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# Možnosti horizontálního přenosu genů antibiotické rezistence v pěstování plodin – One Health EJP FED-AMR

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# OHEJP FED-AMR

- **GRANT:**
  **European Joint Programme**
  **One Health EJP - Horizon 2020**
  **Grant č. 773830**
- 2019 - 2022
- 44 partnerů
- Konvenční zemědělství / HOAL (Hydrological Open Air Laboratories)

- Kultivace (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis/faecium*, MRSA - meticilin-rezistentní *Staphylococcus aureus*, *Salmonella* spp., klostridia
- Sekvenace genomů
- Prvková analýza
- Analýza antimikrobiotik
- Analýza herbicidů

**FED-AMR: The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain**

Start: 1 January 2020
Duration: 2.5 Years
Domain: Antimicrobial Resistance
Key Words: Bacterial transformation, free extracellular DNA, antimicrobial resistance, horizontal gene transfer, ecosystem boundaries.
Contact: Werner Ruppitsch (AGES)

**The Project #FEDAMR**

Over the course of this project, the relevance of horizontal antimicrobial resistance gene (ARG) transfer on free extracellular DNA (exDNA) over ecosystem boundaries relative to bacterial conjugation will be evaluated. ExDNA is widely present in natural environments and sufficiently stable to constitute an important reservoir for ARGs. The dissemination of AMR on exDNA over ecosystem boundaries will be monitored under controlled but naturally occurring environmental conditions in an open-air agricultural research area: The Hydrology Open Air Laboratory (HOAL) in Petzenkirchen, Austria. ARG concentrations, diversity, dynamic variability, mobility and bacterial biodiversity will be determined in an annual longitudinal study covering a crop growing period, different fertilisation and land management techniques and various different – but interlinked – environmental compartments along the route: pig farm –> manure –> soil –> crop/food/feed –> ground/surface water –> pig farm (and other associated human compartments). The results obtained from HOAL will be compared with data retrieved from equivalent compartments in Northern, Eastern and Southern Europe. Movement of ARGs over ecosystem barriers will be inferred by sequence comparisons and construction of phylogenetic trees of ARGs and ARBs. The linkage between human and non-human reservoirs of AMR will be investigated exemplarily with *C. difficile* as ARG transfer platform over ecosystem boundaries and conjugation as means for HGT.

The prevailing selection pressure in each tested habitat during the longitudinal study will be determined by quantifying antimicrobials, herbicides and trace elements in the tested compartments. Environmental conditions which may induce or inhibit the expression of competence genes that are necessary to enable the uptake of free extracellular DNA by bacteria will be identified in soil microcosms and in a pig gut model. The impact of transformation relative to conjugation will be evaluated using *Acinetobacter* sp. (transformation) and *C. difficile* (conjugation) as model organisms in these experiments.

Experimental data obtained during the project will be used to feed and tune probabilistic modelling of the emergence of AMR in target bacteria and to delineate the relative contribution of transformation and conjugation to ARG acquisition in soil environments. Mechanistic models will address key questions regarding the spatio-temporal changes observed in microbial communities and shall support the identification of critical control points for intervention to reduce the spread of AMR from environmental sources

**Project Events**

FED-AMR Kick-off meeting- 27th January 2020

One Health EJP Project Kick-off meeting- 13th November 2019, Berlin, Germany

https://onehealthejp.eu/projects/antimicrobial-resistance/jrp-fed-amr

# OHEJP FED-AMR

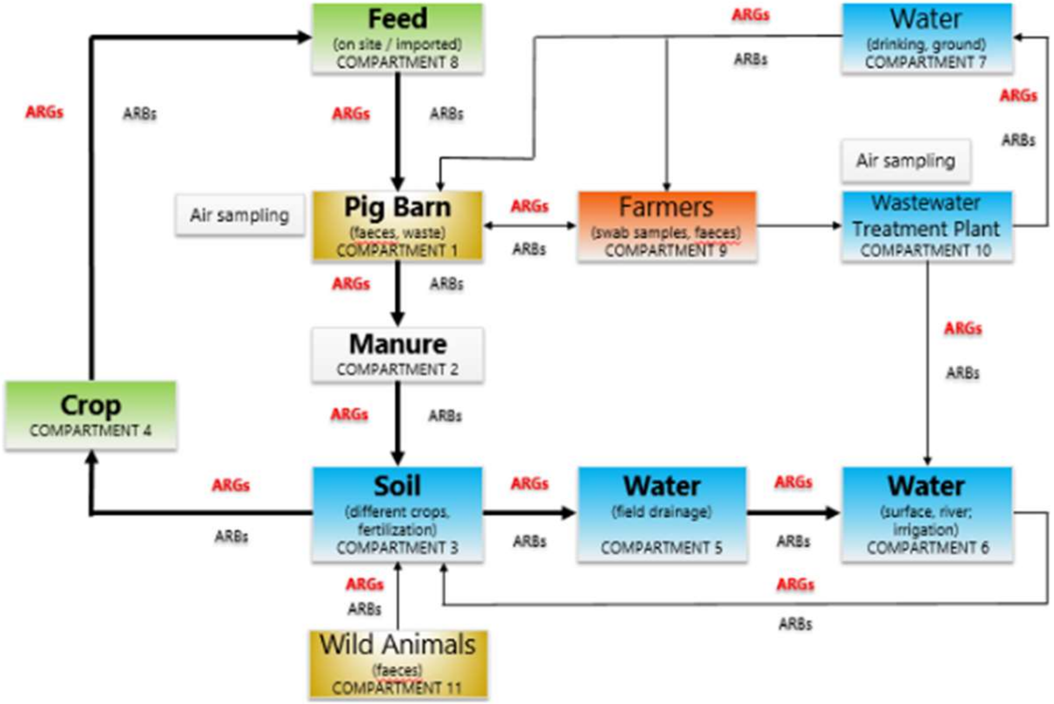


*Fig.1. Potential pathways for ARG dissemination over environmental ecosystem barriers.* Bold *letters/arrows: monitored compartments and pathways for ARG. ARBs: antimicrobial resistant bacteria. Animal compartments: gold. Human compartments: red. Plant-associated compartments: green. Genuine environmental compartments: blue.*

- × Odpadní voda
- × Drenáž
- × Farmáři
- × Volně žijící zvířata

- ✓ Pandemie COVID-19
- ✓ Vzorkování v roce 2020
- ✓ Alternativní plán

A. Cabal Rosel, K. Rathammer, F. Allerberger , M. Canica, M. Chambers , A.B. De Menezes , Z. Drahosova , M. Gbylik-Sikorska , S. Maurischat , C. Mazuet, M. Oleastro, S. Persson, K. Rainer, C. Seyboldt, S.S. Mo, A. Telke, T. Tenson, M. Wögerbauer, W. Ruppitsch: FED-AMR: The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain, dostupné on-line https://fed-amr.ages.at/fileadmin/Redakteure_FED-AMR/Publications/Poster_OHEJP_ASM_Prague; **One Health EJP Annual Scientific Meeting – Prague, Czech Republic, květen 2020, pouze on-line.**

# Podmínky projektu

- Konvenční zemědělství (Česko, Velká Británie, Norsko) x HOAL (Estonsko, Rakousko, Portugalsko)
- Sever x jih x východ x západ Evropy
- Plodiny: pšenice, kukuřice, řepka
- Hnojiva: kejda, hnůj, anorganická hnojiva
- *Clostridium difficile* - modelový organismus pro model střevní transformace bakterií (prasečí/lidský střevní model)

- Jedno vegetační období (ČR jaro - léto - podzim - zima)
- Volná exDNA obohacuje environmentální vzorky až o 55% (historická DNA = historická biodiverzita)
- Analýza exDNA ze vzorků prostředí umožňuje vhled do dynamiky a historie biomu prostředí
- Přirozeně kompetentní mikrobi jsou schopní přijímat volnou nebo vázanou DNA z prostředí, v němž se vyskytují

# Výsledky...

- Kultivace: *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis/faecium,* ~~MRSA - meticilin-rezistentní *Staphylococcus aureus, Salmonella* spp.,~~ klostridia

- Identifikace izolátů (MALDI, testy citlivosti, genome enrichment)

- Sekvenace genomů/metagenomů - analýzy příbuznosti, analýzy genů rezistence (AGES + ARESGenetics) - MEGARes 2.0 database, ACLAME (ARGs), pro odpadní vody byla využita databáze http://repares.vscht.cz; datasety rezistomů ze sekvenací ale vykazovaly mnohem méně ARGs při porovnání s qPCR - nutno kombinovat molekulární metody
  - ARESupa: universal pathogenome assay for culture-free resistome analysis (panel pokrývá > 9000 markerů AMR)

- Celkem 475 vzorků, v nichž bylo detekováno 7252 genů rezistence, 89 klinicky relevantních (49 *E. coli*, 27 *E. faecalis*, 7 *K. pneumoniae*, 6 *E. faecium*) převážně z komartmentu „wildlife“ a z odpadních vod
- 33% izolátů neslo alespoň 1 gen rezistence, v případě *E. coli* to bylo průměrně 48,6 ARGs; u *K. pneumoniae* 20,6 ARGs, *E. faecium* 3,3 ARGs a *E. faecalis* 2,4 ARGs
- Nejvyšší zastoupení (ve 343 vzorcích) *tet*W
- z kompartmentu „wildlife“ byly detekovány geny rezistence ke 30 třídám antibiotik, kde nejvíce zastoupeny byly β-laktamy>tetracykliny >aminoglykosidy >fluorochinolony
- rezistence k těžkým kovům „wildlife“: Cu >Hg >As > Ni-Co >Cr > Ag

Více na https://www.labroots.com/es/webinar/fed-amr-role-free-extracellular-dna-dissemination-antimicrobial-resistance-ecosystem-boundaries-foo

# Výsledky…

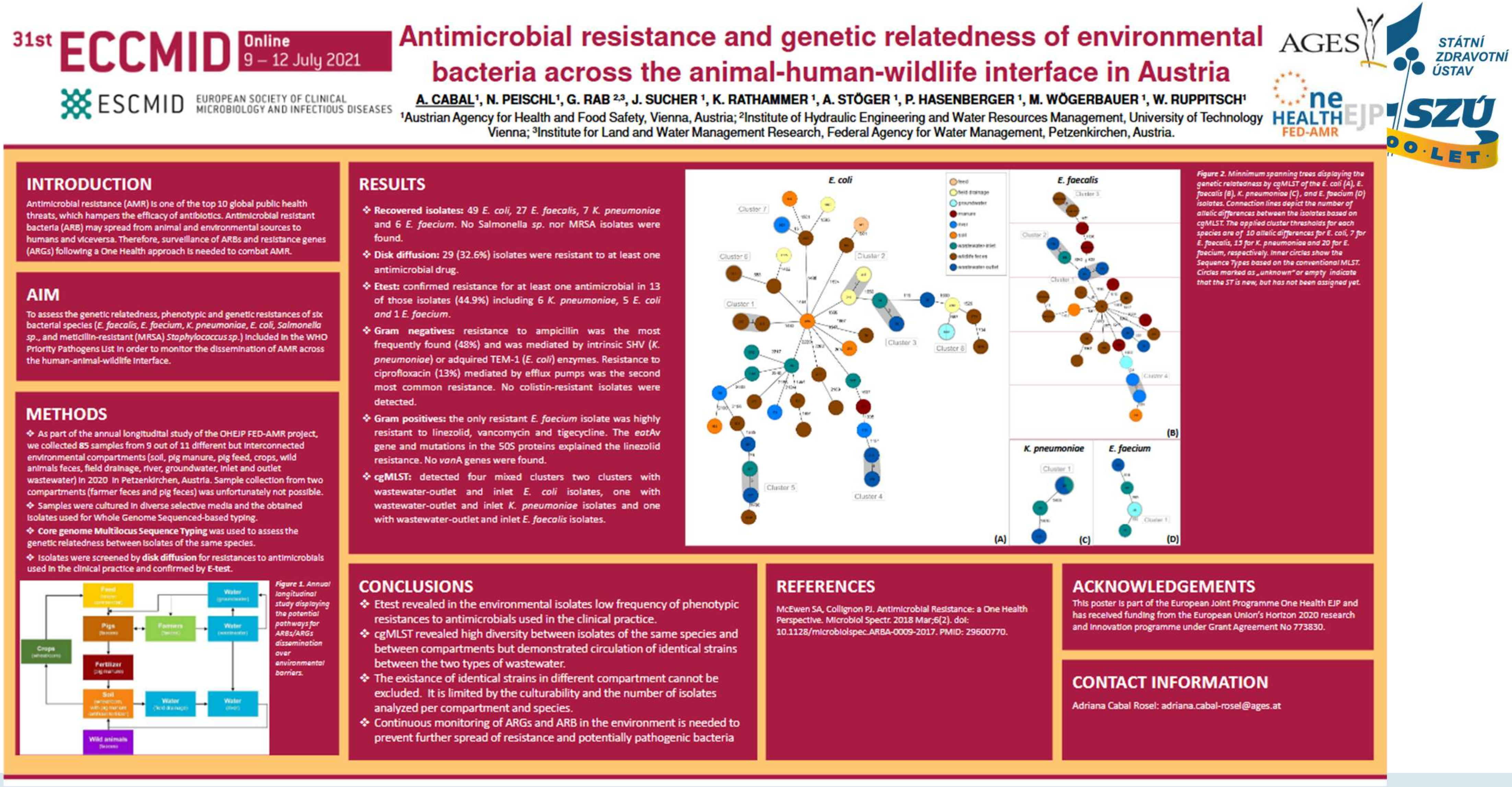
- Prvková analýza - Universita Surrey (GB)

multivariantní analýza kovů a metaloidů prokázala, že koncentrace Cu, Zn a Mn jsou vhodnými ukazateli dopadu používání kejdy/hnoje na životní prostředí

minimální koselektivní koncentrace (MCC) byla překročena o několik řádů u mědi a zinku v některých vzorcích kejdy a odpadní vody, tyto koncentrace by mohly vést ke zvýšení bakteriální rezistence k antibiotikům

jak umělá hnojiva, tak kejda/hnůj zvýšily biologickou dostupnost Cu, Zn a Pb v půdách, mezi umělými hnojivy a kejdou nebyl statisticky významný rozdíl ve výskytu kovů a metaloidů

- Analýza antimikrobiálních látek - PIWET (PL)- jediné antibiotikum doxycyklin (tetracyklinové ATB) bylo detekováno ve vzorcích z prostředí s aplikací prasečí kejdy a kravského hnoje (8 vzorků)
- Analýza herbicidů - AGES (AT)



# Antimicrobial resistance and genetic relatedness of environmental bacteria across the animal-human-wildlife interface in Austria

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## INTRODUCTION

Antimicrobial resistance (AMR) is one of the top 10 global public health threats, which hampers the efficacy of antibiotics. Antimicrobial resistant bacteria (ARB) may spread from animal and environmental sources to humans and viceversa. Therefore, surveillance of ARBs and resistance genes (ARGs) following a One Health approach is needed to combat AMR.

## AIM

To assess the genetic relatedness, phenotypic and genetic resistances of six bacterial species (*E. faecalis, E. faecium, K. pneumoniae, E. coli, Salmonella sp.*, and meticillin-resistant (MRSA) *Staphylococcus sp.*) included in the WHO Priority Pathogens List in order to monitor the dissemination of AMR across the human-animal-wildlife interface.

## METHODS

- ❖ As part of the annual longitudital study of the OHEJP FED-AMR project, we collected **85** samples from 9 out of 11 different but interconnected environmental compartments (soil, pig manure, pig feed, crops, wild animals feces, field drainage, river, groundwater, inlet and outlet wastewater) in 2020 in Petzenkirchen, Austria. Sample collection from two compartments (farmer feces and pig feces) was unfortunately not possible.
- ❖ Samples were cultured in diverse selective media and the obtained isolates used for Whole Genome Sequenced-based typing.
- ❖ **Core genome Multilocus Sequence Typing** was used to assess the genetic relatedness between isolates of the same species.
- ❖ Isolates were screened by **disk diffusion** for resistances to antimicrobials used in the clinical practice and confirmed by **E-test**.

*Figure 1. Annual longitudinal study displaying the potential pathways for ARBs/ARGs dissemination over environmental barriers.*

## RESULTS

- ❖ **Recovered isolates:** 49 *E. coli*, 27 *E. faecalis*, 7 *K. pneumoniae* and 6 *E. faecium*. No Salmonella *sp.* nor MRSA isolates were found.
- ❖ **Disk diffusion:** 29 (32.6%) isolates were resistant to at least one antimicrobial drug.
- ❖ **Etest:** confirmed resistance for at least one antimicrobial in 13 of those isolates (44.9%) including 6 *K. pneumoniae*, 5 *E. coli and* 1 *E. faecium*.
- ❖ **Gram negatives:** resistance to ampicillin was the most frequently found (48%) and was mediated by intrinsic SHV (*K. pneumoniae*) or adquired TEM-1 (*E. coli*) enzymes. Resistance to ciprofloxacin (13%) mediated by efflux pumps was the second most common resistance. No colistin-resistant isolates were detected.
- ❖ **Gram positives:** the only resistant *E. faecium* isolate was highly resistant to linezolid, vancomycin and tigecycline. The *eat*Av gene and mutations in the 50S proteins explained the linezolid resistance. No *van*A genes were found.
- ❖ **cgMLST:** detected four mixed clusters two clusters with wastewater-outlet and inlet *E. coli* isolates, one with wastewater-outlet and inlet *K. pneumoniae* isolates and one with wastewater-outlet and inlet *E. faecalis* isolates.

*Figure 2. Minnimum spanning trees displaying the genetic relatedness by cgMLST of the E. coli (A), E. faecalis (B), K. pneumoniae (C), and E. faecium (D) isolates. Connection lines depict the number of allelic differences between the isolates based on cgMLST. The applied cluster thresholds for each species are of 10 allelic differences for E. coli, 7 for E. faecalis, 15 for K. pneumoniae and 20 for E. faecium, respectively. Inner circles show the Sequence Types based on the conventional MLST. Circles marked as „unknown" or empty indicate that the ST is new, but has not been assigned yet.*

## CONCLUSIONS

- ❖ Etest revealed in the environmental isolates low frequency of phenotypic resistances to antimicrobials used in the clinical practice.
- ❖ cgMLST revealed high diversity between isolates of the same species and between compartments but demonstrated circulation of identical strains between the two types of wastewater.
- ❖ The existance of identical strains in different compartment cannot be excluded. It is limited by the culturability and the number of isolates analyzed per compartment and species.
- ❖ Continuous monitoring of ARGs and ARB in the environment is needed to prevent further spread of resistance and potentially pathogenic bacteria

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## CONTACT INFORMATION

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https://zenodo.org/records/5499916#.YTs2z-dCR4E

# FED-AMR

- Hypotéza: exDNA (volná DNA, environmentální DNA) volně přechází mezi kompartmenty a je přijímána kompetentními organismy
- (např. Yanping Shen, Yibo Liu, Yutong Du, Xu Wang, Jiunian Guan, Xiaohui Jia, Fukai Xu, Ziwei Song, Hongjie Gao, Baiyu Zhang, Ping Guo: Transfer of antibiotic resistance genes from soil to wheat: Role of host bacteria, impact on seed-derived bacteria, and affecting factors, Science of The Total Environment, Volume 905, 2023, 167279, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2023.167279. (https://www.sciencedirect.com/science/article/pii/S0048969723059065)

- **Závěry:**
- z výsledků jednotlivých skupin analytů nelze vyvozovat komplexní závěry
- oproti předpokladu bylo v prostředí detekováno pouze jediné antibiotikum
- metagenomika se jevila jako nejlepší přístup do chvíle, kdy:

…bylo nutné redukovat počty vzorků - FINANCE
…bylo nutné analyzovat obrovské množství dat ze sekvenací - LIDSKÉ ZDROJE (nedostatek kvalifikovaných data-analytiků)
…konečné výsledky zatím nejsou (publikovaná komplexní analýza dat pouze Rakousko)
…hloubka čtení exDNA 6-7 Gb datový výstup není dostatečný, je potřeba alespoň 2Tb

- Studie zahrnující jedno vegetační období (1 rok) je příliš krátká
- Nejmodernější neznamená vždy nejvhodnější a nejvýhodnější
- **Nutná udržitelnost výstupů projektů - STAKEHOLDERS a post-projektové financování (navazující podpora)**

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- …………..

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# Děkuji za Vaši pozornost!

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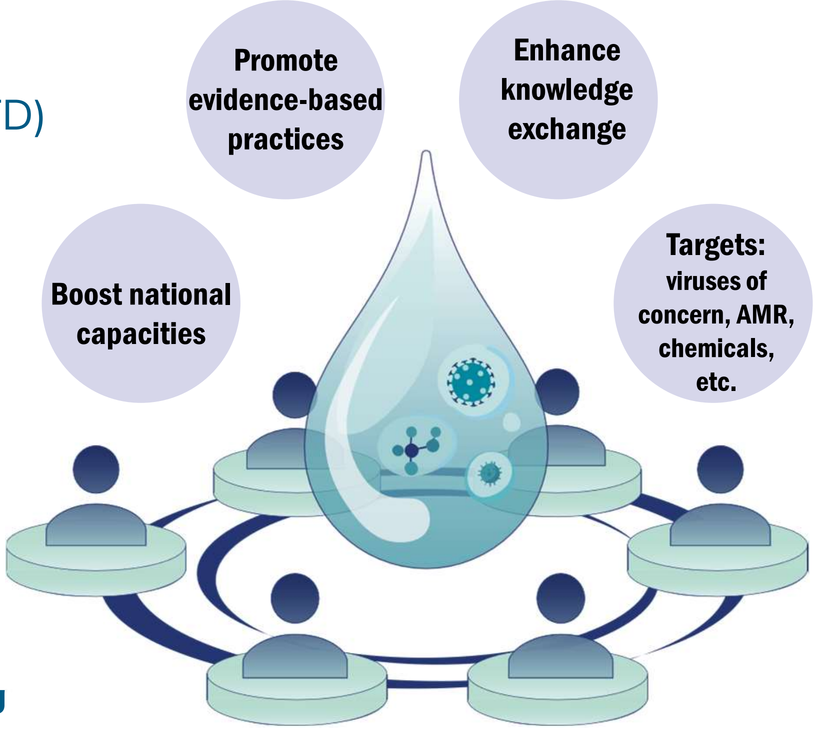
- Direct Grant **H**ealth **E**mergency Preparedness and **R**esponse **A**uthority (Grant Agreement no 101140460)
- Direct Reaction for Urban Wastewater Treatment Directive (UWTD) Directive (EU) 2024/3019



Promote evidence-based practices

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Boost national capacities

**Targets:** viruses of concern, AMR, chemicals, etc.

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