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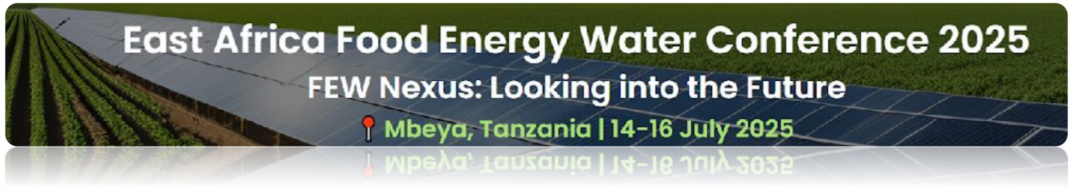
Agricultural Research Organization

Institute of Soil, Water
& Environmental Sciences

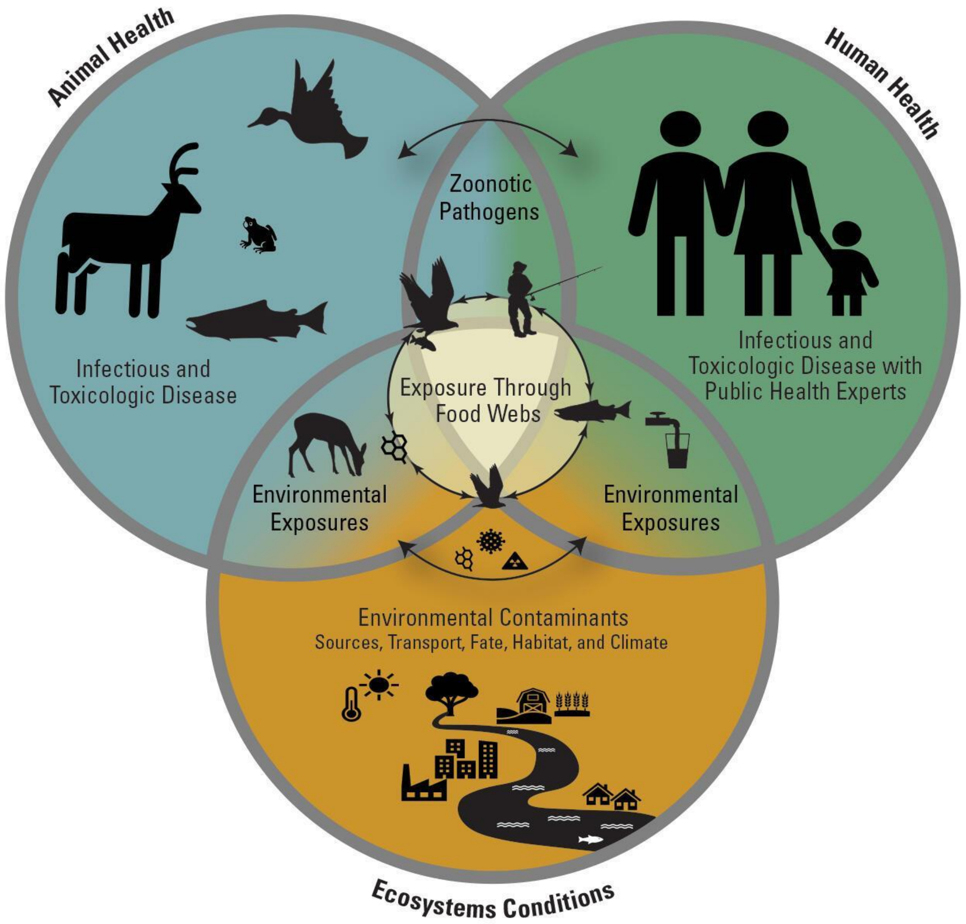
Application of "under the radar" approaches to identify anthropogenically derived pathogen and AMR indicators in produce and soil

Eddie Cytryn

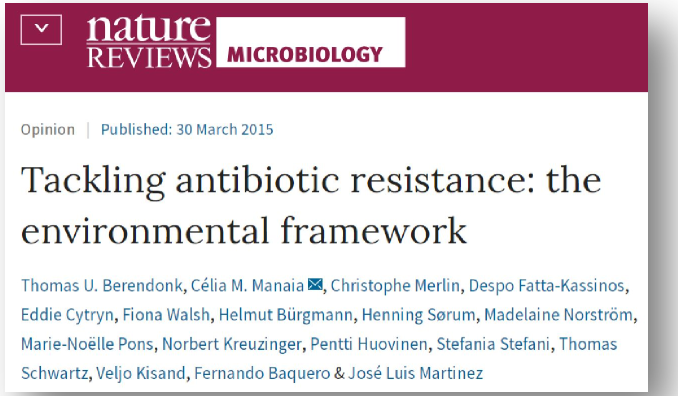


Bring
Them
Home

“One Health” and environmental dimensions of antimicrobial resistance (AMR)



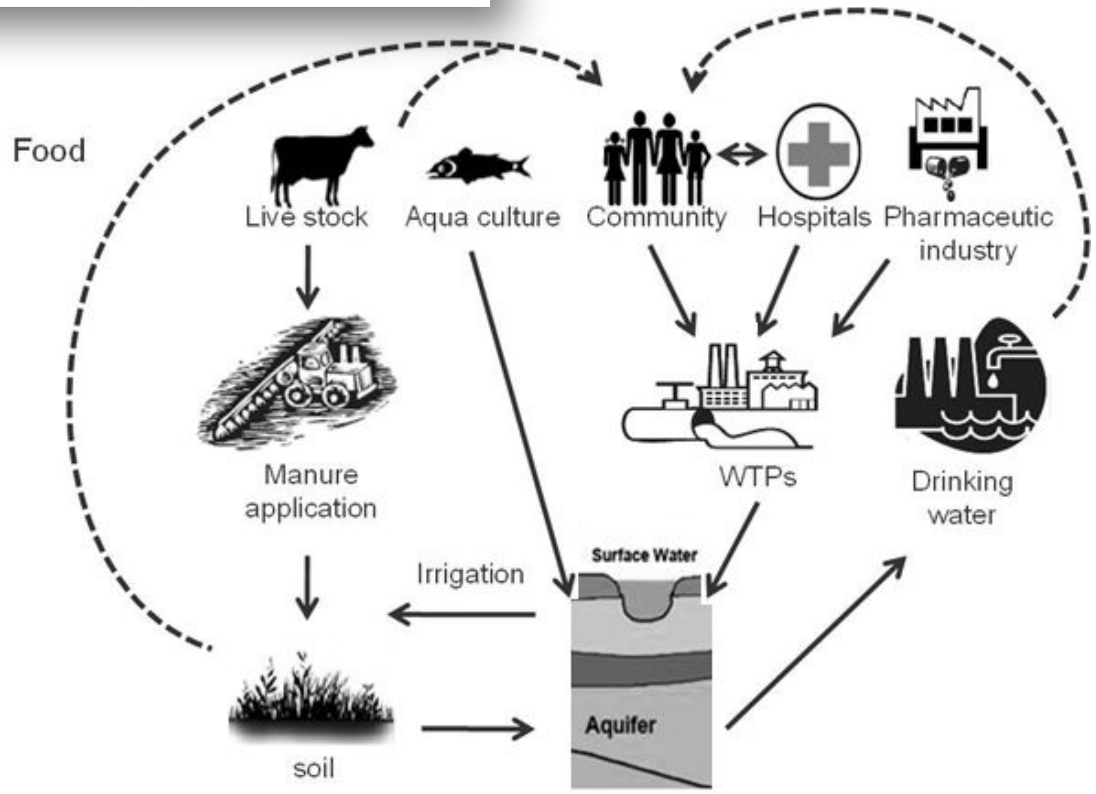
https://www.usgs.gov/


nature REVIEWS MICROBIOLOGY

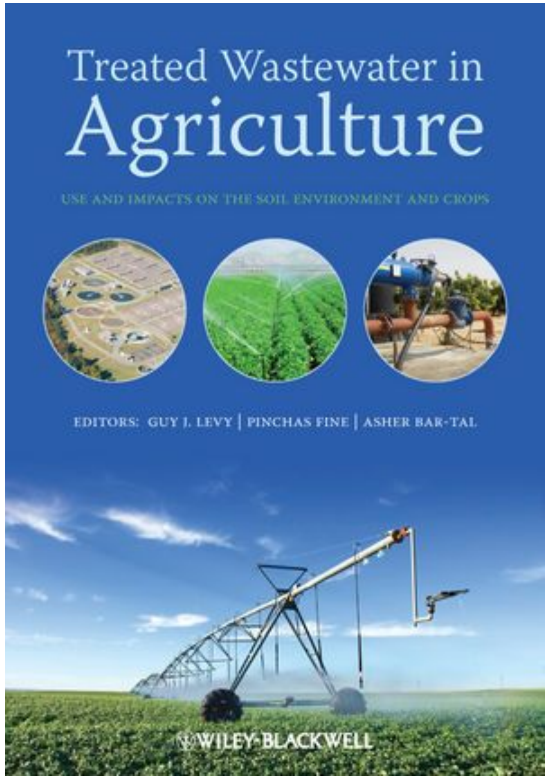
Opinion | Published: 30 March 2015

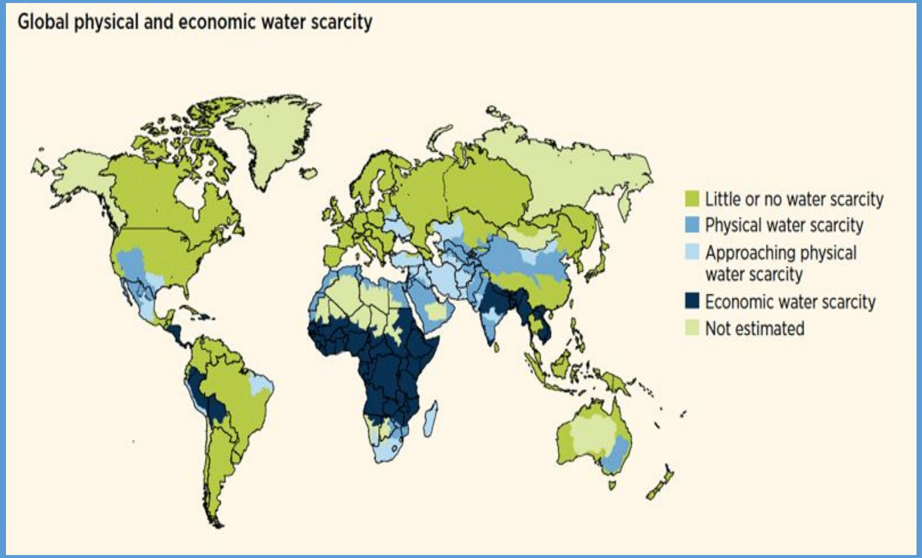
Tackling antibiotic resistance: the environmental framework

Thomas U. Berendonk, Célia M. Manaia, Christophe Merlin, Despo Fatta-Kassinos, Eddie Cytryn, Fiona Walsh, Helmut Bürgmann, Henning Sørum, Madelaine Norström, Marie-Noëlle Pons, Norbert Kreuzinger, Pentti Huovinen, Stefania Stefani, Thomas Schwartz, Veljo Kisand, Fernando Baquero & José Luis Martinez



Wastewater Reuse in Agriculture: a growing phenomenon





United Nations World Water Assessment Programme (WWAP), UN-Water, March 2012.

Over 500 MCM* of sewage is produced in Israel every year, of which ~**93% is treated in sewage treatment plants**. Some **87% is reused** for irrigation in agriculture.

The scope of **agricultural lands irrigated with reclaimed wastewater** in Israel is estimated at some **1.3 million dunams** (130,000 hectares).

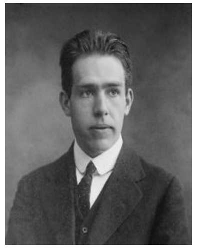
Water scarcity and increasing demand for food has resulted in global expansion of wastewater reuse for irrigation. In many regions irrigation water is untreated sewage….

Water Authority, "Reclamation of Wastewater and Low-Quality Water," accessed 15 January 2018

Treated wastewater (TWW) irrigation: Blessing or curse?

- Alternative water sources for irrigation have become a global necessity due to reduced precipitation, warmer climates, and growing food demand.

- Irrigation using marginal water sources can contaminate food with pathogenic microorganisms, such as parasites, viruses, and bacteria- including strains that are highly resistant to antibiotics.



The opposite of a fact is falsehood, but the opposite of one profound truth may very well be another profound truth. (Niels Bohr)

TWW irrigation: Blessing or curse?

Contents lists available at ScienceDirect

Water Research X

journal homepage: www.sciencedirect.com/journal/water-research-x

ELSEVIER

Check for updates

Mitigating risks and maximizing sustainability of treated wastewater reuse for irrigation

David Yalin[a,#], Hillary A. Craddock[b,#], Shmuel Assouline[c], Evyatar Ben Mordechay[d], Alon Ben-Gal[e], Nirit Bernstein[c], Rabia M. Chaudhry[f,†], Benny Chefetz[d], Despo Fatta-Kassinos[g], Bernd M. Gawlik[h], Kerry A. Hamilton[i], Leron Khalifa[c], Isaya Kisekka[j], Iftach Klapp[k], Hila Korach-Rechtman[l], Daniel Kurtzman[c], Guy J. Levy[c], Roberta Maffettone[h], Sixto Malato[m], Célia M. Manaia[n], Kyriakos Manoli[o], Orah F. Moshe[p], Andrew Rimelman[q], Luigi Rizzo[r], David L. Sedlak[s], Maya Shnit-Orland[t], Eliav Shtull-Trauring[c], Jorge Tarchitzky[d], Venus Welch-White[f,†], Clinton Williams[u,†], Jean McLain[v,#], Eddie Cytryn[c,#,*]

We need to balance the two!
The Solution: **Research** Technology & Regulation

Microbial criteria for wastewater reuse

NEW STANDARDS FOR TREATED WASTEWATER REUSE IN ISRAEL

Yossi Inbar
Senior Deputy Director General, Industries
Ministry of Environmental Protection, Jerusalem, 95464 Israel

M.K. Zaidi (ed.), Wastewater Reuse–Risk Assessment, Decision-Making and Environmental Security, 291–296.

Table 1. Proposed new Israeli standards for effluent (average levels) *

Parameter	Units	Unrestricted Irrigation*	Rivers
Electric conductivity	dS/m	1.4	n/a
BOD	mg/l	10	10
TSS	mg/l	10	10
COD	mg/l	100	70
N-NH4	mg/l	20	1.5
Total nitrogen	mg/l	25	10
Total phosphorus	mg/l	5	1.0
Chloride	mg/l	250	400
Fluoride	mg/l	2	n/a
Sodium	mg/l	150	200
Faecal coliforms	Unit per 100 ml	10	200
Dissolved oxygen	mg/l	>0.5	>3
pH	mg/l	6.5–8.5	7.0–8.5
Residual chlorine	mg/l	1	0.05
Anionic detergent	mg/l	2	0.5
Mineral oil	mg/l	n/a	1
SAR	(mmol/L)0.5	5	n/a
Boron	mg/l	0.4	n/a
Arsenic	mg/l	0.1	0.1
Mercury	mg/l	0.002	0.0005
Chromium	mg/l	0.1	0.05
Nickel	mg/l	0.2	0.05
Selenium	mg/l	0.02	n/a
Lead	mg/l	0.1	0.008
Cadmium	mg/l	0.01	0.005
Zinc	mg/l	2	0.2
Iron	mg/l	2	n/a
Copper	mg/l	0.2	0.02
Manganese	mg/l	0.2	n/a
Aluminum	mg/l	5	n/a

European Commission

Update on AMR-related issues in several environmental policy contexts

Unit ENV C1 – Sustainable Freshwater Management
DG Environment

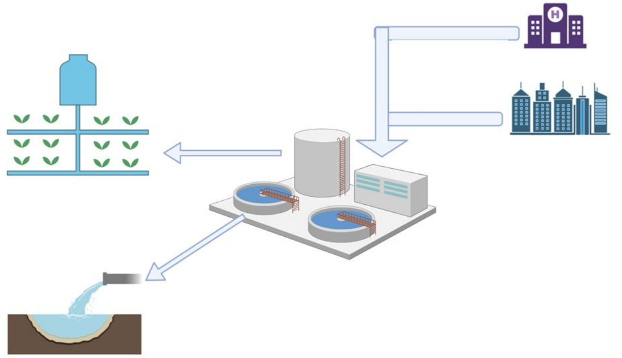
AMR One Health Network 29 February 2024

Urban Waste Water Treatment Directive

- Actions on Wastewater Treatment in the Strategic Approach: Invest in removal of pharmaceuticals and ARGs; investigate feasibility of upgrading selected UWWTPs to more advanced treatment technologies; support development of "greener" pharmaceuticals that degrade more readily to harmless substances
- Proposal to revise the UWWTD adopted by COM in October 2022 https://environment.ec.europa.eu/topics/water/urban-wastewater_en, including:
  - For all agglomerations of 100 000 p.e. and more, MS to monitor AMR at the inlets and outlets of UWWTPs at least twice/year, to increase knowledge/support further action.
  - Obligation on MS to conduct a risk assessment (human health/environment) and apply additional treatment where necessary.
  - Extended producer responsibility for pharmaceuticals and personal care products to contribute to treatment costs.

European Commission

But how do we monitor AMR in wastewater and irrigated crops?



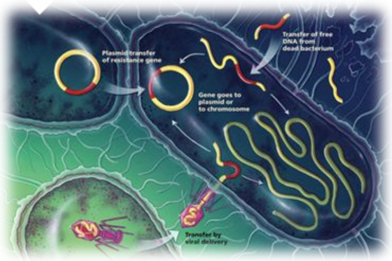
Culturomics

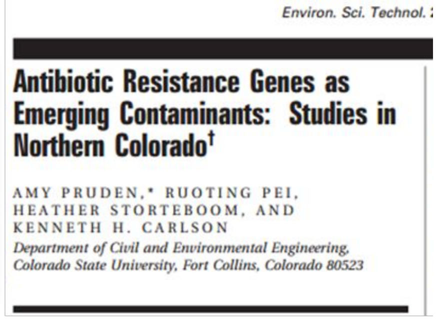
Selective media targeting total and antibiotic resistant coliforms/*E. coli*)

WHO integrated global surveillance on ESBL-producing *E. coli* using a "One Health" approach

qPCR

Quantitative evaluation of specific Bacteria/ARGs

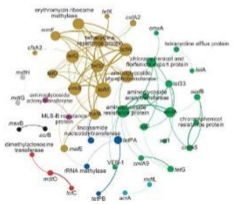



Environ. Sci. Technol.

Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado†

AMY PRUDEN,* RUOTING PEI, HEATHER STORTEBOOM, AND KENNETH H. CARLSON

Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, Colorado 80523



Metagenomics

Next-gen sequencing of DNA directly extracted from environmental samples (microbiome/resistome)

nature COMMUNICATIONS

An omics-based framework for assessing the health risk of antimicrobial resistance genes

An-Ni Zhang[1,2], Jeffry M. Gaston[3], Chengzhen L. Dai[2], Shijie Zhao[2], Mathilde Poyet[2,4,5], Mathieu Groussin[2,4,5], Xiaole Yin[1], Li-Guan Li[1], Mark C. M. van Loosdrecht[6], Edward Topp[7], Michael R. Gillings[8], William P. Hanage[9], James M. Tiedje[10], Katya Moniz[2], Eric J. Alm[2,4,5] & Tong Zhang[1,11,12]

- What indicators do we monitor?
- What are the epidemiological cutoffs?

ESBL MDR *E. coli* are abundant inWWTP effluents

Cephalosporins

CTX-M-15
OXA-1
TEM-126

Sulfonoamides

sul1
sul3

Aminoglycosides

APH(3'')-Ib
APH(6)-Id
APH(4)-Ia
aadA
AAC(3)-IIc
rmtB

Fluoroquinolones

AAC(6')-Ib-cr
qacH

Macrolides

mphA

Glycopeptides

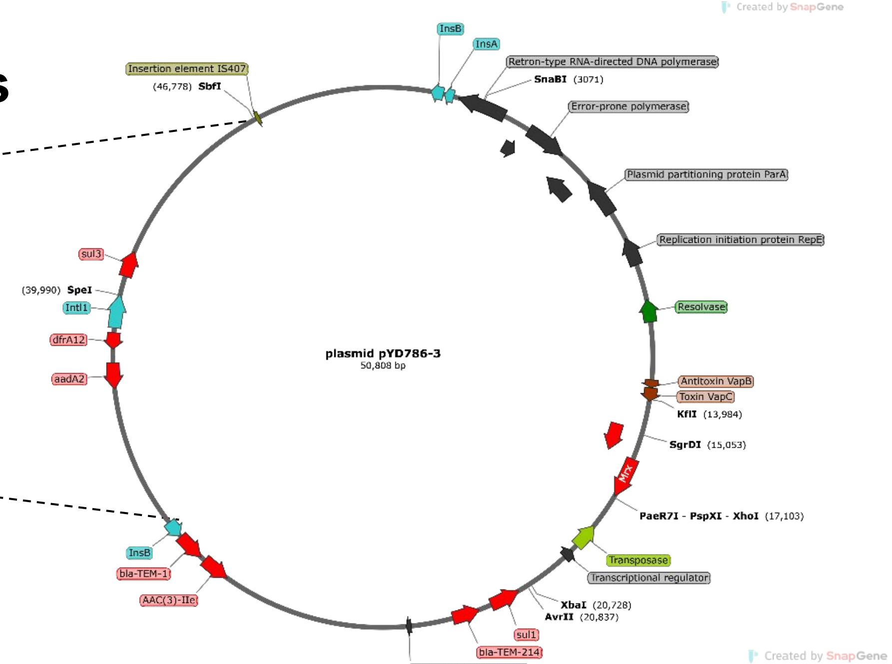
BRP(MBL)

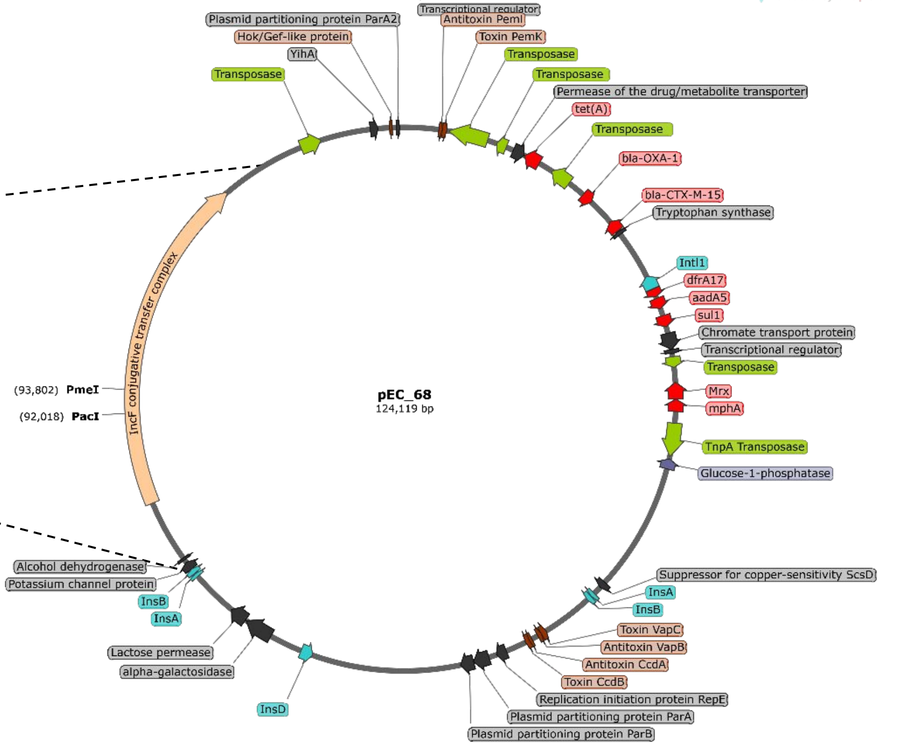
Chloramphenicol

floR
mef(B)1

Tetracycline

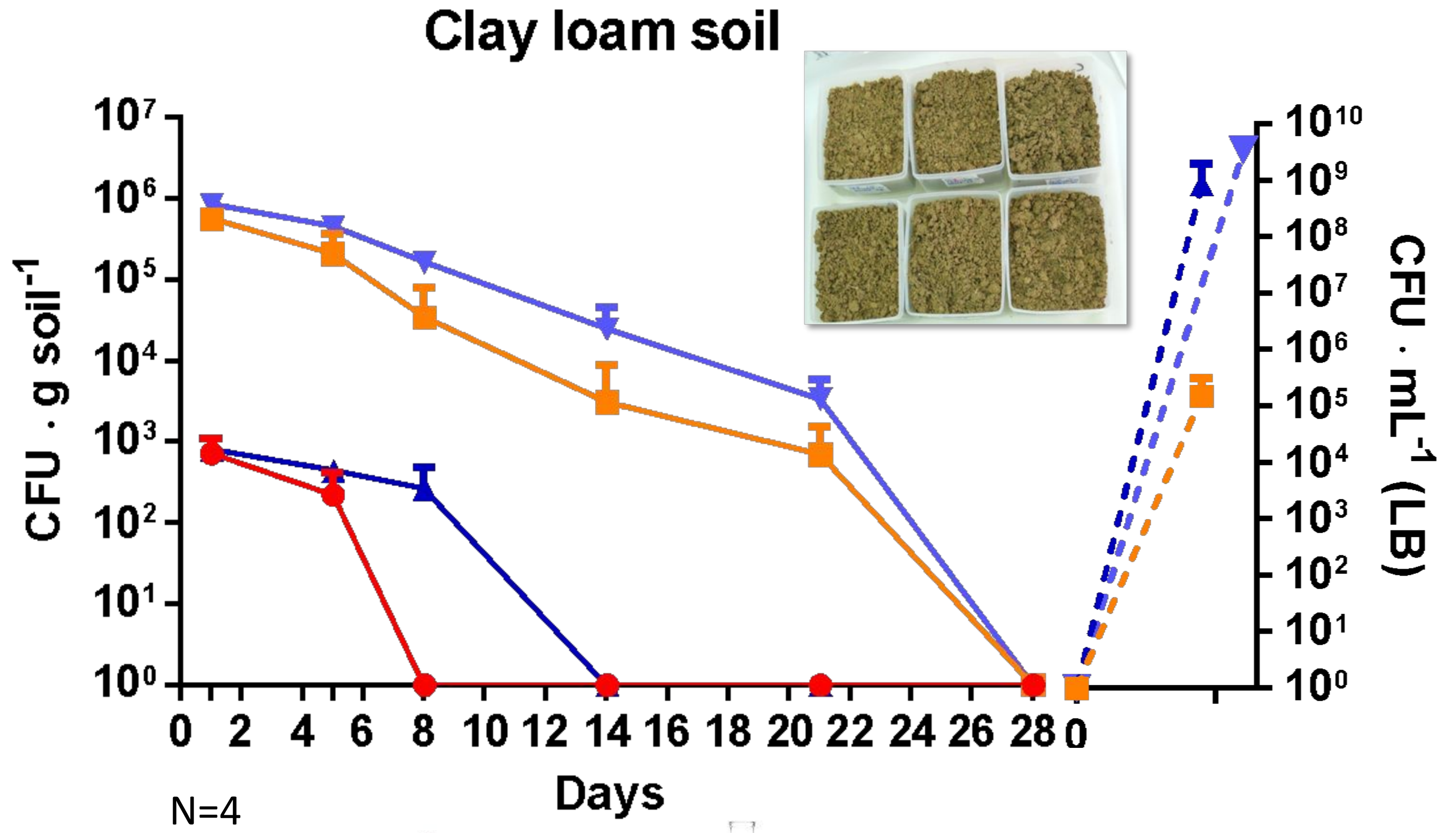
tet(A)

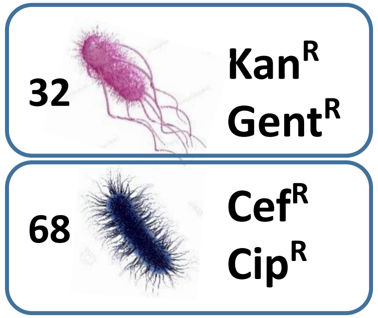


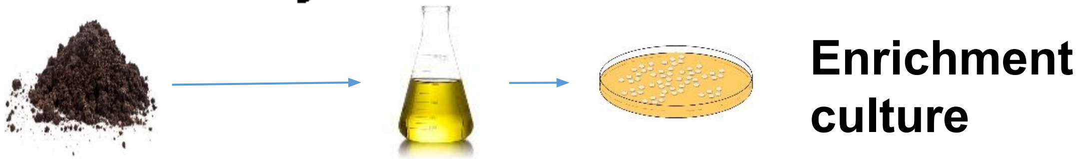


Assembly of hybrid Illumina/Oxford Nanopore whole genome sequencing

Persistence of MDR E. coli in soil





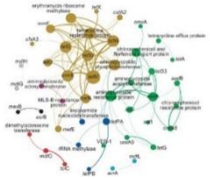


"UNDER THE RADAR"

not detected does not mean not present!

Marano *et al.*, 2021, *Environmental Science and Technology*

Enrichment platform to detect “UNDER THE RADAR” microbiomes and resistomes that potentially proliferate under “gut-like conditions



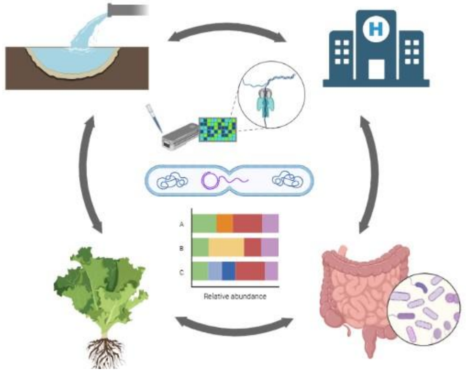
ISME

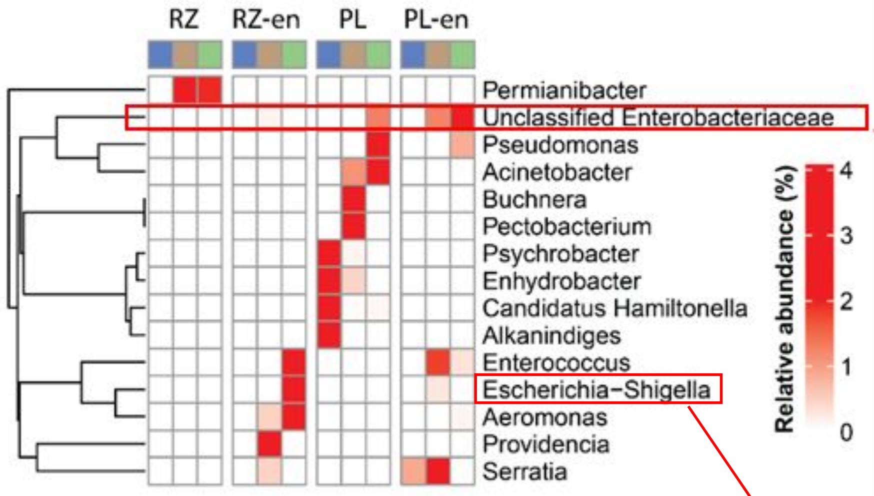
Original Article

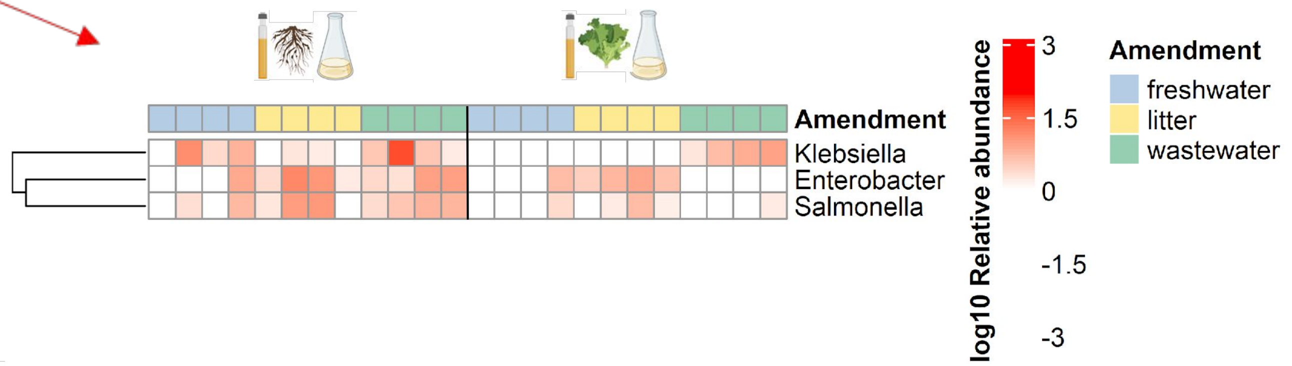
Occurrence of “under-the-radar” antibiotic resistance in anthropogenically affected produce

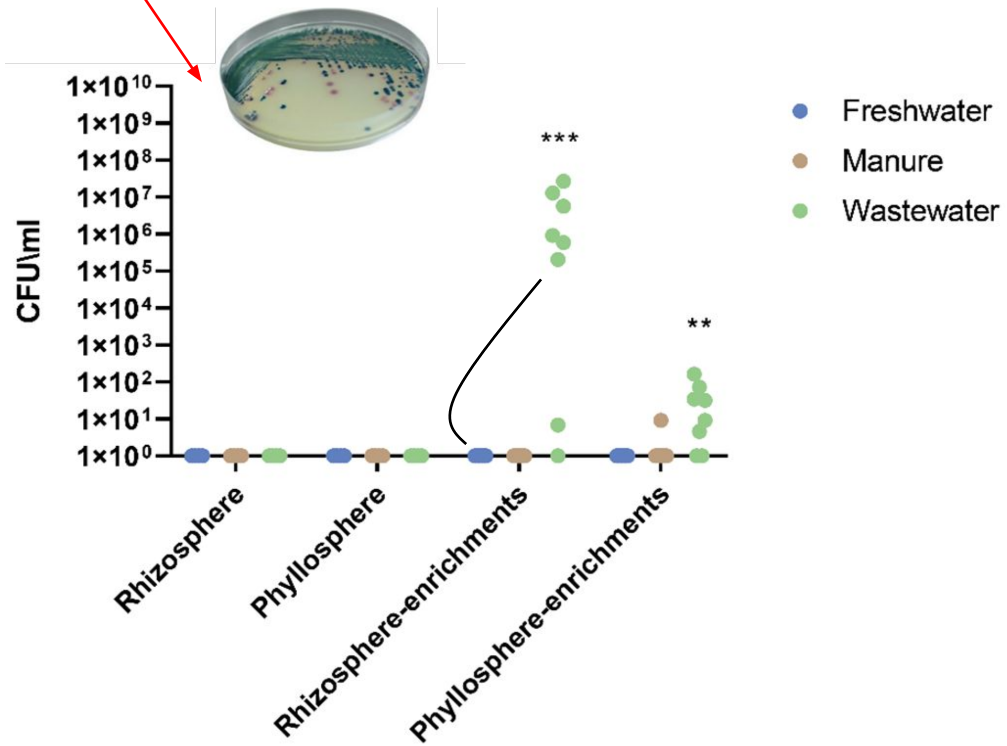
- Samples enriched for 16 hrs in BHI media under aerobic & anoxic conditions
- Bacteria = total and cefotaxime resistant *E. coli*/other fecal coliforms

Gut-like enrichment facilitates the detection of clinically relevant pathogens



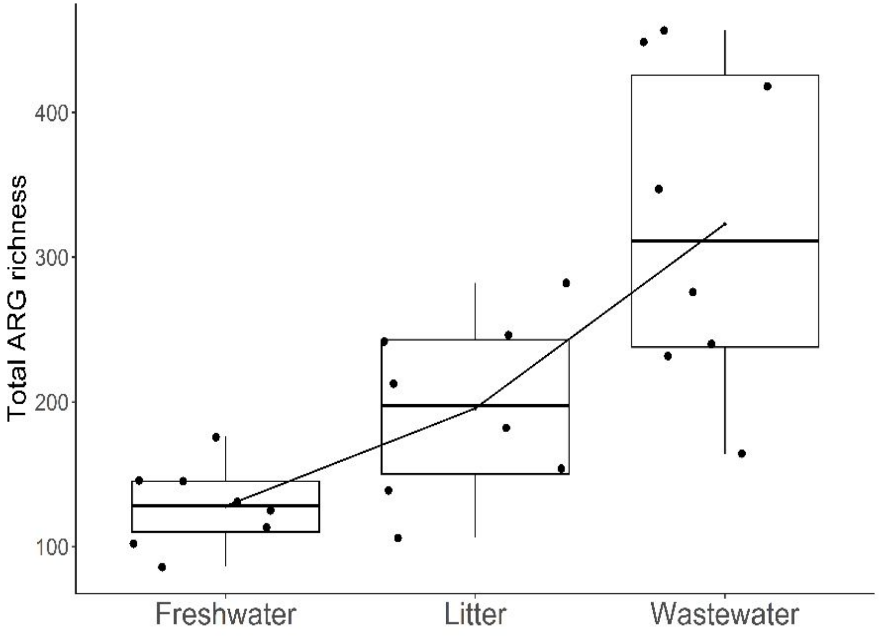


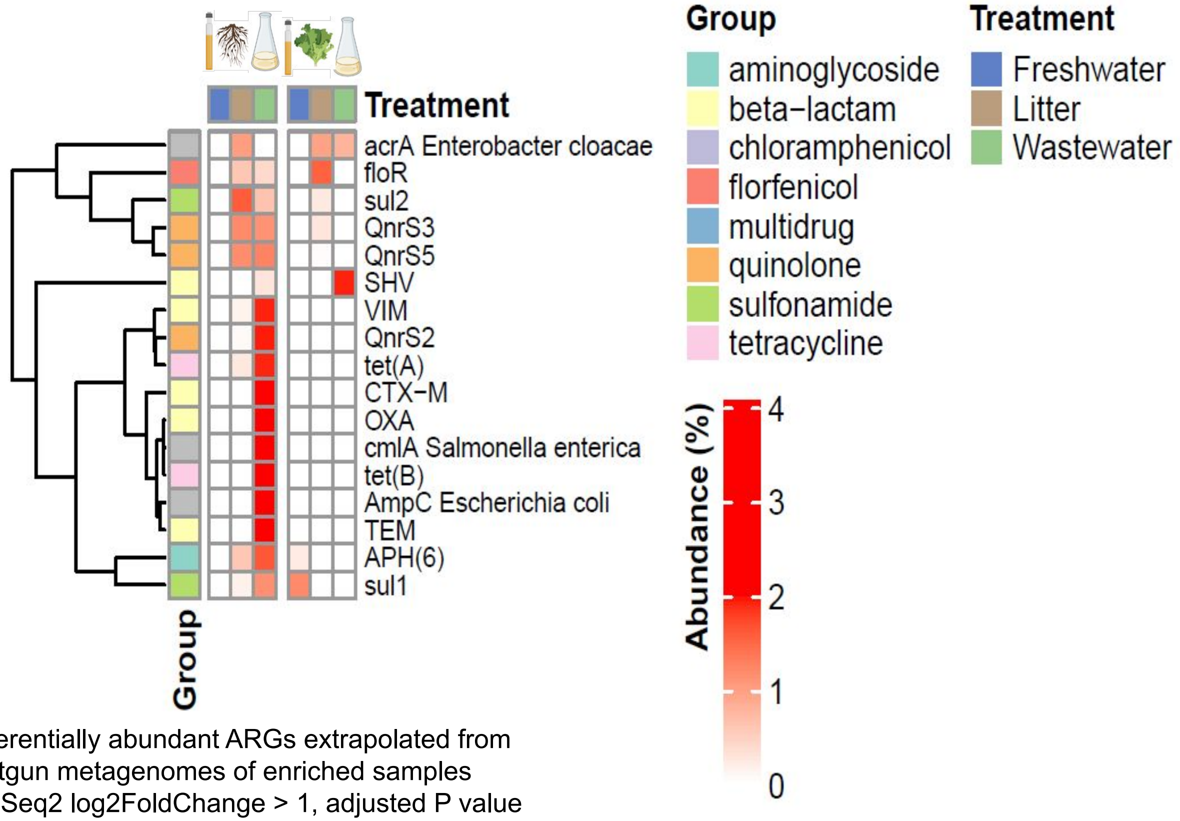




Shotgun-metagenomic data

Gut-like enrichment selects for clinically relevant ARGs in "anthropogenically-impacted" samples





Differentially abundant ARGs extrapolated from shotgun metagenomes of enriched samples (DESeq2 log2FoldChange > 1, adjusted P value < 0.01) in enriched rhizosphere and phyllosphere samples

Davidovitch *et al.,* ISME J.

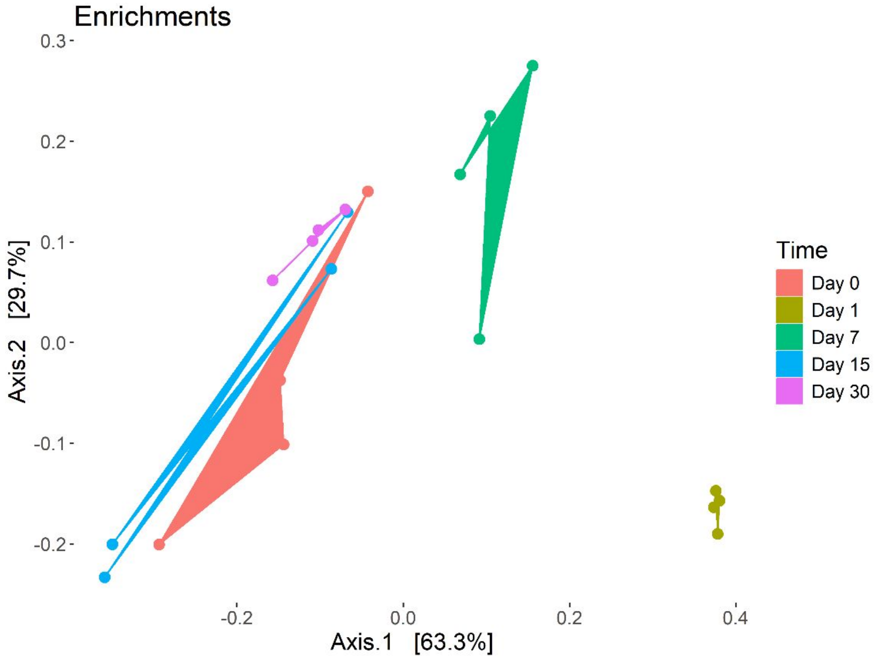
Persistence of sewage-derived pathogens in soil

Microbiome and resistome dynamics

1 day — 30 days

0 — 7 days — 15 days

❖ Sewage added to soil till maximum water holding capacity, and subsequently soil was kept at constant humidity at 30°C

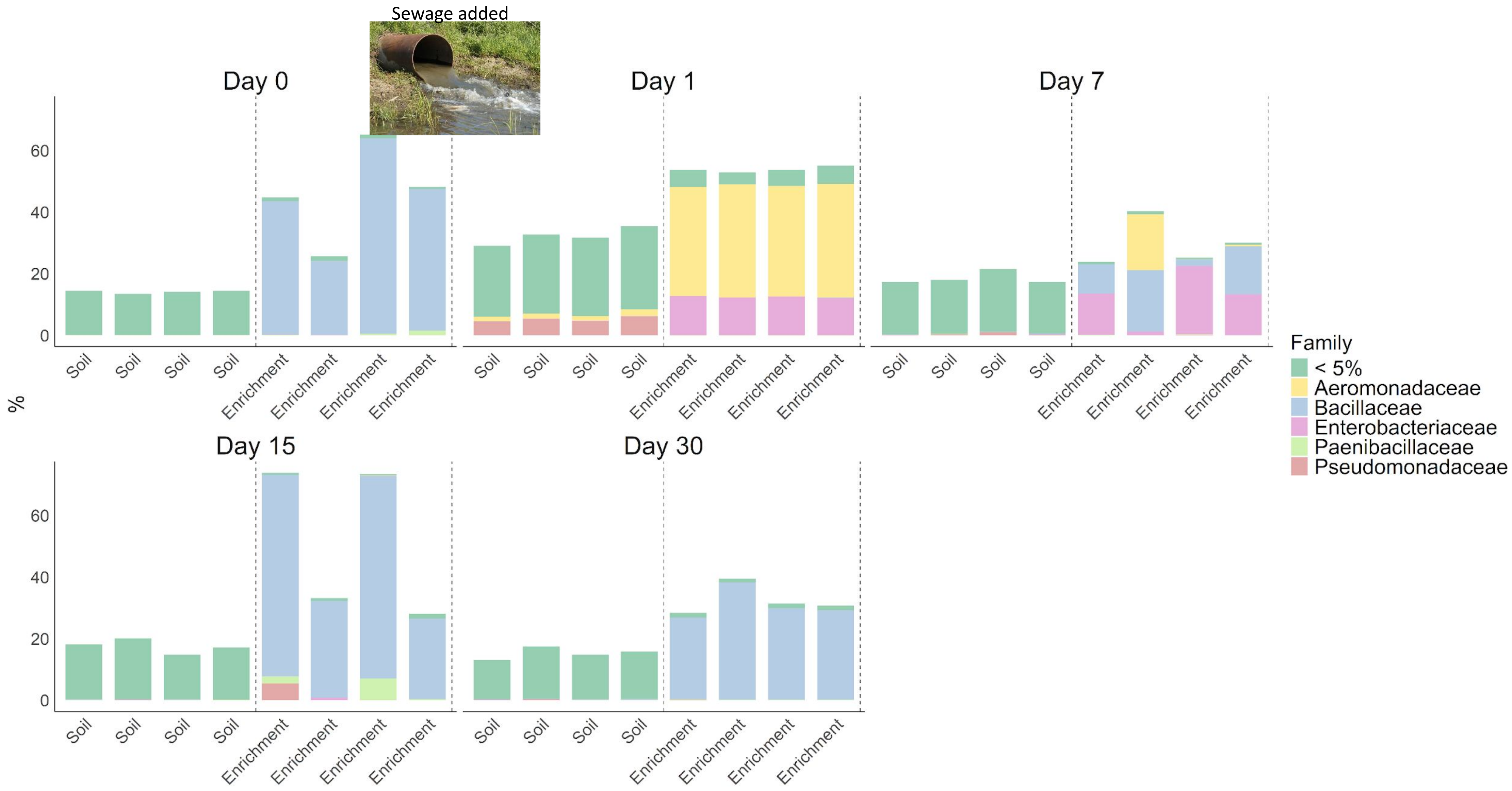


Ecosystem Resilience !

In ecology, resilience is the capacity of an ecosystem to respond to a perturbation or disturbance by resisting damage and subsequently recovering.

Source: Wikipedia

Persistence of sewage-derived bacteria in soil



Conclusions

Under the radar pathogens: Enrichment can expose **viable** pathogen and AMR indicators in anthropogenically impacted soil and crops that are not detected in direct analyses

Ecological barriers: TWW-derived ARB and ARGs dissipate in irrigated soils and produce due to the resilience of the soil microbiome

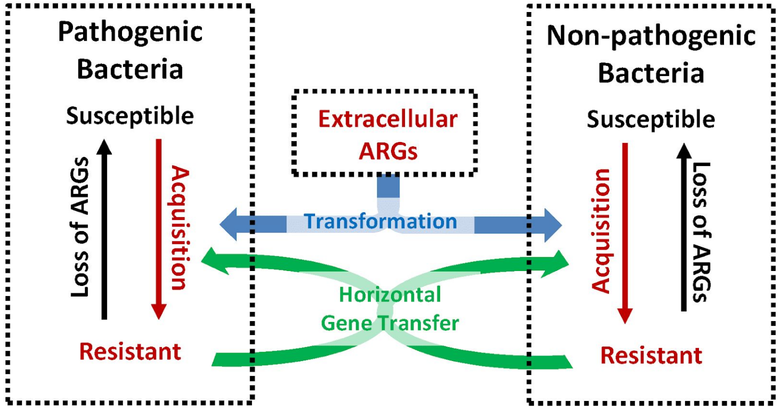
- ***By combining empirical studies, risk assessment tools and ecological models we can devise irrigation regimes that take advantage of soil resilience to reduce pathogen contamination of irrigated produce, even when low quality water is used!***

Future directions

Integration of empirical data from enrichment-based/HGT studies in quantitative microbial risk assessment (QMRA) models to assess risks of AMR and pathogens in TWW-irrigated produce

I. Hazards

- *To what extent do ARB, ARGs, and MGEs contribute to spread of antibiotic resistant pathogenic bacteria?*
- *To what extent do ARGs carried by non-pathogenic bacteria contribute to spread of antibiotic resistant pathogenic bacteria?*



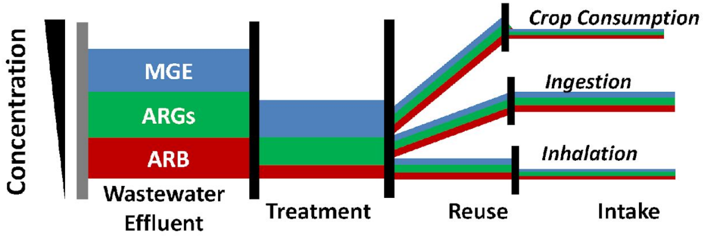
$$\frac{ds_s}{dt} = \mu s_s\left(1 - \frac{(s_s + s_r)}{N}\right) - \gamma s_s s_r + \kappa s_r - (\delta)s_s$$

$$\frac{ds_r}{dt} = \alpha_{fit}\mu s_r\left(1 - \frac{(s_s + s_r)}{N}\right) - \gamma s_s s_r + \kappa s_r - (\delta)s_r$$

Hong et. al., 2018

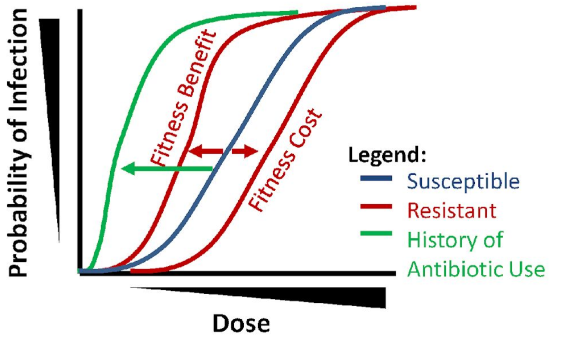
II. Exposure Assessment

- *What are wastewater effluent concentrations of ARB, ARGs, and MGEs?*
- *How do treatment processes influence concentrations?*



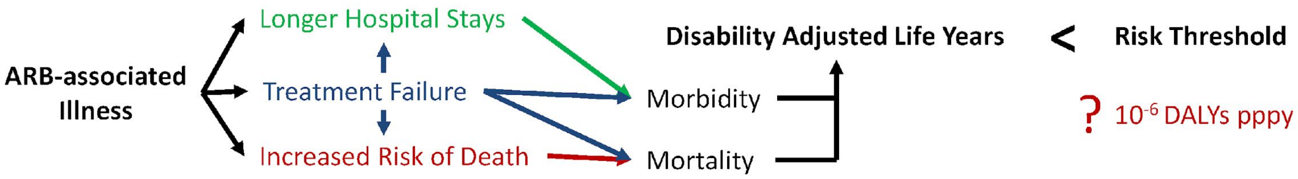
III. Dose-Response

- *Do antibiotic resistant strains have different dose-response relationships due to fitness costs or benefits of ARGs?*
- *How does a history of antibiotic use influence dose-response relationships for susceptible and resistance strains?*



IV. Risk Characterization

- *What is the impact of antibiotic resistant infections, relative to susceptible infections, on morbidity and mortality.*
- *Are the current risk thresholds for enteric infections appropriate for antibiotic resistant infections?*



Institute of Soil, Water & Environmental Sciences

Acknowledgments

Yong-Guan Zhu

Collaborators

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ISRAEL NATURE AND PARKS AUTHORITY

David Weinberg
Shiri Biran
Amnon Gorodenchick
Idan Bar-Lev

IUE
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ASU
Arizona State University

Kerry Hamilton
Hunter Quon

UTS
Australian Institute for Microbiology & Infection

Ethan Wyrsch
Steven Djordjevic

DSWAP

Funding

National Natural Science Foundation of China
1986

הקרן הלאומית למדע
المؤسسة الإسرائيلية للعلوم
Israel Science Foundation

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