- A method to make many copies of (amplify) small sections of DNA or a gene to get enough material to study in detail



<https://pixabay.com>

PUBLICATIONS

- Comprehensive screening of genomic and metagenomic data reveals a large diversity of tetracycline resistance genes (Berglund et al., 2020:
<https://doi.org/10.1099/mgen.0.000455>)
- SARS-CoV-2 in wastewater: potential health risk, but also data source (Lodder & de Roda Husman, 2020:
[https://doi.org/10.1016/S2468-1253\(20\)30087-X](https://doi.org/10.1016/S2468-1253(20)30087-X))

Wastewater surveillance of SARS-CoV-2

In The Netherlands, measurements in raw wastewater from WWTPs has been used as a tool to gain insight in the spread of SARS-CoV-2. SARS-CoV-2 can be detected in sewage, because approximately 40% of the carriers shed virus with feces. At the start of the Dutch outbreak, two municipal WWTPs were investigated that were located in the region where the first COVID-19 patients were recorded, as well as the WWTPs from the main Dutch airport, Schiphol. Over the course of the year, the number of investigated treatment plants was increased step-wise, and as of 7 September 2020, all 316 Dutch municipal WWTPs (and the Schiphol WWTP) are sampled weekly. As in the Netherlands human surveillance (so far) only includes people with symptoms, wastewater surveillance is a valuable addition, because the virus in sewage is derived from all carriers (who shed virus with feces), including people without symptoms or prior the onset of symptoms. Also, wastewater measurements can potentially serve as an early warning method, since shedding of virus occurs before the onset of symptoms. This hypothesis is currently being validated through comparison of wastewater and human surveillance data.

UPCOMING EVENTS IN 2021

BEIJING, CHINA, SEP 12 – 16, 2021

13th IWA Conference on Instrumentation, Control and Automation
<http://iwa-mia.org/upcoming-events/>

GOTHENBURG, SWEDEN, MAY 29 – JUN 3, 2022

EDAR 6: 6th International Symposium on the Environmental Dimension of Antibiotic Resistance
<https://www.gu.se/en/care/edar-6-gothenburg-2021>



PARTICIPATING



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport



SUPPORTING



AWARE (Antibiotic Resistance in Wastewater: Transmission Risks for Employees and Residents around Wastewater Treatment Plants) is supported by the European Commission (JPI-EC-AMR ERA-Net Cofund grant no 681055).

