Tracking and Assessing the Risk from Antibiotic Resistance Genes using Chip Technology in Surface Water Ecosystems (TRACE)

Wolfgang Fritzsche

Final Evaluation Meeting of the Water JPI Pilot Call Projects

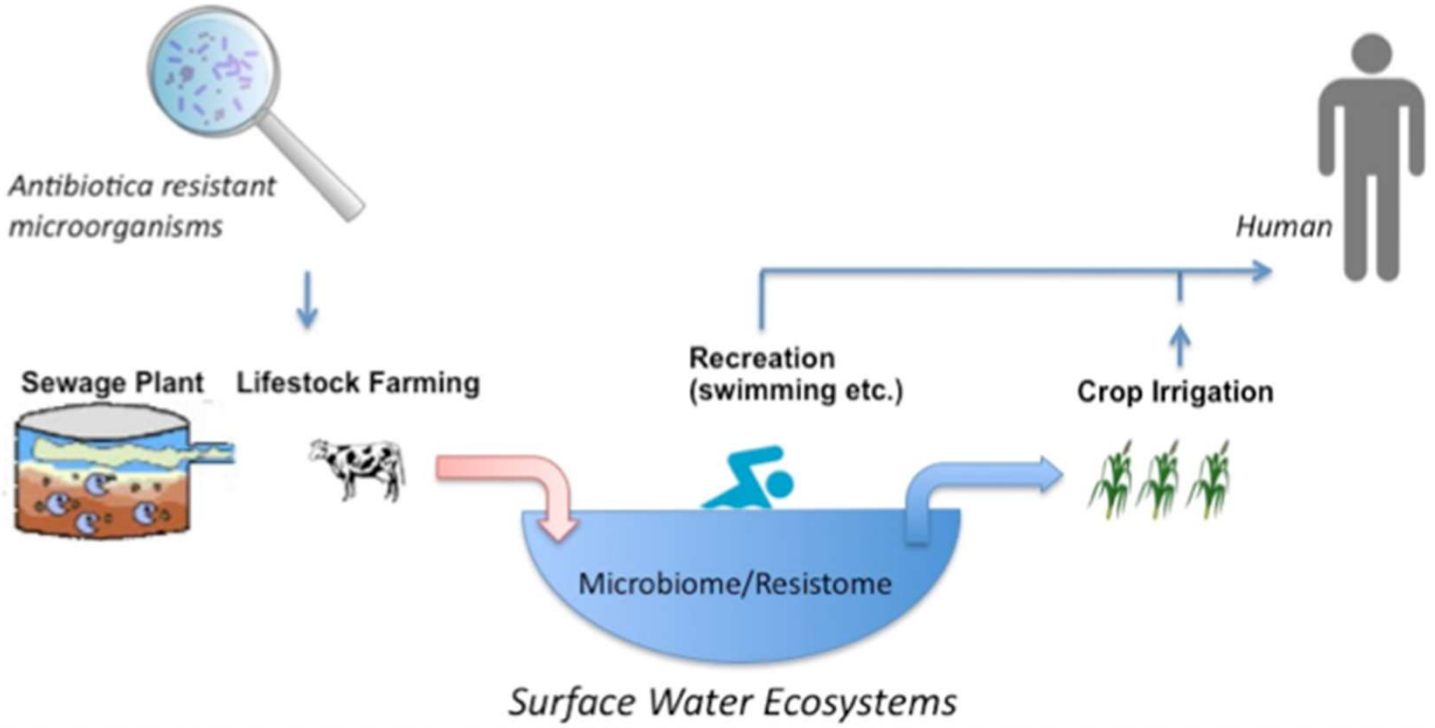
"Impact to Science and to Society"

Marina Congress Centre, Helsinki, Finland

June 4th 2018

Focus of TRACE

- Understand the sources and behaviour of antibiotic resistance in natural surface waters and infection routes
- Development of a novel detection technologies as (1) a on-site fast assay as well as (2) a chip-based solution to detect a panel of antibiotic resistance genes (ARG) for waterborne microorganisms, allowing time- and cost-efficient evaluation of AR patterns and the associated risk for human health



Partners

UCD DUBLIN

• Risk assessment

LEIBNIZ-INSTITUT für PHOTONISCHE TECHNOLOGIEN
ipht jena

• DNA chip design & test
• Chip optimization

UNIVERSIDADE NOVA DE LISBOA

• Probe design & optimization
• Lab tests

Food Analytik & Consulting

• Identification of marker organisms and sequences
• one-target assay

ICRA Institut Català de Recerca de l'Aigua

• Antibiotic pollution
• ARGs quantification/screening
• Selection of target ARGs

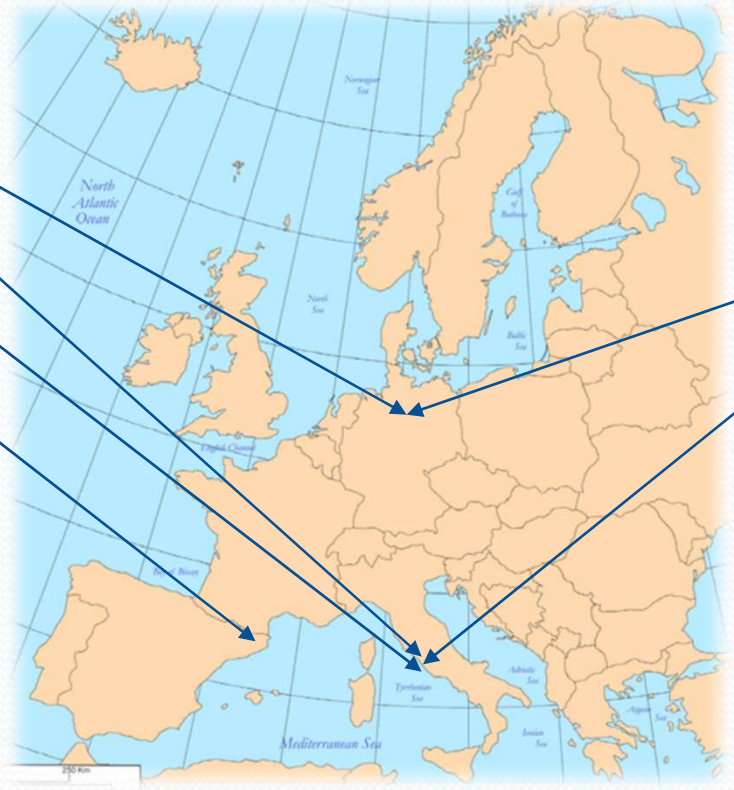
SAPIENZA UNIVERSITÀ DI ROMA

• ARB isolation and characterization

Scientific and technological results -
Characterization of impacted sites

5 study sites:

- Rivers
  - Saale (GE)
  - Arrone (IT)
  - Tiber (IT)
  - Ter (SP)



- Other
  - Luetsche lake (GE)
  - Ostia Beach (IT)

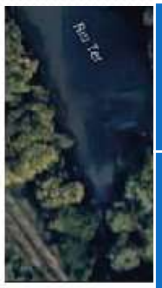
Scientific and technological results - Identification of biomarker genes

- Selected Genes

Gene	Product	Resistance to / Mode of action	Number of Variants	Target variant
qnrS	Pentapeptide repeat family, which protects DNA gyrase from inhibition by quinolones	Fluoroquinolones / DNA replication	5	qnrS1
bla$_{TEM}$	Class A ß-lactamases that hydrolyse penicillin and related antibiotics	Beta-lactam antibiotics / Cell wall synthesis	Many	bla$_{TEM-1}$
intI1	Class I integron integrase	Gene capture	None	Anthropogenic pollution
uidA	ß-glucuronidase	Hydrolysis of glucoronides	E. coli (≈97%) Shigella Salmonella	Fecal contamination of water

ARGs

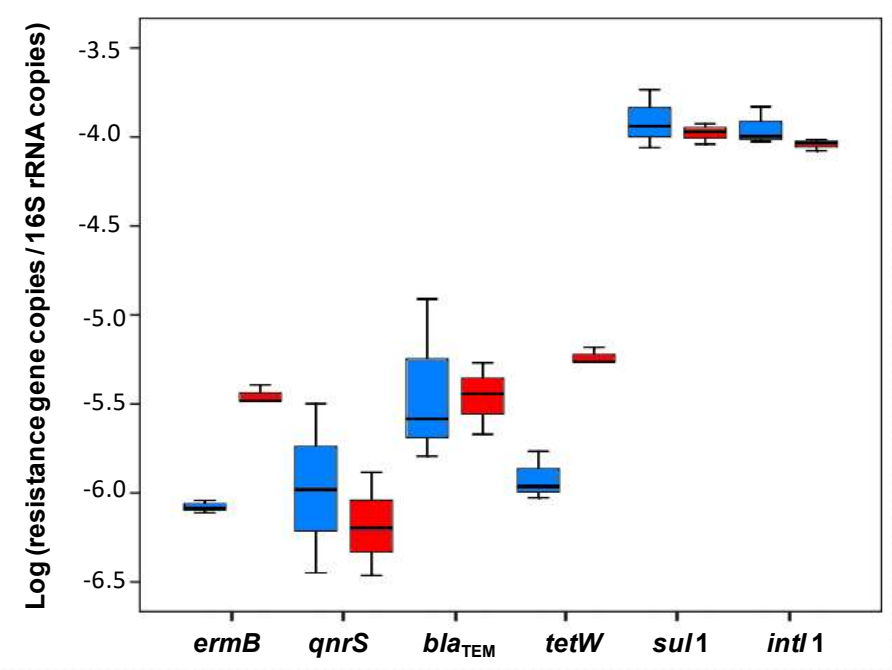
Scientific and technological results -
Characterization of impacted sites



System	Location	Date	Analyses[1]
Ter	Girona (Spain)	07/10/2015	AB, ARB, ARG
		15/04/2016	ARB, ARG, MTG
		12/07/2016	ARB
		13/12/2016	ARB
Saale	Jena (Germany)	30/07/2015	AB, ARG
		13/10/2015	
		11/01/2016	
		14/03/2016	
		09/05/2016	
		20/07/2016	
Lago Luetsche		03/06/2015	AB, ARG
Tíber	Roma (Italy)	07/09/2016	AB, ARG
		12/12/2016	
		25/04/2017	
		12/07/2017	
Arrone		24/08/2016	AB, ARG
		05/12/2016	
		18/04/2017	
		18/07/2017	

Scientific and technological results –
Quantification of ARGs

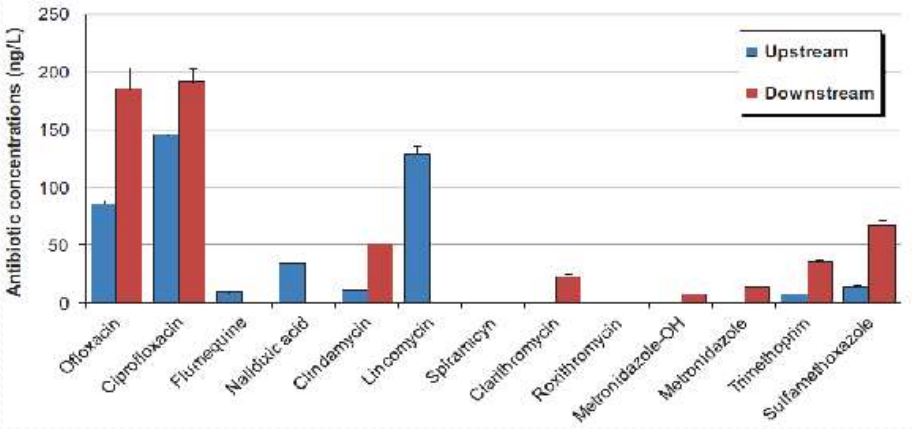
Relative concentration of ARGs in water samples from impacted (red) and non-impacted (blue) sites at the Luetsche Lake.



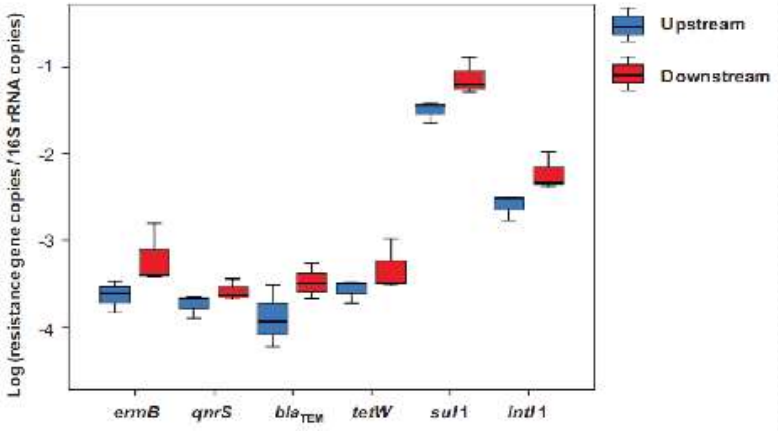
Scientific and technological results – Quantification of ARGs

- Antibiotic pollution and abundance of ARGs in river Ter

Antibiotic exposition in river Ter samples collected upstream and downstream sites

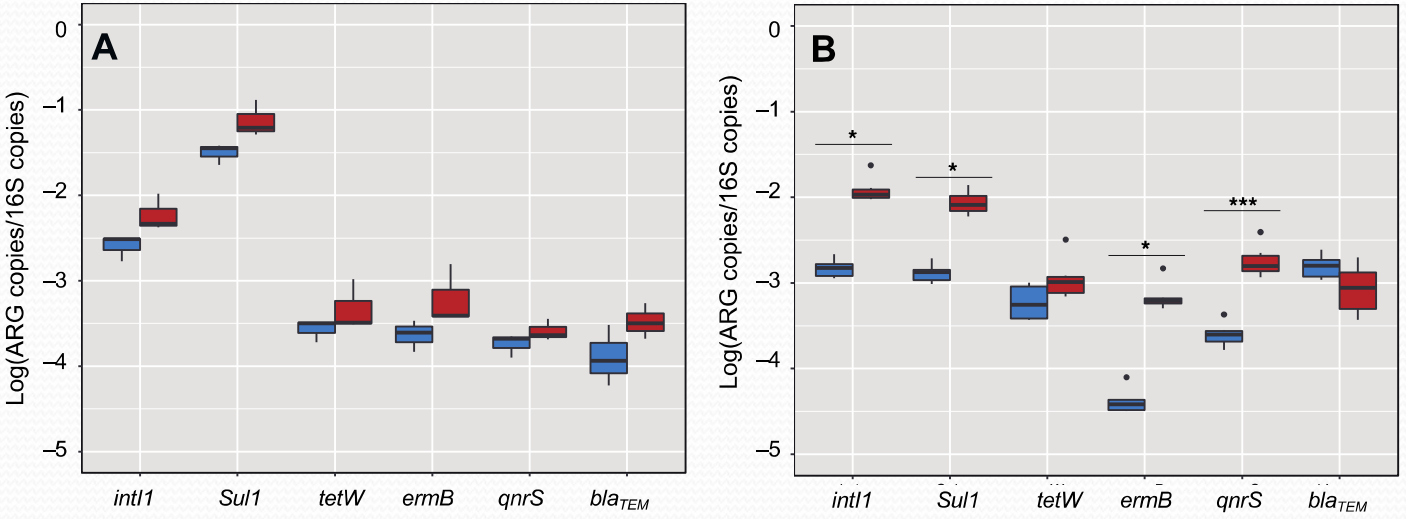


Relative concentration of ARGs in river Ter



Scientific and technological results –
Quantification of ARGs

Normalized concentration of target ARGs in water samples from river Ter collected upstream (blue) and downstream (red) the WWTP effluent discharge point in October 2015 (A) and April 2016 (B).

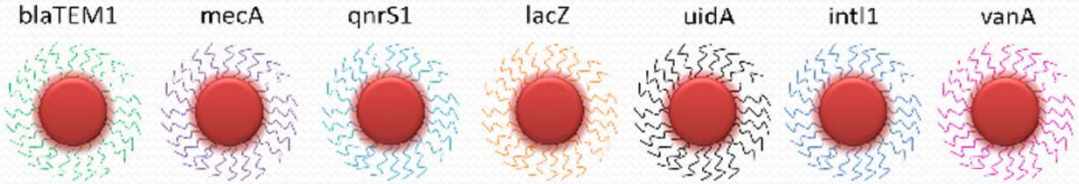


Scientific and technological results –

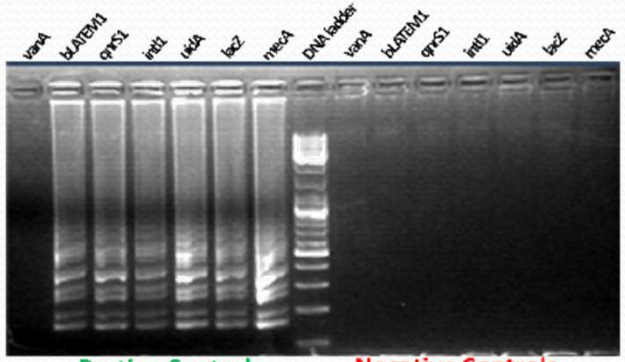
On-site detection technology development – A: Fast Assay

- Simple semi-quantitative assay for point-of-need: gold nanoprobes-based colormetric assay for marker pathogen and ARG identification

Gold Nanoprobes developed for the detection of ARGs

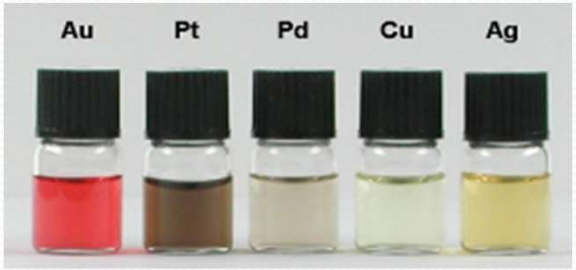


LAMP design and development for each of the ARGs



Colorimetric assay

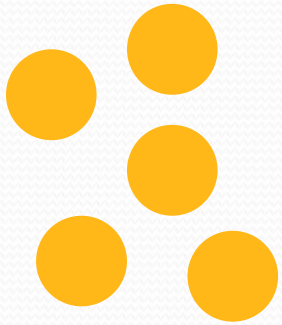
Localized Surface Plasmon Resonance (LSPR)



composition

size

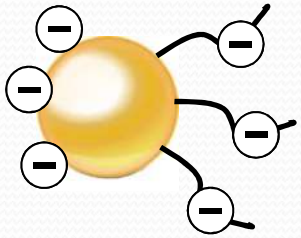
geometry

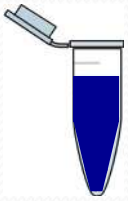


aggregation

+ salt

electrostatic *steric*

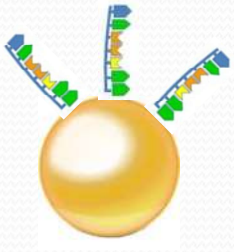




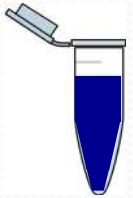
stabilization

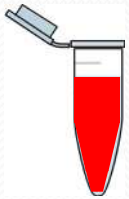
DNA sensing by LSPR

no target DNA

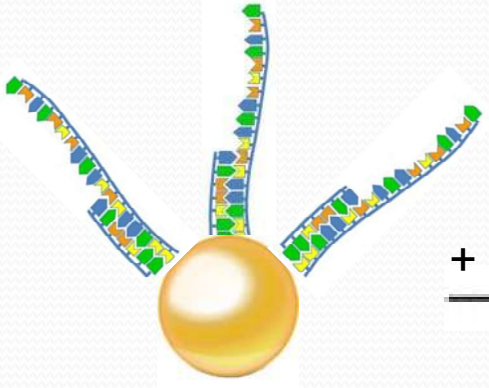


+ salt

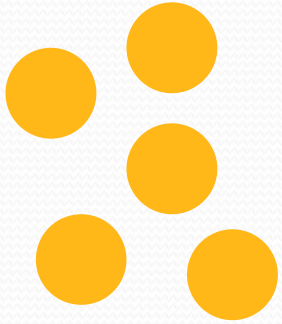


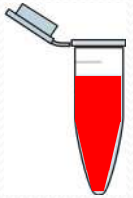


target DNA



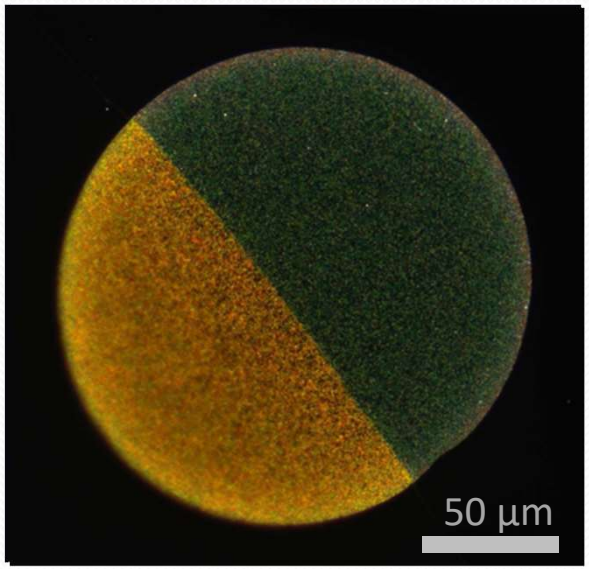
+ salt

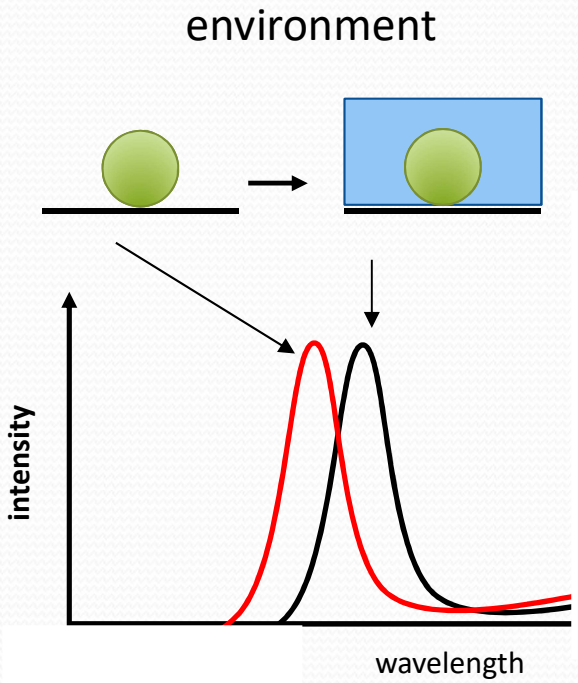




Scientific and technological results –

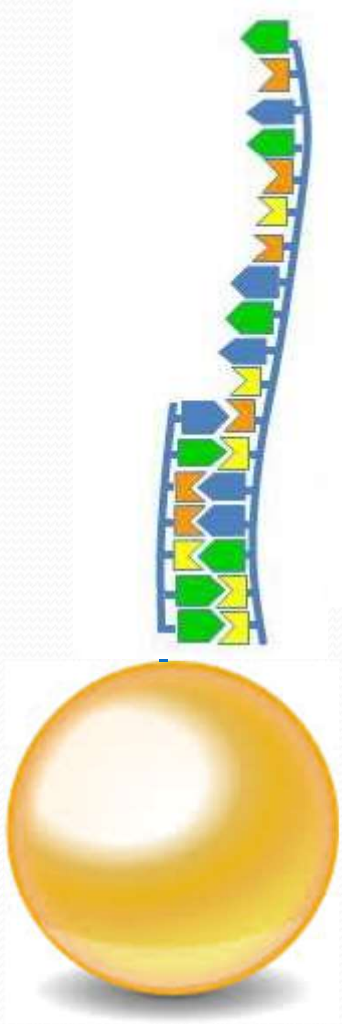
On-site detection technology development – B: Microarray





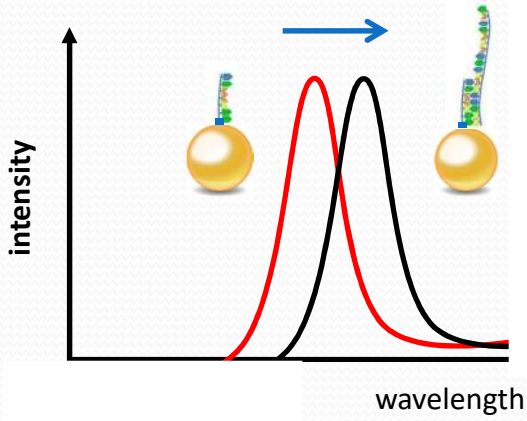
DNA sensing by LSPR

probe DNA (surface attached): capture DNA



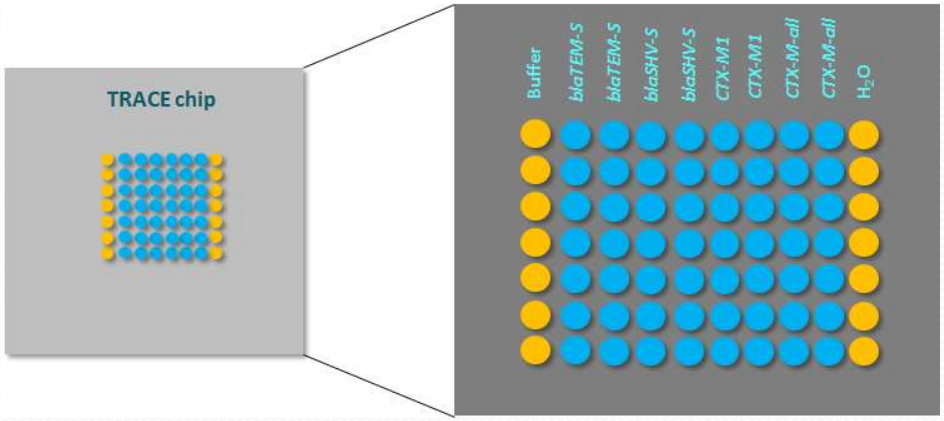
target (analyte) DNA

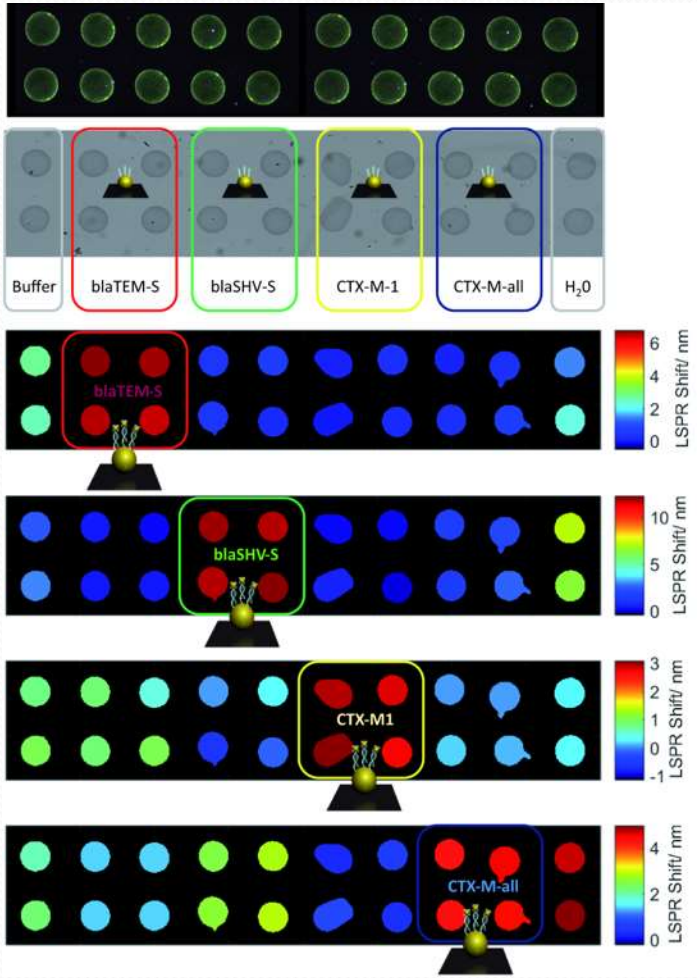
signal: wavelenght shift



Scientific and technological results –
On-site detection technology development - B

- Microarray chip for 4 ARGs



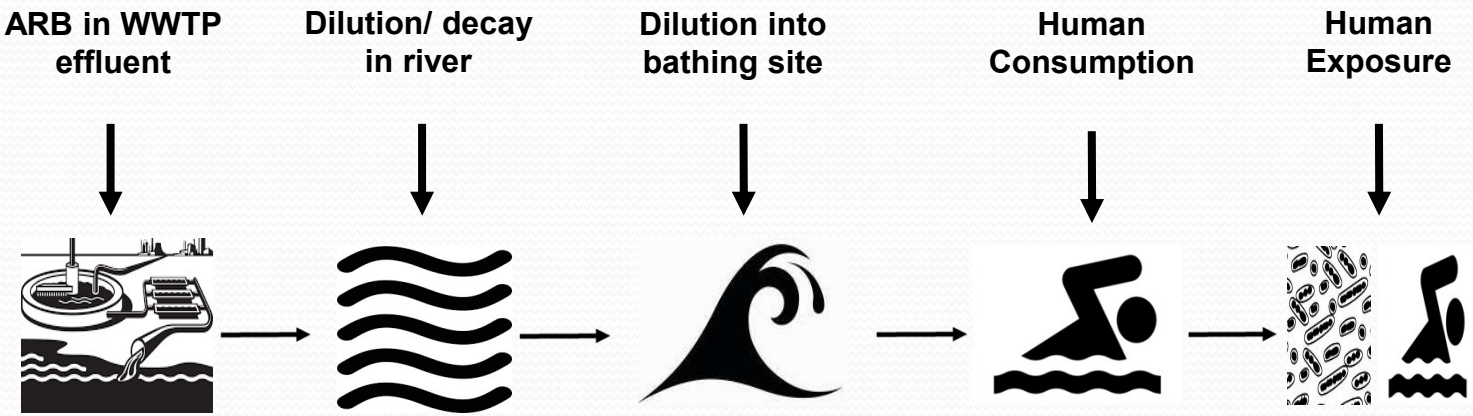


Scientific and technological results

Human Health Risk Assessment Model

Risk models for the prediction of environmental behaviour of antibiotic-resistant microorganisms in surface waters

- Drinking water model
- Recreational water model
- Irrigations model



Collaboration, coordination, mobility – Interactions in Consortium

- FFCT and ICRA developed a straight collaboration during this period for the evaluation of the ARGs selected by the consortium
- Sample sharing, e.g. FOOD provides samples from Germany to ICRA and to IPHT
- Ongoing site characterization for risk analysis with close collaboration between UCD, ICRA, Uniroma
- All the necessary FASTA files for sequence alignment in order to develop LAMP primers and probes sequences were provided by ICRA to FFCT
- DNA templates and biological samples were provided by ICRA to FFCT for LAMP amplification and posterior detection with Au-nanoprobes.
- Exchange of PhD students between Uniroma and ICRA was done on May 2017. Particularly, one student from Uniroma carried out a short stay (1-month) to learn the basics of qPCR technique for the quantification of ARGs in samples collected at the Italian systems (Ostia beach, rivers Tiber and Arrone).
- IPHT and FOOD are in direct contact related to practical protocols and PCR samples to validation.
- ICRA and UNL provide samples for validation by FOOD/IPHT.
- IPHT hold strong cooperation with all partners and coordinated the work as well as dissemination: home-page, joint poster presentation, etc.
- Four joint meetings were organized (ICRA, Uniroma, UCD and IPHT) with the participation of all project partners and external attendees. The project was finished with a workshop with internal as well as external presentations.

→ Strong interactions in consortium, both scientific as coordinative, synergies

Collaboration, coordination and mobility - Consortium Meetings

N°	Date	Location	Attending partners	Purpose
1	15/06/2015	Girona	All	**Kick-Off meeting**: Coordination of project activities and research plan, Sampling schedule of model ecosystems Coordination issues, Agreement on firsts deliverables, creation of project website.
2	26/06/2016	Rome	All	**Mid-Term meeting**: Pre discussion, - Milestones activities and project dela Coordination issues, Disse conferences, stakeholder engagement, etc.)
3	29-30/06/2017	Dublin	All	**Last-Term meeting**: Presentat stakeholders, General discussion delays, Coordination issues, Diss publications, conferences, stakeh
4	7-8/12/2017	Jena	All	**Final meeting**: Presentation of final results to partners and stakeholders, pending tasks and problems unsolved, coordination issues, publication and dissemination strategy.

Collaboration, coordination and mobility - Conferences and Workshops, dissemination

- Participation on
  - 19 conferences and workshops, national and international
- Home page: http://jpi-trace.eu/
- Linkedin group: https://www.linkedin.com/groups/8385238
- Local actions adressing the general public

Stakeholder engagement

- The UCD-team has **6 monthly project meetings** and has a project steering group with relevant stakeholders included. This includes representatives from the Irish EPA and Irish water (water utility company in Ireland responsible for all water services including water treatment and distribution).

- **TRACE workshop 2017 (Dublin):** A workshop event focusing AR was organised by UCD project partners at the University College Dublin on June 29th 2017. The event was attended by stakeholders (Irish water, HSE), companies (H2Ozone, Quest Utility Services, Humanist Times), general public, steering committee (EPA, King's College London, Brunel University), researchers (University Limerick, NUI Maynooth, NUI Galway, Ulster University, Dublin Institute of Technology, Limerick Institute of Technology iCRAG, Trinity College Dublin) and TRACE project partners (58 participants).

- **TRACE workshop 2018 (Jena):** A workshop event was organized by IPHT on 7th December 2017. This event was organized from the project coordinator with participation of researcher (project partners as well as clinical and federal research institutes) and public administrations (e.g. ,Thuringian water reservoir administration).

Impact and knowledge output

- 1. O'Flaherty, E., C.M. Borrego, J.L. Balcázar and E. Cummins (2017) Human exposure assessment to antibiotic-resistant Escherichia coli through drinking water. Sci. Total Environ. Vol. 616-617, pp.1356-1364. doi.org/10.1016/j.scitotenv.2017.10.180
- 2. Subirats, J., X. Triadó-Margarit, L. Mandaric, V. Acuña, J.L. Balcázar, S. Sabater and C.M. Borrego (2017) Wastewater pollution differently affects the antibiotic resistance gene pool and biofilm bacterial communities across streambed compartments. Molecular Ecology, 26: 5567–5581.
- 3. Lekunberri I., M. Villagrasa, J.L. Balcázar and C.M. Borrego (2017) Contribution of bacteriophage and plasmid DNA to the mobilization of antibiotic resistance genes in a river receiving treated wastewater discharges. Sci. Total Environ. 601–602: 206-209. DOI: 10.1016/j.scitotenv.2017.05.174.
- 4. Lekunberri, I., J.L. Balcázar and C.M. Borrego (2017) Detection and quantification of the plasmid-mediated mcr-1 gene conferring colistin resistance in wastewater. Internat. J. Antimicrob. Agents. 50(6): 734–736. DOI: 10.1016/j.ijantimicag.2017.08.018.
- 5. Subirats, J., E. Royo, J.L. Balcázar and C.M. Borrego (2017) Real-time PCR assays for the detection and quantification of carbapenemase genes (blaKPC, blaNDM and blaOXA-48) in environmental samples. Environ. Sci. Pollut. Res. 24:6710–6714. DOI: 10.1007/s11356-017-8426-6.
- 6. Lekunberri, I., J. Subirats, C.M. Borrego and J.L. Balcázar (2017) Exploring the contribution of bacteriophages to antibiotic resistance. Environ. Pollut. 220(Pt B):981–984. DOI: 10.1016/j.envpol.2016.11.059
- 7. Subirats, J., A. Sànchez-Melsió, C.M. Borrego, J. L. Balcázar and P. Simonet (2016) Metagenomic analysis reveals that bacteriophages are reservoirs of antibiotic resistance genes. Internat. J. Antimicrobial Agents 48: 163-167. DOI: 10.1016/j.ijantimicag.2016.04.028.
- 8. O'Flaherty E and Cummins E. (2017a). Antibiotic resistance in surface water ecosystems: presence in the aquatic environment, prevention strategies and risk assessment. Human and Ecological Risk Assessment Vol. 23, No. 2, pp. 299–322. doi.org/10.1080/10807039.2016.1247254
- 9. Li, G., D. Zopf, G. Schmidl, W. Fritzsche, and O. Stranik. "Concentric Dot-Ring Metal Nanostructures Prepared by Colloidal Lithography." Applied Physics Letters 109, no. 16 (2016): 163101.
- 10. Thiele, Matthias, Andrea Knauer, Daniell Malsch, Andrea Csaki, Thomas Henkel, J. Michael Kohler, and Wolfgang Fritzsche. "Combination of Microfluidic High-Throughput Production and Parameter Screening for Efficient Shaping of Gold Nanocubes Using Dean-Flow Mixing." Lab on a Chip 17, no. 8 (2017): 1487-95.
- 11. Kosman, Joanna, Jacqueline Jatschka, Andrea Csaki, Wolfgang Fritzsche, Bernard Juskowiak, and Ondrej Stranik. "A New Strategy for Silver Deposition on Au Nanoparticles with the Use of Peroxidase-Mimicking Dnazyme Monitored Via a Localized Surface Plasmon Resonance Technique." Sensors 17, no. 4 (2017): 849.

Under review:

- O'Flaherty E., Membré JM. and Cummins E. (2018a) Meta-analysis of the reduction of sensitive and antibiotic resistant Escherichia coli as a result of low and medium pressure UV lamps
- O'Flaherty E., Solimini A. Pantanella F. and Cummins E. (2018b). Human exposure assessment to antibiotic resistant Escherichia coli through the irrigation of lettuce.

Manuscripts in preparation

- 1. Lekunberri, I., M. Villagrasa, J.L. Balcázar, B. Giese, J. Müller and C.M. Borrego. Seasonal variations of ARGs in the Saale River (Germany).
- 2. Lekunberri, I., G. Venutto, J. Subirats, A. Solimini, J.L. Balcázar and C. M. Borrego. Antibiotic resistance in two Italian rivers impacted by wastewater treatment plant discharges.
- 3. Ferreira, C.F., A.S. Matias, C. Roma-Rodrigues, J. Subirats, I. Lekunberri, J.L. Balcázar, C.M. Borrego and P.V. Baptista. Au-nanoprobes coupled to isothermal amplification for screening antibiotic resistance genes in surface waters.
- 4. O'Flaherty E., Solimini A. Pantanella F. and Cummins E. (2018c). Human exposure assessment to antibiotic resistant Escherichia coli through recreational water.
- 5. Zopf, D., Pittner A., Dathe A., Grosse N., Csáki A., Fritzsche W., Stranik O. Pathogen identification and detection by plasmonic microarray
- 6. Solimini, Venuto, Gagliardi, Schippa, De Giusti, Pantanella. Quantification of antibiotic resistant E. coli in two river systems of Central Italy

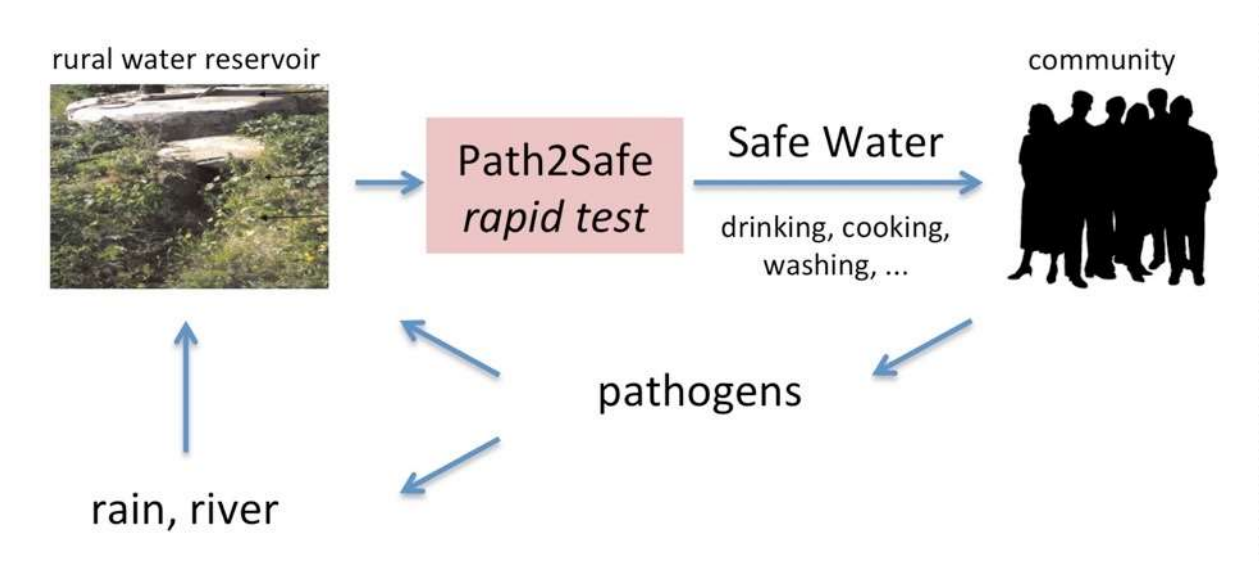
Impact and knowledge output

- Better knowledge on the impact of wastewater discharges on water systems (rivers Ter and Saale). Antibiotic pollution in both rivers and the impact of the anthropogenic pollution on the river resistome and mobilome were evaluated.
- Effect of wastewater discharges on the concentration of virulent and antibiotic-resistant *E. coli* in rivers Ter, Tiber and Arrone → risk to human health → elaboration of appropriate risk assessment models.
- The Meta-analysis model can be used to improve accuracy of risk assessment models investigating the effect of UV treatment on AR or AS *E. coli*. In particular risk assessment models examining the human exposure to AR *E. coli* through drinking water or examining the impact of a WWTP located near a recreational site could use this data to predict AR or AS *E. coli* concentrations found after UV treatment. The Drinking water model can provide guidelines to water management entities on the best water treatment combinations to use to provide the lowest human exposure to AR *E. coli*. The results can help set acceptable levels of AR *E. coli* in source water for DWTP. This study shows how research and risk assessment can help improve water regulations. The Irrigation model could help set local guidelines for producers on maximum permissible contamination levels in irrigation water. The results provide recommendations on the most suitable post-harvest treatment to use to reduce the human exposure to AR *E. coli*. The Recreational model also provides information on the possible human exposure levels to AR *E. coli* through recreational water.
- The nanoprobe based systems still require optimization but may be used for fast screening of the relevant ARGs in water beds. LAMP is a robust, yet simpler, alternative to PCR based approaches and the Au-nanoprobes potentiate specificity of amplification analysis. The system might be of use for the fast screening at point of need without the need for cumbersome equipment. Possible translation to a SME is under evaluation.
- The (nano)technological development of a plasmonic-array chip platform for multiplex molecular detection with optical readout allows for an effective discrimination of several targets in one assay, surpassing limitations of established analytical approaches in that field.

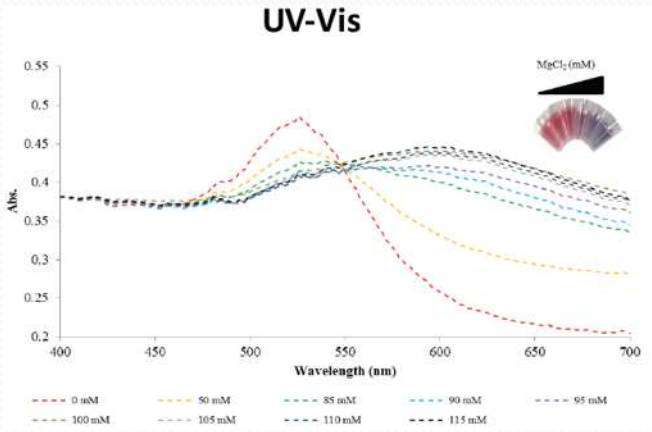
Continuation of the work in the future

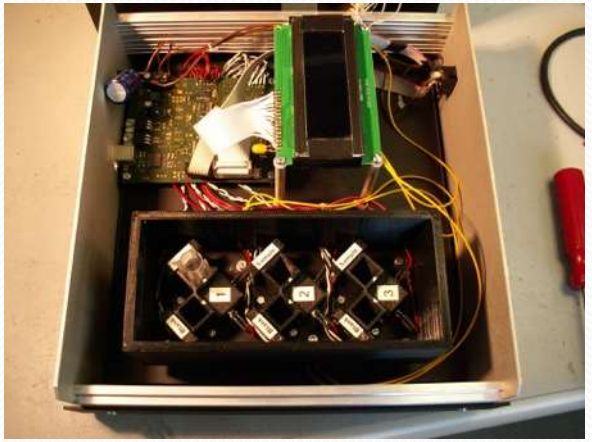
- Application "A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - **Path2Safe**" submitted in the research funding initiative PRIMA (partnership for research and innovation in the Mediterranean area) in March 2018.

A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - Path2Safe

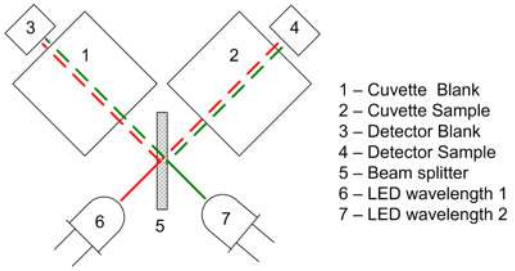


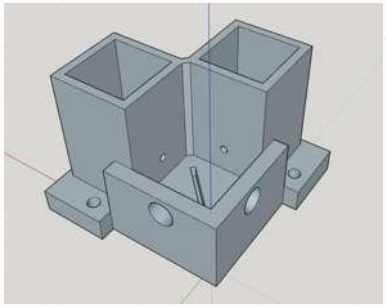
A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - Path2Safe

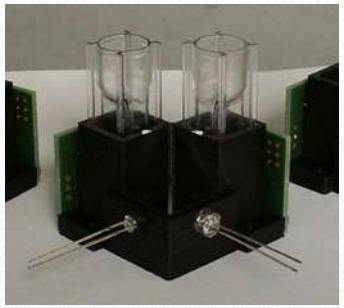




Pedro Baptista, Lisbon







Matthias Urban

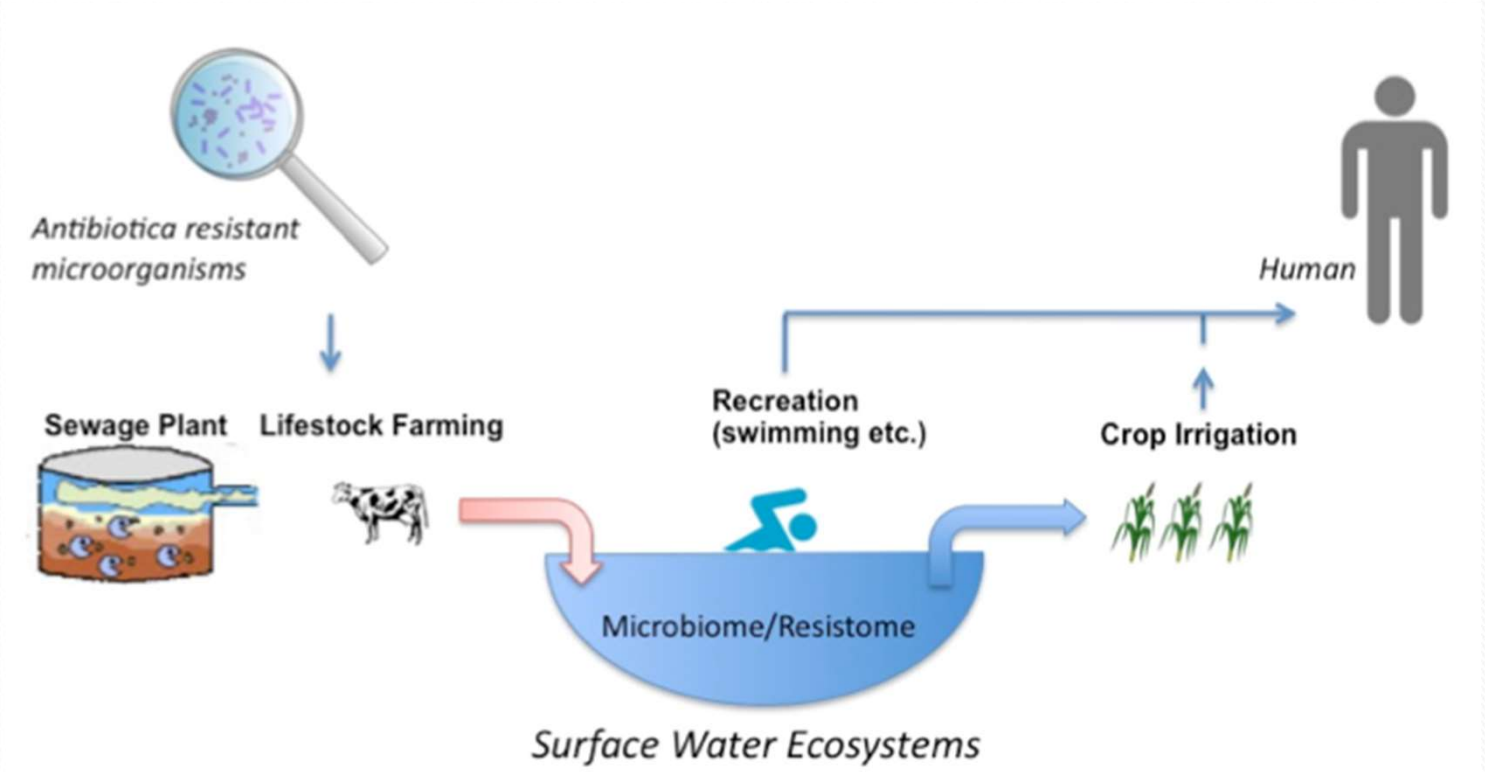
Continuation of the work in the future

- Application "A sustainable strategy for the self-management of water safety in low resource settings using a rapid pathogen screening - **Path2Safe**" submitted in the research funding initiative PRIMA (partnership for research and innovation in the Mediterranean area) in March 2018.

- An international project "One Platform- Multiple biomarker detection of Rheumatoid Arthritis - **RA-detect"** between the partners from Lisbon and Jena on antibody-based multiplex detection (with the microarray analyser of TRACE) is running.

- DAAD proposal "**Plasmonic nanoarray for detection of prostate cancer biomarkers**" between UNL and IPHT is starting at 2018.

- Additionally, several bilateral communication channels between the project partners were established und continued in future, as the joint participation conferences and meetings.


Antibiotica resistant microorganisms
Human
Sewage Plant
Lifestock Farming
Recreation (swimming etc.)
Crop Irrigation
Microbiome/Resistome
Surface Water Ecosystems


Food
Water JPI