WBE AND ENVIRONMENTAL MONITORING OF ANTIMICROBIAL RESISTANCE IN EUROPE AND AFRICA

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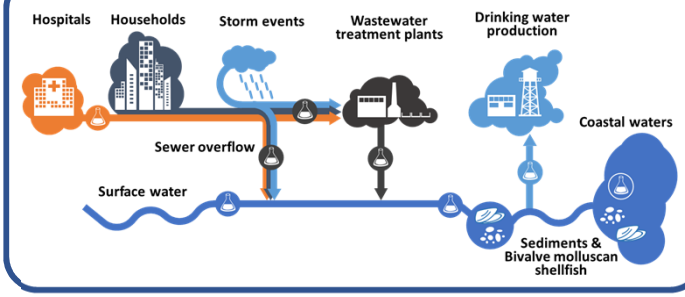
BACKGROUND

Wastewater-based epidemiology approach as used for the monitoring of the prevalence and spread of COVID-19 in communities can also be used for antimicrobial resistance (AMR) monitoring. AMR is a growing global public health threat because it significantly reduces the effectiveness of antibiotics in treating infections. By analysing wastewater, information on the types of antibiotic resistant bacteria and antibiotic resistance genes present in the wastewater of a particular community or region can be obtained. Furthermore, wastewater has been identified as an important pathway for the spread of antimicrobial resistance in the environment. Antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are environmental contaminants that circulate among humans, animals and the environment. ARB can self-replicate and disseminate ARGS through horizontal gene transfer. The international research project SARA within the AquaticPollutants Joint Transnational Call 2020 determines the prevalence of ARB and ARGs in wastewater, WWTP effluents and surface water.

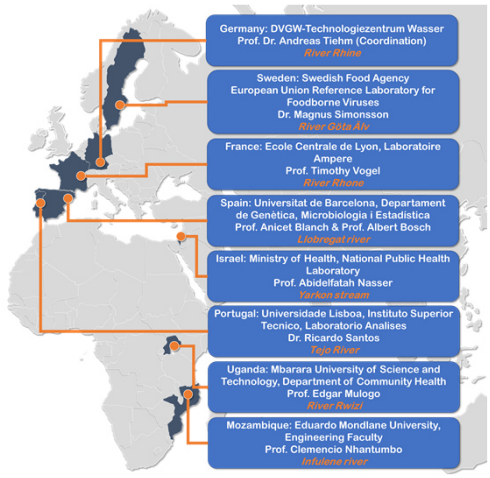
AIMS

- Harmonization of methods and training of European and African partners
- Detection of AMR in raw sewage
- Monitoring ARB an ARGs in surface waters
- Determining the impact of climate and extreme weather events
- Microbial risk assessment

SAMPLING POINTS



PARTNERS & MODEL SITES



RESULTS

Harmonisation of methods

Cultivation
- *E. coli*
- ESBL-producing *E. coli*
- Somatic coliphages
- F-specific coliphages

DNA/RNA
- Isolation of bacterial DNA
- Isolation of viral DNA and RNA

PCR
- SARS-CoV-2, AdV, NV, HAV, HEV, EV
- Human MST markers (CrAssphage, HF183, HMBif)
- Antibiotic resistance genes
- Metagenomics

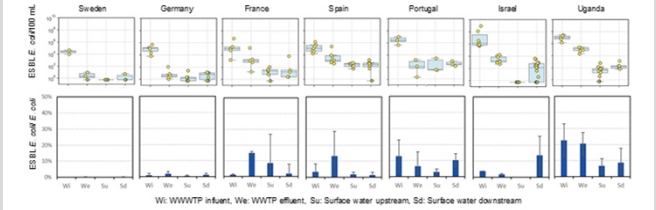
Analyses are carried out by **all partners** and have therefore been **harmonised**

➡ Booklet available on SARA website (www.sara-project.info)

Nucleic acid extracts are **exchanged** and analyzed in **specialized laboratories**

Quantification of antibiotic resistant bacteria

Extended spectrum beta-lactamase (ESBL) producing *E. coli* have been selected as indicators of antibiotic resistant bacteria.



➡ The ratio of ESBL *E. coli* to *E. coli* shows high variance depending on the model site and sampling location.

Initial screening of antibiotic resistance genes

Metagenomic analysis

In total, 229 ARGs were identified. 10 most abundant genes (normalized to sequencing depth) were:

- ATP-binding cassette antibiotic efflux pump genes *msbA*, *macB* and *novA* (multidrug resistance)
- Resistance-nodulation-cell division antibiotic efflux pump genes *muxB*, *mdtC*, *mexK*, *muxB*, *mexW* and *mdtC* (multidrug resistance)
- Phosphoethanolamine transferase gene *ugd* (peptide antibiotic resistance)

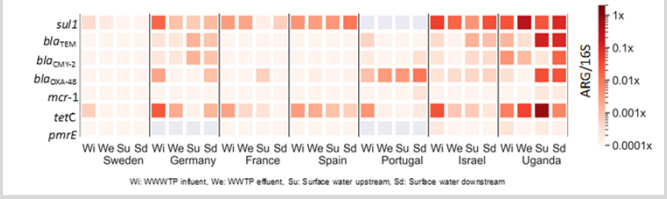
Quantitative PCR

ARGs examined by qPCR could be grouped into three categories:

Frequently detected in high concentration	*sul1* (sulfonamide resistance) bla_{TEM} (beta-lactamase) *tetC* (tetracycline resistance) *ermB* (macrolide resistance)
Frequently detected in medium concentration	bla_{CMY-2} (beta-lactamase) bla_{OXA-48} (beta-lactamase) $bla_{TEM-52,-52}$ (beta-lactamase) *tetM* (tetracycline resistance)
Not detected or detected with low frequency in low concentration	bla_{NDM-1} (beta-lactamase) bla_{KPC-1} (beta-lactamase) *mcr-1* (peptide antibiotic resistance) *vanA* (glycopeptide resistance)

➡ Selection of key ARGs for intensive monitoring

Relative abundance of antibiotic resistance genes



FUTURE WORK

In the further course of the project the monitoring will be continued under inclusion of extreme events. Assembly-based approaches will be used to associate ARGs to their carrying ARB and plasmids and microcosm-based studies will provide insights on the persistence of ARB and ARGs in aquatic environments.

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