JDS4 Microbiology Program

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Organization JDS 3

Institutional Contributions

JRC ISPRA
(Metagenomics)

BOKU, LUNZ
(DOC quality)

MEDIZINISCHE UNIVERSITÄT WIEN

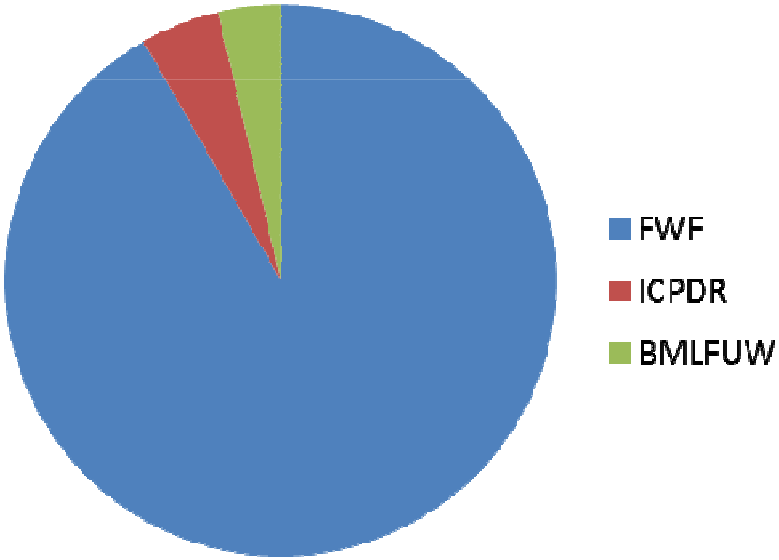
Medizinische Universität Graz

ICC Water & Health

TU WIEN

SOLUTIONS
(anthropogenic markers)

Financial Contributions



Total Sum: 214.000 Euro + Logistics

CENTRAL GOAL

- an **ambitious and scientifically innovative** microbiology program

- By the synergistic combination of different thematic and methodic microbiological approaches a comprehensive picture of the microbiology as regards patterns of **microbial faecal pollution**, **antibiotic resistances** and the **total bacterial population** (**microbiome** and **standing stock** analysis) will be developed.

- The **input of the national experts** (parallel measurements, connection to national microbiology programs) and additional data made available by other expert groups (antibiotic concentrations, heavy metals, chemical anthropogenic wastewater markers, etc) will deliver **important background information for a better interpretation** of the observed microbiological patterns.

- This program shall combine:
  - standard faecal pollution monitoring,
  - microbial faecal source tracking,
  - antibiotic resistance analysis
  - bacterial microbiome analysis,
  - microbial ecology

Papers published so far on Antibiotic resistance in the Danube (from JDS 3)

Kittinger C, Kirschner A, Lipp M, Baumert R, Mascher F, Farnleitner AH, Zarfel GE (2018) Antibiotic Resistance of **Acinetobacter spp**. Isolates from the River Danube: Susceptibility Stays High. Int J Environ Res Public Health. 15(1); doi: 10.3390/ijerph15010052

Kittinger C, Lipp M, Folli B, Kirschner A, Baumert R, Galler H, Grisold AJ, Luxner J, Weissenbacher M, Farnleitner AH, Zarfel G (2016) **Enterobacteriaceae** Isolated from the River Danube: Antibiotic Resistances, with a Focus on the Presence of ESBL and Carbapenemases. PLoS One 11(11): e0165820

Kittinger C, Lipp M, Baumert R, Folli B, Koraimann G, Toplitsch D, Liebmann A, Grisold AJ, Farnleitner AH, Kirschner AKT, Zarfel G (2016). Antibiotic resistance patterns of **Pseudomonas spp.** isolated from the River Danube. *Front. Microbiol. 7: 586*

- Based on qualitative results (presence/absence) only
- No DNA based approach applied
- Organisms only cultured post-hoc from frozen samples

FINANCING

- **BMNT:** 15.000 € (standard faecal pollution microbiology & specific logistics)

- **ICPDR:** general basic logistics

- **OEAD:** 7.000 € (pre-JDS-preparation)

“Harmonization of the microbiological methods for the assessment of the Danube River water quality”

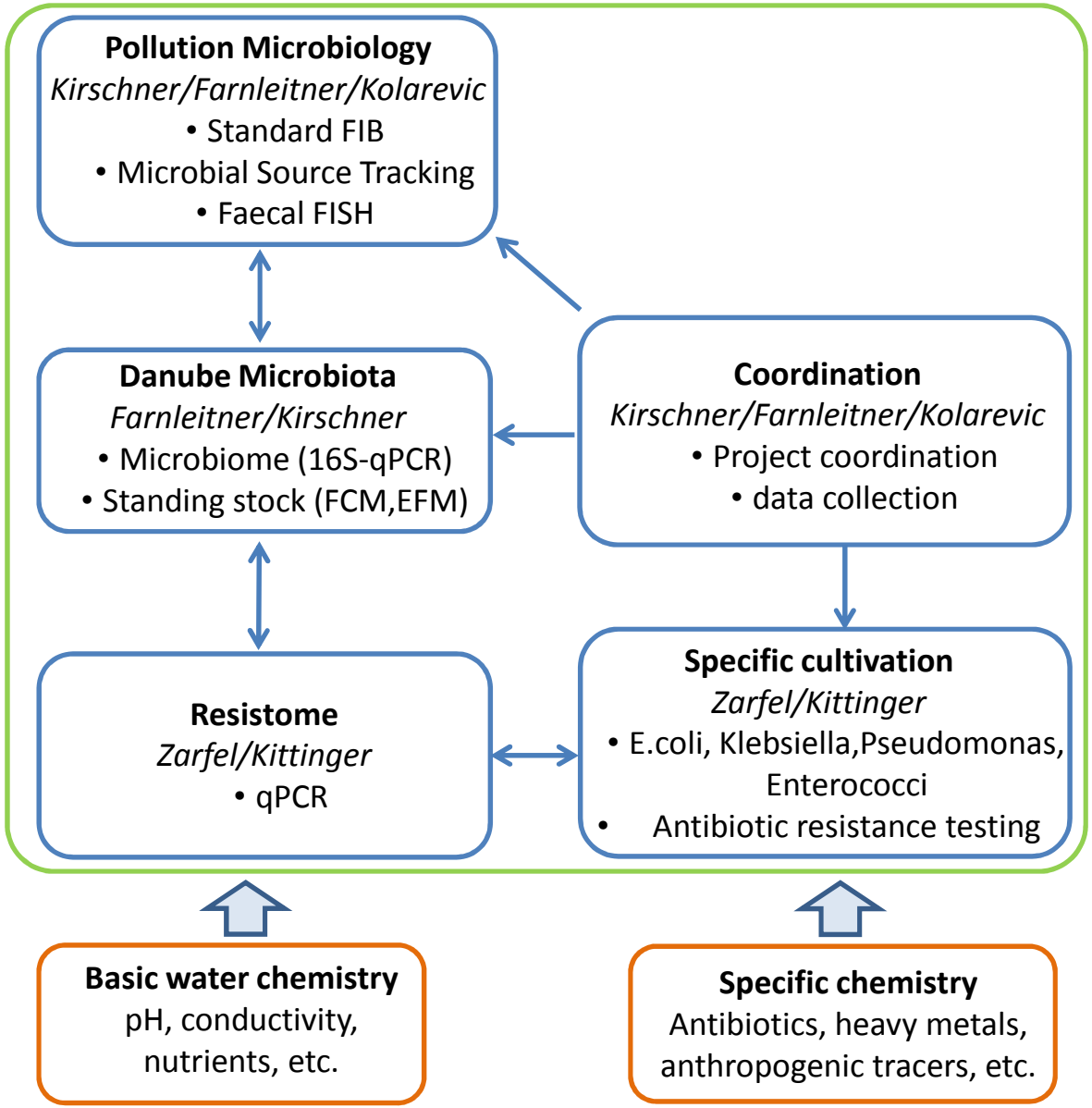
- **FWF-Austrian Science Fund:** 400.000 €

„Understanding fecal pollution sources as propagation pathways of antimicrobial resistance in the Danube River: Establishing a quantitative whole river approach “

Submission: May 2018, Decision: October 4, potential resubmission: November 2018

Final decision: March 2019

JDS4 MICROBIOLOGY PROGRAM & TEAM



Core-team:
A Kirschner (AUT)
S Kolarevic (SRB)

Suggested selection of sampling sites for the JDS4 microbiology program

- To be adjusted with ICPDR, eDNA Team and others

Site nr	JDS site name	river km	comment	work day	l/m/r combined	ARB cultivation	Sediment	parking space for caravan & lab bus	sample transport to laboratory
1	Kelheim, gauging station	2415	highest faecal pollution levels left river bank, JDS 2013	1	l/m/r	E.coli/Klebs/Pseud		Regensburg	
2	Geisling power plant	2354	impoundment in Germany	1	combined	E.coli	**Sediment**		
3	Mühlau	2258	little polluted reference site	2	combined			Linz	
4	Inn	2225	important tributary	2	combined	E.coli			
5	US dam Abwinden-Asten	2120	important impoundment, after WWTP inflow Linz	2	combined	E.coli/Klebs/Pseud	**Sediment**		
6	Oberloiben	2008	high pollution levels found left river bank JDS 2013	3	l/m/r	E.coli/Klebs/Pseud		Vienna	transport
7	Nussdorf	1935	reference site us Vienna	3	combined				
8	Downstream Vienna	1930	directly after inflow of WWTP Vienna	3	l/m/r	E.coli/Klebs/Pseud			
9	Bratislava	1869	important capital with expected wastewater	4	l/m/r	E.coli/Klebs/Pseud		Bratislava	
10	Medvedov / Medve	1806	low pollution reference site	4	combined				
11	Moson Danube (tributary)	1794	pollution from Györ	4	combined	E.coli			
12	US Budapest - Megyeri Bridge	1660	low pollution reference site us Budapest	5	combined	E.coli		Budapest	
13	DS Budapest - M0	1632	after inflow Budapest WWTP	5	l/m/r	E.coli/Klebs/Pseud			
14	Dunaföldvar	1560	high pollution levels in 2013, midstream	5	l/m/r				
	break day			6				Budapest	transport
15	US Drava	1384	reference site to Drava and ds Drava	7	l/m/r	E.coli/Klebs/Pseud		Novi Sad	
16	Drava (tributary)	1379	important tributary	7	combined	E.coli			
17	DS Drava (Erdut/Bogojevo)	1367	ds important tributary	8	l/m/r	E.coli		Novi Sad	
18	DS Novi Sad	1252	ds important city with high pollution	8	l/m/r	E.coli/Klebs/Pseud			
19	Tisa (tributary)	1215	important tributary	9	combined	E.coli		Belgrade	transport
20	DS Tisa / US Sava (Belegis)	1200	ds/us important tributary	9	l/m/r	E.coli/Klebs/Pseud			
21	Sava (tributary)	1170	important tributary	10	combined	E.coli		Belgrade	
22	Upstream Pancevo	1159	ds Belgrade and Sava, high pollution	10	l/m/r	E.coli/Klebs/Pseud			
	break day			11				Belgrade	
23	Banatska Palanka	1071	moderate pollution reference site	12	combined	E.coli		to be defined	
24	IGR Golubac/Koronin	1040	important reservoir	12	combined	E.coli/Klebs/Pseud	**Sediment**		
25	IGR Tekija/Orsova	954	important reservoir	12	combined				
26	Vrbica/Simijan	926	high pollution levels during JDS 3, left and right	13	l/m/r	E.coli/Klebs/Pseud		to be defined	transport
27	Upstream Timok	849	low pollution reference site us Timok	13	combined	E.coli			
28	Timok	845	high heavy metal loads	14	combined	E.coli		to be defined	
29	Pristol / Novo Salo	834	ds Timok	14	l/m/r	E.coli/Klebs/Pseud			
30	Downstream Zimnicea/Svistov	550	high pollution levels left/right	15	l/m/r	E.coli/Klebs/Pseud		Ruse	
31	Downstream Jantra	532	moderate pollution reference site	15	combined	E.coli			
	break day			16				Ruse	transport
32	Russenski Lom (tributary)	498	extremely high pollution levels	17	combined	E.coli		Ruse	
33	Downstream Ruse	488	high pollution levels, right river side	17	l/m/r	E.coli/Klebs/Pseud			
34	Arges (tributary)	432	extremely high pollution levels	18	combined	E.coli		to be defined	
35	Downstream Arges	429	high pollution levels, left river side	18	l/m/r	E.coli/Klebs/Pseud			
36	Giurgeni	235	low pollution reference site	19	combined	E.coli		to be defined	transport
37	Reni	130	after Siret/Prut tributaries	19	l/m/r	E.coli/Klebs/Pseud			
38	St.Gheorge arm	104	one representative Danube arm in the Delta	20	l/m/r	E.coli/Klebs/Pseud		to be defined	

LOGISTICS

PLAN A - Sampling together with eDNA team (start: June 29, 2019, 3 weeks)

Selected microbiology team members will have to travel down the Danube

A camper van or laboratory bus will be hired allowing basic on-site laboratory work (filtration of samples, cultivation of selected organisms (E. coli/Klebsiella, & Pseudomonas), incubation at 37°C and 44°C, short-term storage of samples at 4°C and -20°C, running an on-line flow cytometer) and accommodation for two people.

Most probably, a 3 person team will trvel down the Danube (partly change in Belgrade)

- Sampling has to be performed with the help of the e-DNA team (providing sampling boats and having the permission to sample across borders
- Short-term stored samples (4°C, -20°C) have then to be transported via ICPDR logistics to the main laboratories in Austria (Vienna, & Graz) a.s.a.p.(at least every 2 days)
- **Total survey time:** 3 weeks

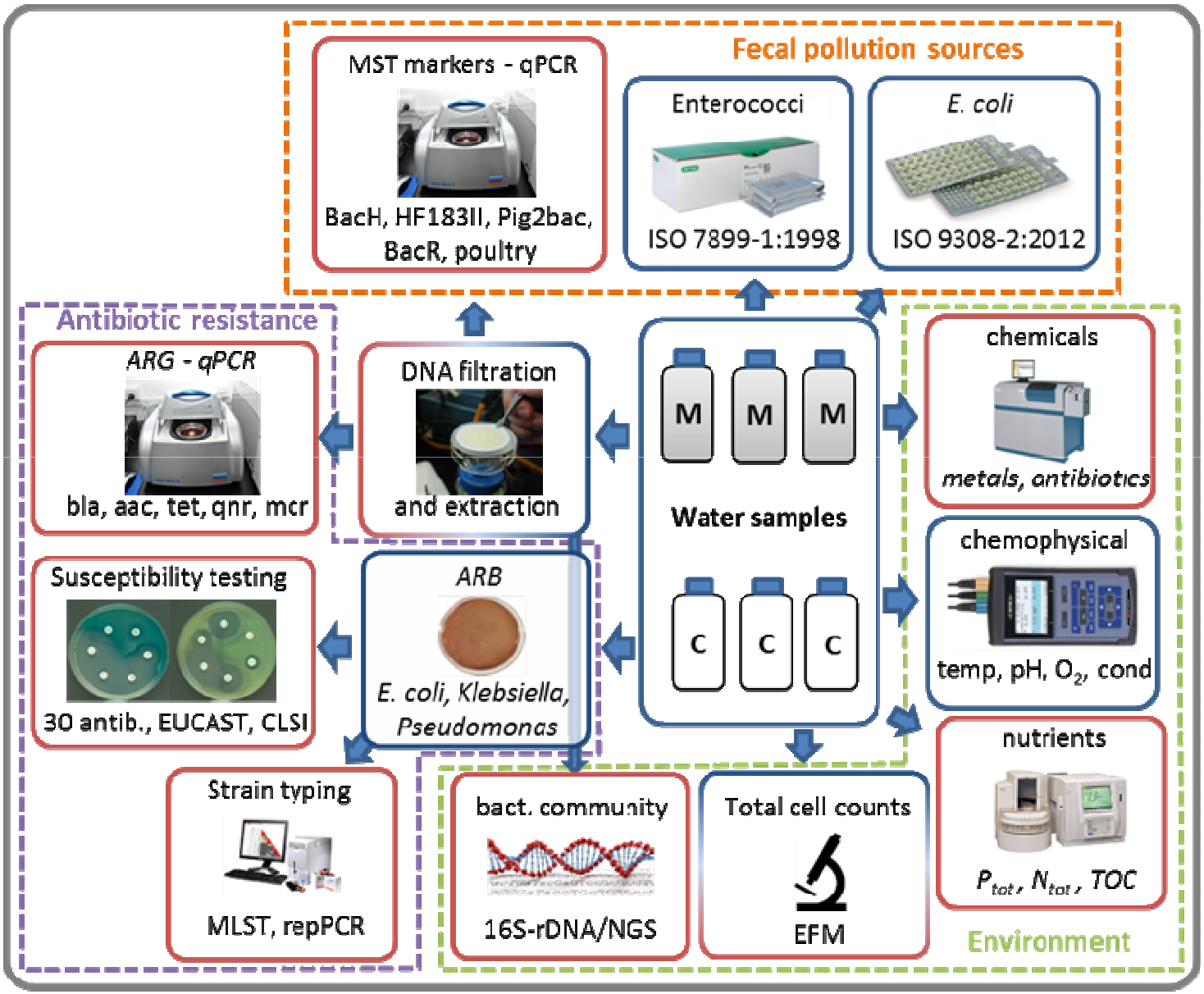
PLAN B - Sampling together with Momir Paunovic (start: end of July, after JDS, 3 weeks)

- Same conditions as above, ICPDR logistics possible?

PLAN C - Only if no funding by FWF is granted (only 15.000 Euros available)

- Basic microbiology program (pollution microbiology, DNA filtration)
- Training of national experts for sampling and sample analysis

METHODS PROPOSED



REQUIREMENTS FOR THE JDS4 MICROBIOLOGY PROGRAM

Time requirements:

Approx. 3 weeks June or August

Help of eDNA team:

- Providing **sampling boats and staff for cross-sectional sampling**
- Providing analysis of **critical ancillary environmental parameters** at the time of sampling (see: list of parameters)

Logistic support by ICPDR:

- **Regular (2 days) transport of samples** (4°C, -20°C) from the lab-caravan to the main lab (Vienna)
- **Regular (2 days) transport of lab material** (RT, 4°C, -20°C) from the main lab to the lab caravan
- **Regular (2 days) transport of laboratory waste** (plastic, autoclaved fluids, etc) (can also be done by the national teams)
- **List of safe places**, with electricity, sanitary facilities, in each country where to stay overnight in a caravan along the Danube
- **List of national experts** for potential help

Specimens

- Water (left, middle, right at “Hot-spot” sites, pooled at “basic” sites)
- Biofilms (pooled)
- Wastewater effluents (4 selected, to be coordinated with ICPDR)
- Fine Sediments: only at 3 representative stations (according to sampling plan)

List of ancillary variables

- Hydrological variables: discharge, precipitation
- Chemophysical variables: pH, electrical conductivity, water temperature, oxygen, TSS
- Nutrients: TOC, Ptot, Ntot, NO_3, NH_4
- Biological variables: chlorophyll a, macro-zoobenthos
- Chemical parameters (Selection and co-selection factors):
  - **Heavy metals**: Cr, Cu, As, Cd, Zn, Pb, Ni
  - **Antibiotics (and decay products?)**:
    - Fluoroquinolones (Ciprofloxazin)
    - Sulfonamides (Sulfamethoxazole)
    - Beta-Lactames (Carbapenemes, 2^{nd} and 3^{rd} generation Cephalosporines)
    - Penicillines (Penicillin G und V, Ampicillin und Amoxycillin or total sum)
    - Macrolides (Erithromycin, Clarithromycin, Azithromycin)
    - Aminoglycoside (Gentamycin)
    - Tetracycline, Colistin, Tigecycline , Fosfomycin
  - **Other potential co-selection factors** (pesticides, via non-target screening)