



Antibiotikarückstände, Resistenzgene und resistente Mikroorganismen im aufbereiteten Abwasser

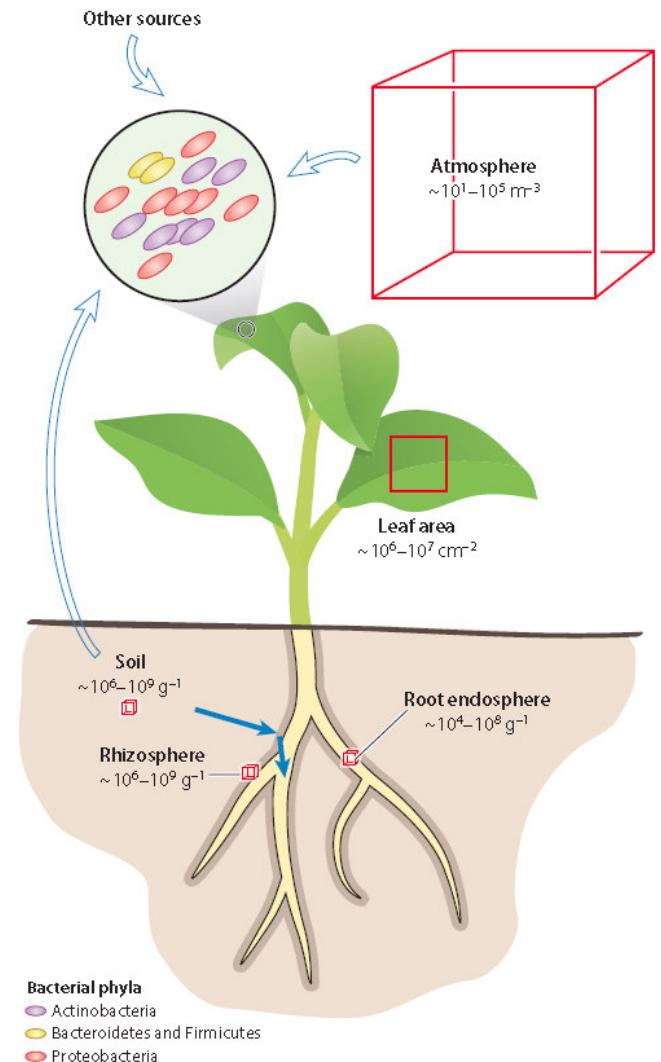
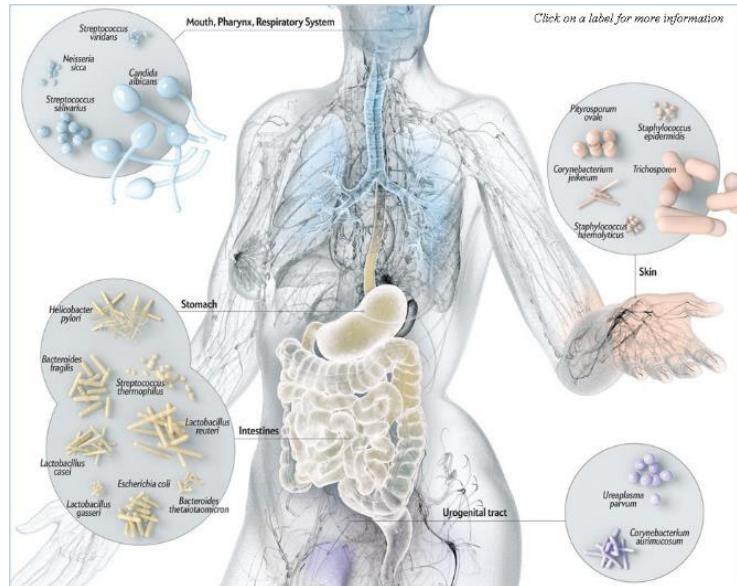


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Dank für die Unterstützung bei der Vorbereitung dieses Vortrags geht an
**Prof. Dr. Eddie Cytryn, Prof. Dr. Thomas Berendonk, Dr. Ioannis
Kampouris & Prof. Dr. Jan Siemens**

Antibiotika-Resistenz ein ökologisches und Umweltphänomen



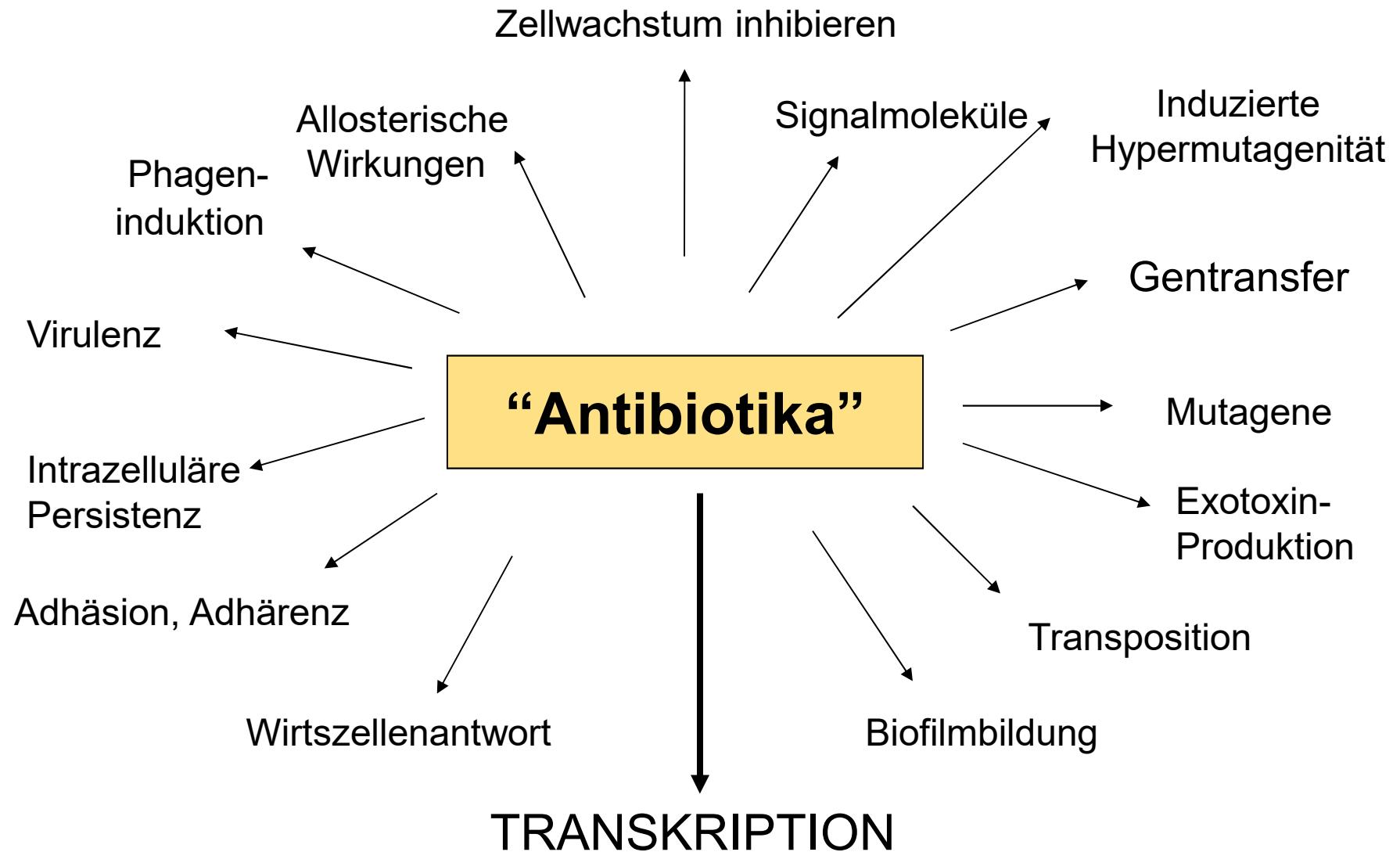
Das natürliche oder intrinsische Resistome

Lack of target, inactivation, low uptake and efflux
(Olivares et al., 2013; Perry & Wright, 2013)

Erworrene Antibiotikaresistenz

Antibiotics, metal and biocide compounds used by humans cause community shifts, select resistance and horizontally acquired resistances

Natürliche Funktionen von Antibiotika: bioaktive Moleküle, die bei sub-inhibitorischen Konzentrationen



Courtesy of J. Davies

Umwelt “hot spots”



- ➔ Abwasser, Klärschlämme, Gülle, Gärreste sind Umwelthabitate mit einer großen Zahl Bakterien mit transferablen Resistenzgenen gegen Desinfektionsmittel, Metall- und Antibiotikaverbindungen.
- ➔ Komplexe Interaktionen zwischen Bakterien in Habitaten mit sub-inhibitorischen Konzentrationen von unterschiedlichsten selektiven Agenzien.

Courtesy M. Gillings

Nutzung von aufbereitetem Abwasser

Belastung von Beregnungswasser mit Mikroschadstoffen, multi-resistanten Bakterien, ARGs, MGEs

Effekte auf

Bodenmikrobiome/Mikroschadstoffe

Pflanzenmikrobiom/Mikroschadstoffe

Grundwassermikrobiom/Mikroschadstoffe

Wichtige Faktoren:

- ➔ Beregnungshäufigkeit
- ➔ Bodeneigenschaften
- ➔ Physikochemische Eigenschaften der Mikroschadstoffe
- ➔ Fähigkeit der Pflanzen Mikroschadstoffe aufzunehmen
- ➔ Mikrobiome des aufbereiteten Abwassers



Contents lists available at [ScienceDirect](#)

Environment International

journal homepage: www.elsevier.com/locate/envint



Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment



Sara Rodriguez-Mozaz^{a,b,*}, Ivone Vaz-Moreira^c, Saulo Varela Della Giustina^{a,b}, Marta Llorca^{a,b,d}, Damià Barceló^{a,b,d}, Sara Schubert^e, Thomas U. Berendonk^e, Irene Michael-Kordatou^f, Despo Fatta-Kassinos^{f,g}, Jose Luis Martinez^h, Christian Elpersⁱ, Isabel Henriques^j, Thomas Jaeger^k, Thomas Schwartz^k, Erik Paulshus^l, Kristin O'Sullivan^l, Katariina M.M. Pärnänen^m, Marko Virta^m, Thi Thuy Doⁿ, Fiona Walshⁿ, Célia M. Manaia^c



Konzentrationen von Antibiotika in aufbereitetem Abwasser von 13 europäischen Kläranlagen

S. Rodriguez-Mozaz, et al.

Environment International 140 (2020) 105733

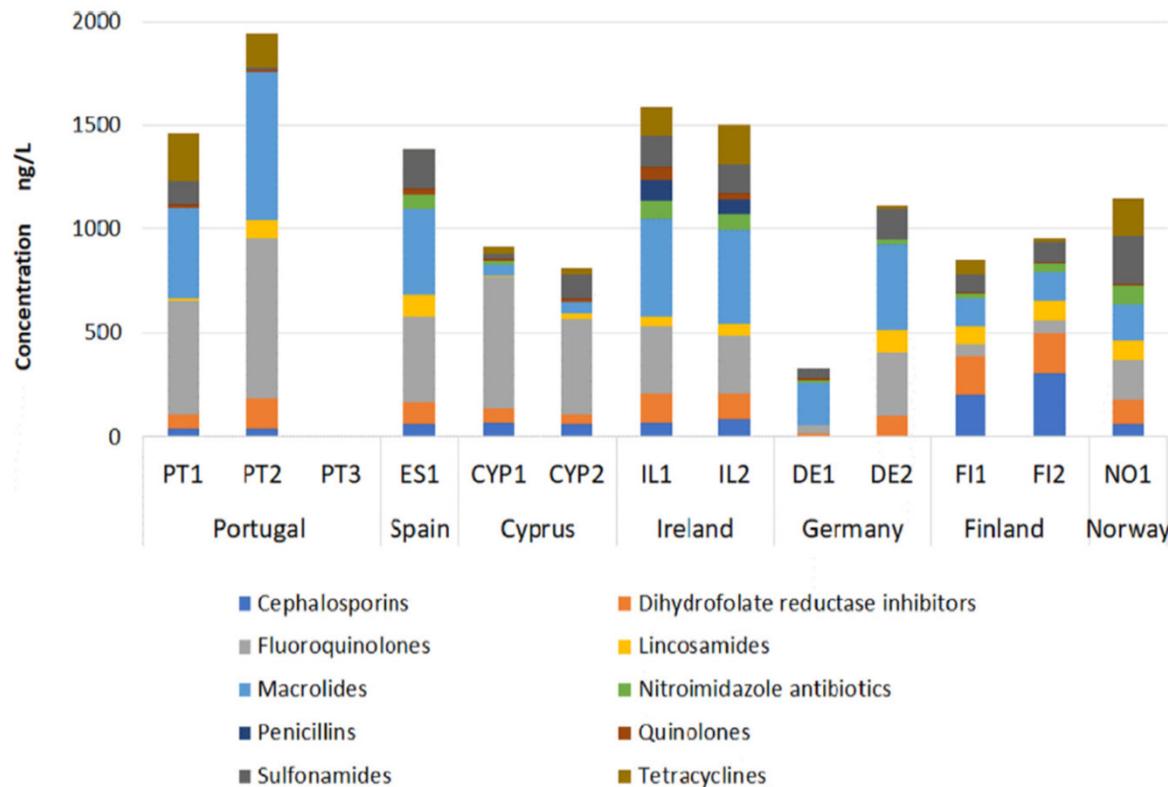


Fig. 3. Antibiotic concentrations profile (ng/L) in the 13 WWTPs under study. Each column represents one WWTP and each of the bars in the column represents the average concentration from the 4 sampling campaigns (March 2015, October 2015, March 2016 and September 2016) for all the antibiotics belonging to a chemical family, except PT1 (average of March 2015, October 2015 and September 2016). PT3 (just one sampling campaign in Spring 2016) is omitted.

Konzentrationen von Ciprofloxacin und Azithromycin in aufbereitetem Abwasser von 13 europäischen Kläranlagen: Frühjahr 2015, Herbst 2015, Frühjahr 2016, Herbst 2016

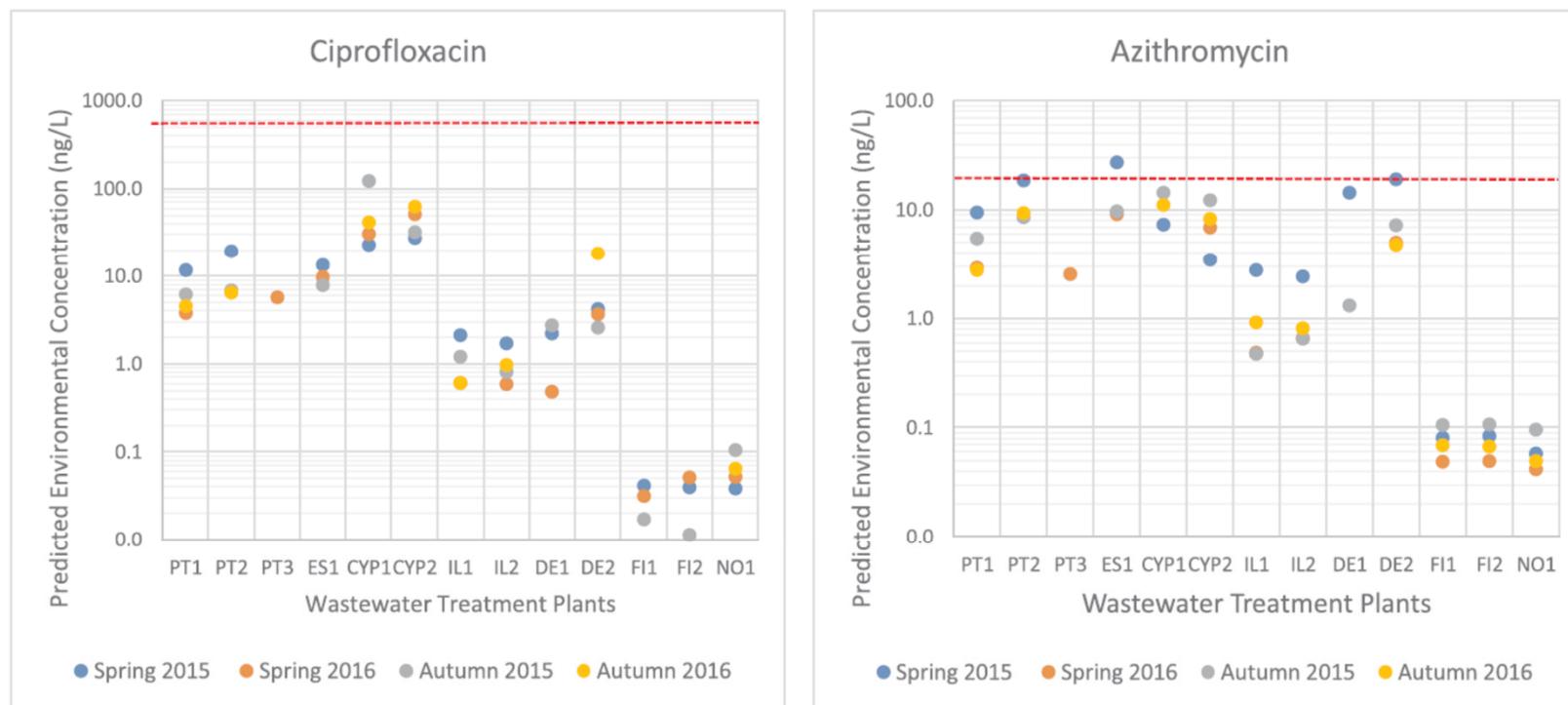


Fig. 5. Predicted Environmental Concentration (PEC) of ciprofloxacin and azithromycin (ng/L) associated with the 13 WWTPs under study in each of the 4 sampling campaigns. Dashed line corresponds to the lower PNEC (ng/L) selected based on Tell et al. 2019; i.e. 60 and 20 ng/L for ciprofloxacin and azithromycin respectively.

Courtesy of T.U. Berendonk

Evaluation of chemical and biological contaminants of emerging concern in treated wastewater intended for agricultural reuse



Nikiforos A. Alygizakis^{a,b,*}, Jakub Urík^c, Vasiliki G. Beretsou^d, Ioannis Kampouris^e, Aikaterini Galani^b, Martina Oswaldova^a, Thomas Berendonk^e, Peter Oswald^a, Nikolaos S. Thomaidis^{b,*}, Jaroslav Slobodník^a, Branislav Vrana^c, Despo Fatta-Kassinos^d

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^b Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece

^c Masaryk University, Faculty of Science, Research Centre for Toxic Compounds in the Environment (RECETOX), Kamenice 753/5, 625 00 Brno, Czech Republic

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^e Environmental Sciences Technische Universität Dresden, Institute for Hydrobiology, Dresden, Germany

Environment International 2020, 138

Table 1

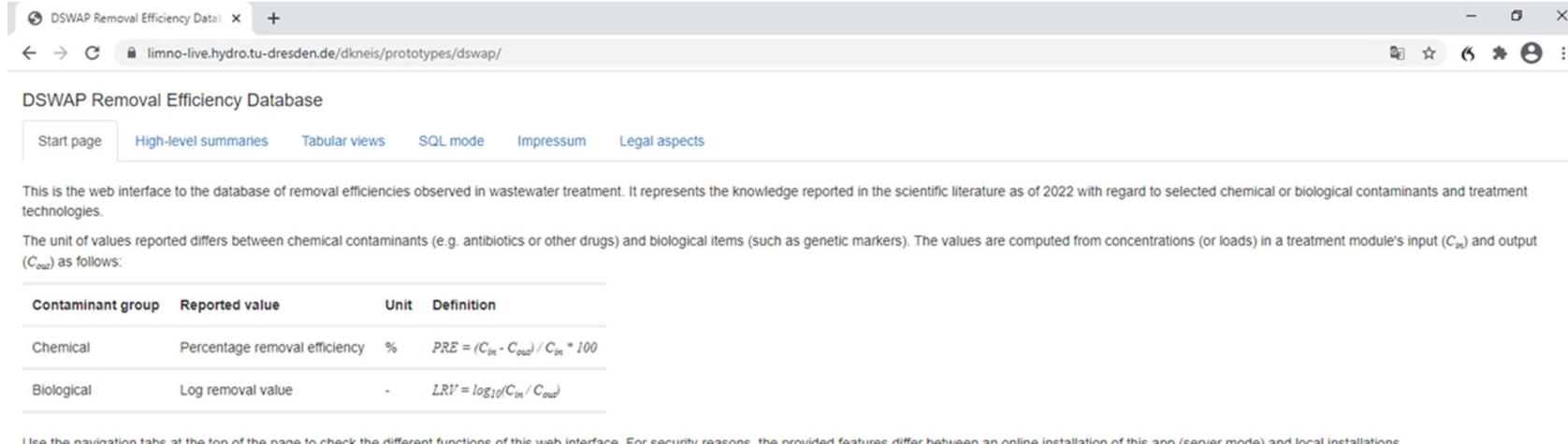
List of the prioritized compounds, their maximum observed concentration ($n = 4$), the provisional no-effect concentration (PNEC) thresholds used and their reference. Additionally, the table represents the frequency of appearance (FoA), the frequency of exceedance (FoE), the extent of PNEC exceedance (EoE) and the risk score for the prioritized compounds.

Compound	Maximum concentration (ng L ⁻¹)	PNEC (ng L ⁻¹)	Reference PNEC	FoA	FoE	EoE	Risk Score
Candesartan	96	3.1	P-PNEC	1.00	1.00	1.00	3.00
Telmisartan	1086	42	P-PNEC	1.00	1.00	0.88	2.88
4-OH-E1 (4-Hydroxyestrone)	90	3.6	EQS-proposal	1.00	1.00	0.87	2.87
Ofloxacin	335	21	PNEC exp. Aquire 80421	1.00	1.00	0.49	2.49
Azithromycin	262	19	EQS-proposal	1.00	1.00	0.40	2.40
Venlafaxine	415	38	EQS-proposal	1.00	1.00	0.29	2.29
Galaxolidone	900	101	P-PNEC	1.00	1.00	0.27	2.27
Ibuprofen	57	10	EQS chronic water	1.00	1.00	0.16	2.16
Lorazepam	334	96	P-PNEC	1.00	1.00	0.09	2.09
Medazepam	512	206	P-PNEC	1.00	1.00	0.04	2.04
Carbamazepine	86	50	PNEC chronic Aquire 152195	1.00	1.00	0.02	2.02
Clarithromycin	166	120	EQS-proposal	1.00	0.50	0.01	1.51
Diclofenac	56	50	EQS-proposal	1.00	0.50	0.00	1.50
Atorvastatin epoxide	11	10	P-PNEC	1.00	0.50	0.00	1.50
Erythromycin	244	200	EQS-proposal	1.00	0.25	0.00	1.25
Meclofenamic Acid	98	97	P-PNEC	1.00	0.25	0.00	1.25

Courtesy of T.U. Berendonk

Data on removal efficiency associated with different treatment technologies is scattered (and scarce)

- ⇒ Data compilation on removal efficiencies observed in cases studies into a simple, extensible database
- ⇒ Accessible via web interface to facilitate the selection of appropriate treatment technology

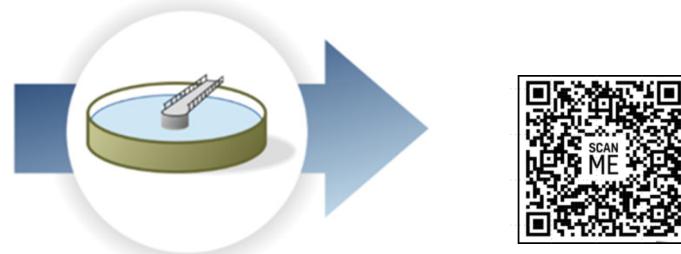


This is the web interface to the database of removal efficiencies observed in wastewater treatment. It represents the knowledge reported in the scientific literature as of 2022 with regard to selected chemical or biological contaminants and treatment technologies.

The unit of values reported differs between chemical contaminants (e.g. antibiotics or other drugs) and biological items (such as genetic markers). The values are computed from concentrations (or loads) in a treatment module's input (C_{in}) and output (C_{out}) as follows:

Contaminant group	Reported value	Unit	Definition
Chemical	Percentage removal efficiency	%	$PRE = (C_{in} - C_{out}) / C_{in} * 100$
Biological	Log removal value	-	$LRV = \log_{10}(C_{in} / C_{out})$

Use the navigation tabs at the top of the page to check the different functions of this web interface. For security reasons, the provided features differ between an online installation of this app (server mode) and local installations.



Courtesy of T.U. Berendonk

DSWAP Datenbank zur Effizienz verschiedener Technologien beim Abbau/Abtrennung von Mikroschadstoffen

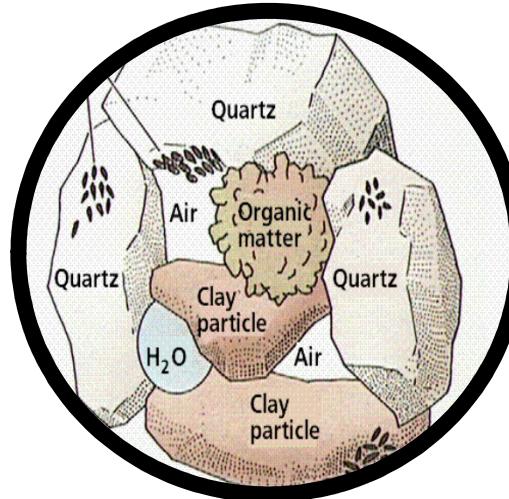
The screenshot shows a web browser window titled "DSWAP Removal Efficiency Data". The URL in the address bar is "limno-live.hydro.tu-dresden.de/dkneis/prototypes/dswap/". The page content is titled "DSWAP Removal Efficiency Database". It features a navigation menu with links to "Start page", "High-level summaries", "Tabular views", "SQL mode", "Impressum", and "Legal aspects". Below the menu, there are three tabs: "Numbers of records" (selected), "Ranges per contaminant", and "Ranges per technology group". A dropdown menu labeled "Select criterion for building the summary" is set to "TechnologyGroup". The main content area displays a table with two columns: "TechnologyGroup" and "Records". The table lists various treatment technologies and their corresponding record counts.

TechnologyGroup	Records
Conventional activated sludge treatment	114
Fenton treatment	5
Forward Osmosis	3
Free Chlorine	25
Granular activated carbon treatment	22
Membrane Bioreactor	46
Microfiltration	6
Nano Filtration	7
Ozonation treatment	68
Ozonation/H ₂ O ₂ treatment	4
Photo Fenton treatment	47
Reverse Osmosis	36
Sand Filtration	15
Sewage treatment plants	36
UV combined with H ₂ O ₂ treatment	94

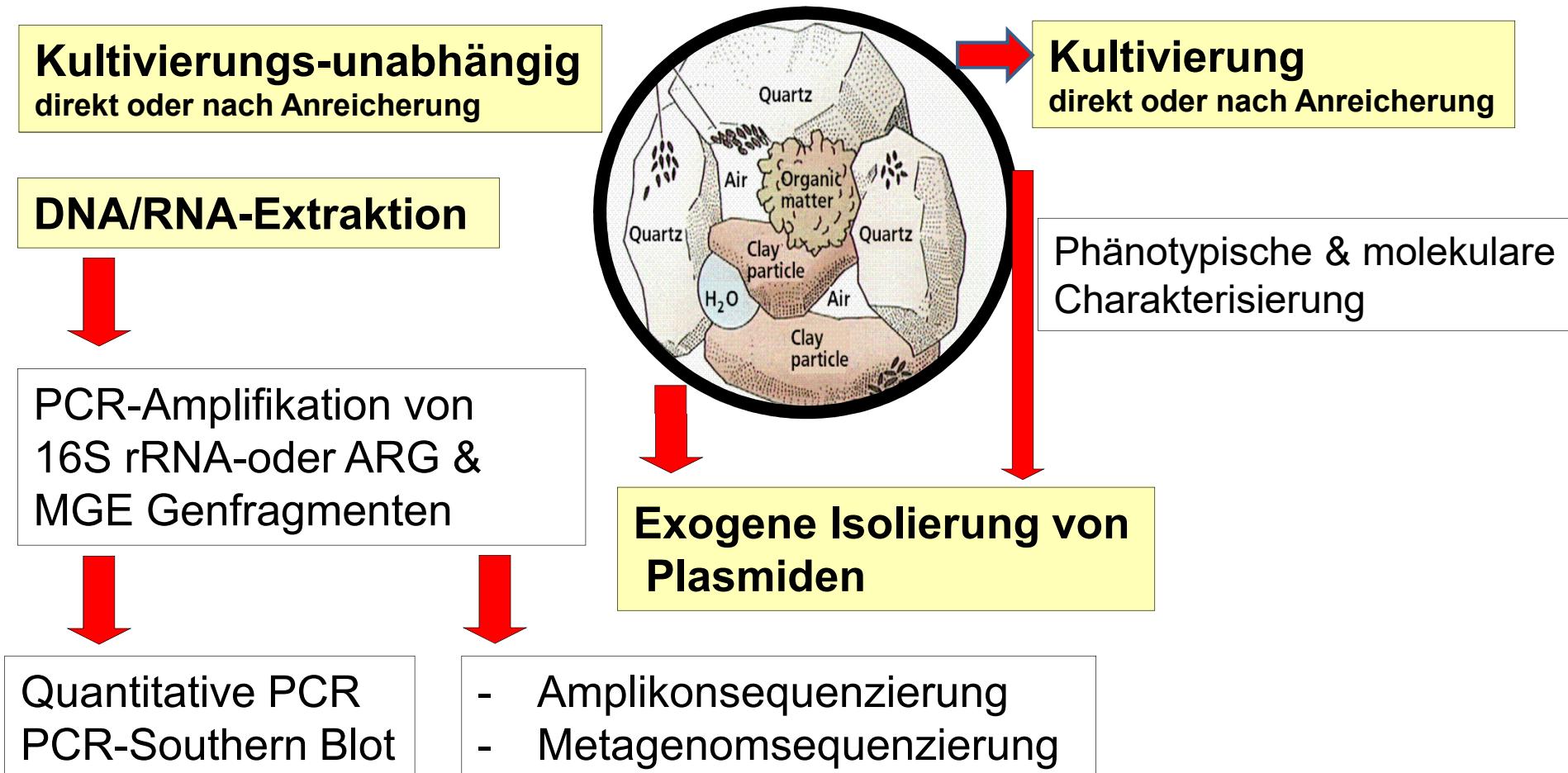
Monitoring der Transmission von Resistenzgenen, Integrons und Plasmiden

Organischer → Boden → Gemüse & Obst → Menschliches
Dünger
Gülle
Gärreste
Klärschlamm

Beregnungswasser



Monitoring von Antibiotikaresistenzgenen und mobilen genetischen Elementen





Contents lists available at [ScienceDirect](#)

Water Research

journal homepage: www.elsevier.com/locate/watres



Antibiotic resistance gene load and irrigation intensity determine the impact of wastewater irrigation on antimicrobial resistance in the soil microbiome

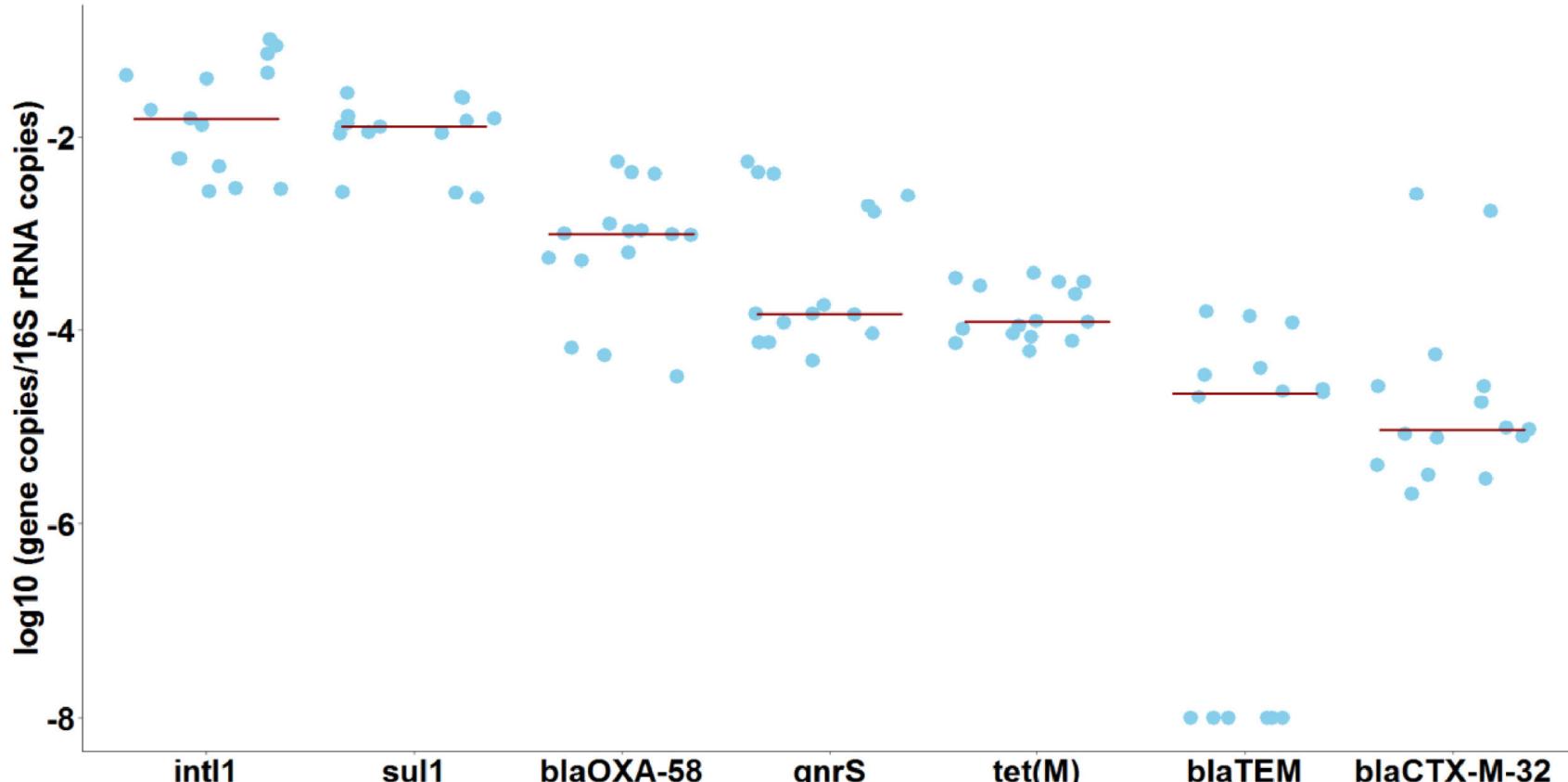


Ioannis D. Kampouris^{a,*}, Shelesh Agrawal^b, Laura Orschler^b, Damiano Cacace^a,
Steffen Kunze^a, Thomas U. Berendonk^a, Uli Klümper^{a,*}

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^bTechnische Universität Darmstadt, Institute IWAR, Chair of Wastewater Engineering, Franziska-Braun-Straße 7, 64287 Darmstadt, Germany

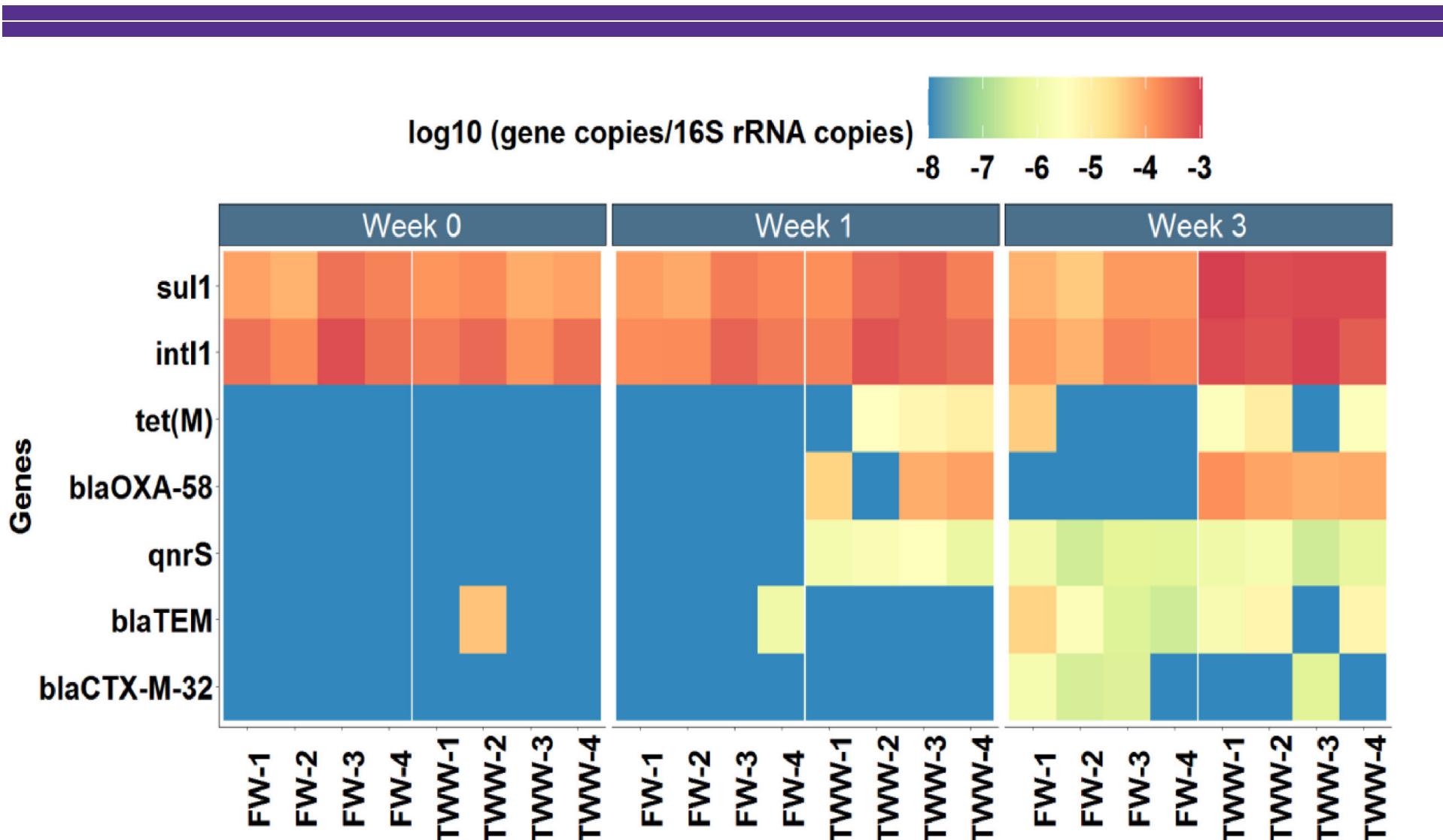
Genes with the highest abundance in TWW irrigation associated with the TWW-irrigated soil



***bla_{TEM}* & *bla_{CTX-M-32}* showed the lowest abundance in the TWW irrigation.**

Courtesy Ioannis Kampouris

TWW irrigation favored higher dissemination for the genes with higher abundance in TWW than FW

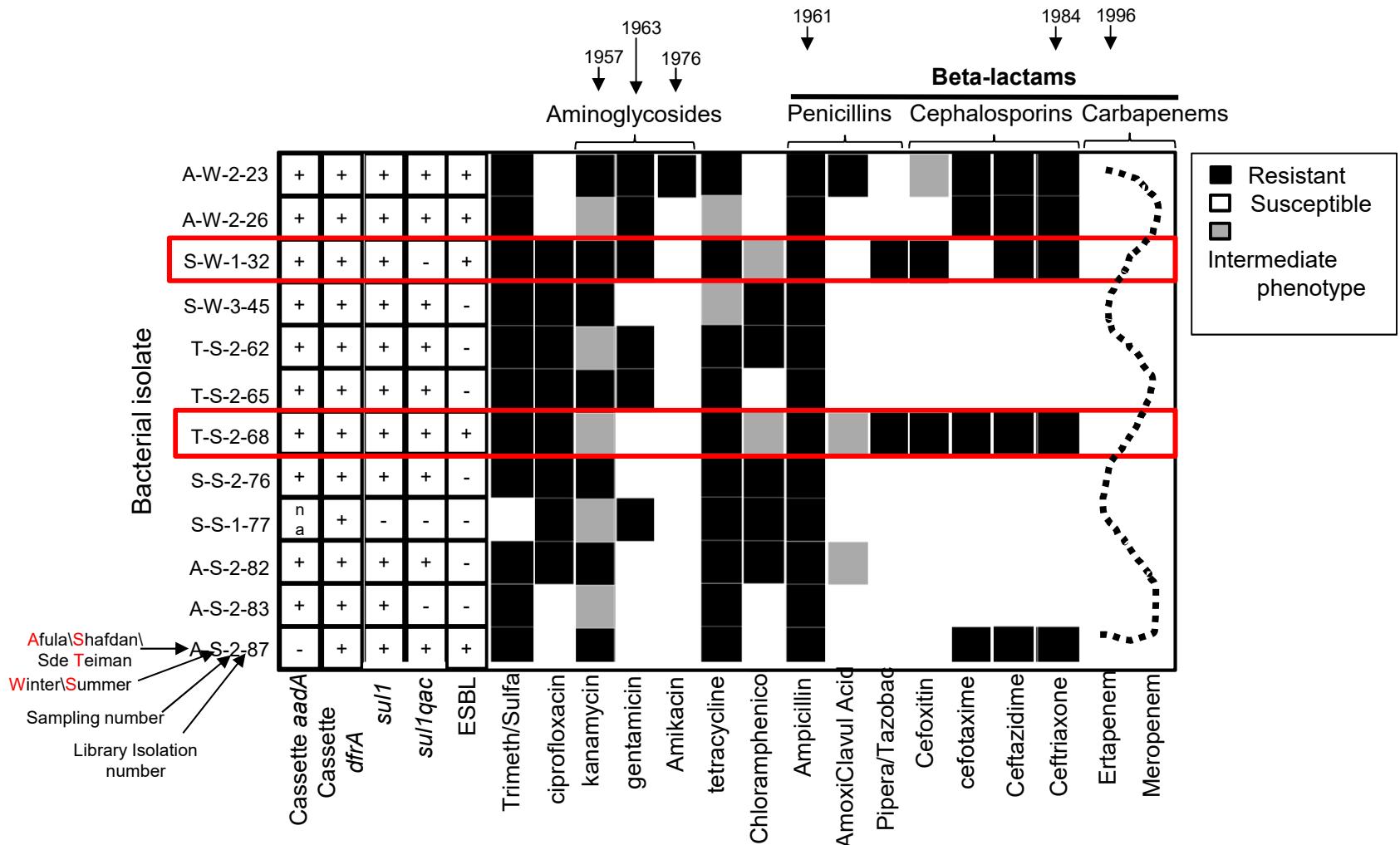


FW: Freshwater-Group, TWW: Treated Wastewater-Group,
PERMANOVA test (Euclidean distance, $R^2 = 0.63$, $p < 0.001$, $n=4$)

Courtesy of Ioannis Campouris & T.U. Berendonk

WWTP effluents contain MDR pathogen indicators

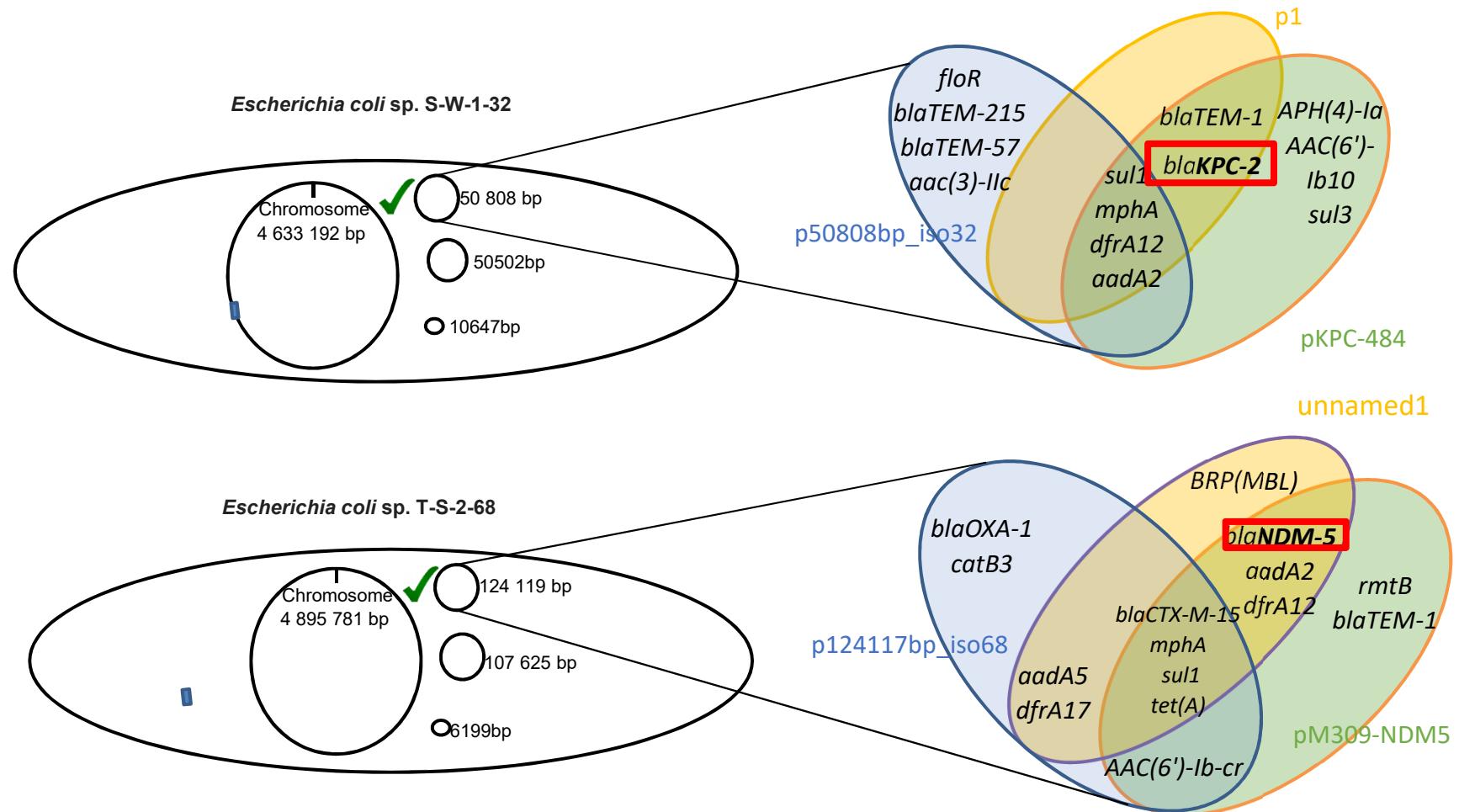
MDR fecal coliforms characterized from three effluents - Two *E.coli* selected for further experiments



Courtesy of Eddie Cytryn

Assembly of hybrid Illumina/Oxford Nanopore whole genome sequencing

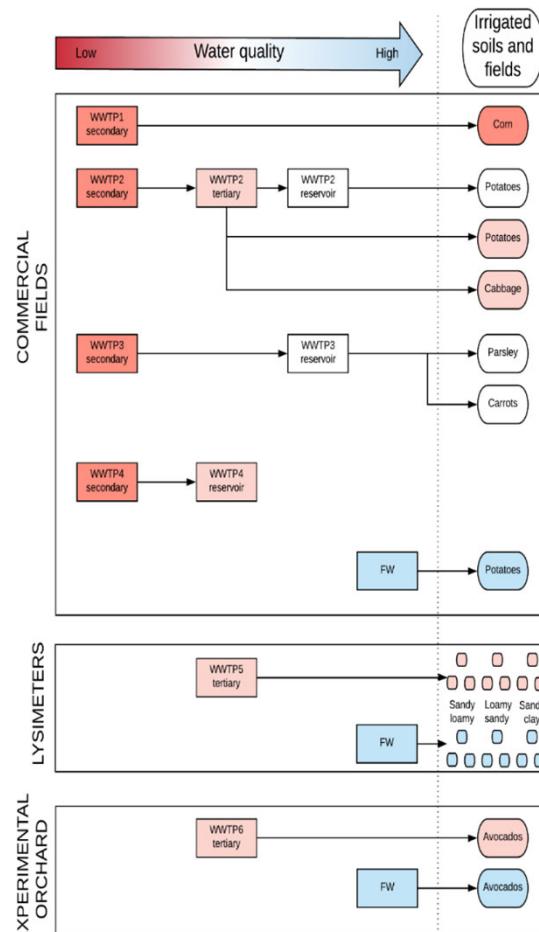
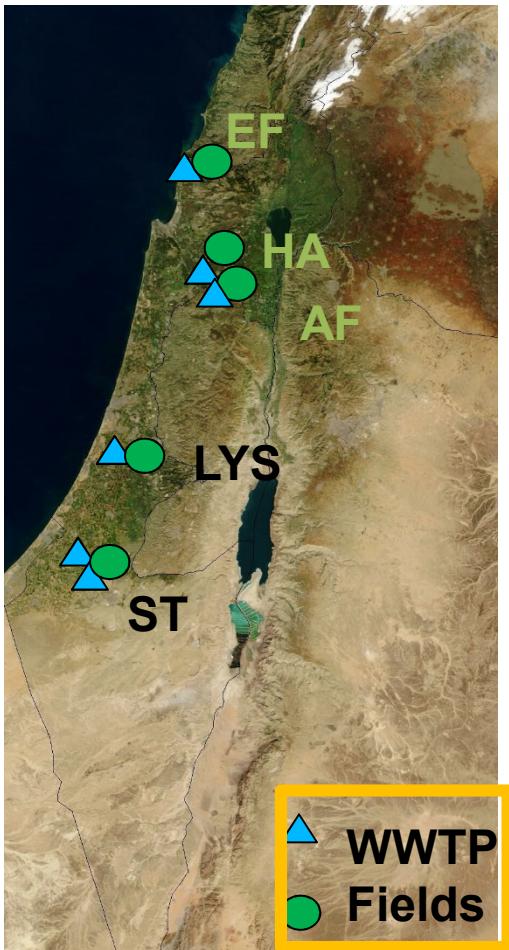
WWTP effluents contain MDR pathogen indicators



Marano et al., 2019 , Water Research

Courtesy of Eddie Cytryn

Source tracking of ARGs in WWTP effluents



Targeted genes

16S rRNA- marker for total bacteria

int1- Class 1 integron integrase gene

qnrS- plasmid associated quinolone resistance gene (proxy for plasmid-associated AR).

bla_{TEM}- common, well established

bla_{CTX-M}- clinically-associated ESBL

bla_{OXA} (variants 2, 10)

bla_{GES}- harbored on integrons & abundant in wastewater environments

Source tracking of ARGs in WWTP effluents

ARG	<i>intI1</i>	<i>qnrS</i>	<i>blaTEM</i>	<i>blaCTX-M</i>	<i>blaOXA-2</i>	<i>blaOXA-10</i>	<i>blaGES</i>
copies/ml							
Effluent samples	10^6 - 10^8	10^2 - 10^4	10^3 - 10^5	ND-DNQ	10^2 - 10^4	10^3 - 10^7	10^3 - 10^5
Freshwater samples	10^1 - 10^4	DNQ	DNQ	ND	DNQ- 10^2	ND	ND

Daily load of ARGs entering (copies/m ²)	<i>intI1</i>	<i>qnrS</i>	<i>blaTEM</i>	<i>blaCTX-M</i>	<i>blaOXA-2</i>	<i>blaOXA-10</i>	<i>blaGES</i>
	10^{10} - 10^{12}	10^6 - 10^8	10^7 - 10^9	ND- 10^3	10^6 - 10^8	10^7 - 10^9	10^5 - 10^7

DNQ- detectable but not quantifiable; ND- not detected

Marano et al., 2019 , Water Research

Courtesy of Eddie Cytryn



Source tracking of ARGs in WWTP effluents

SOIL

Irrigation Water source	Crop type	ARGs (copies g soil ⁻¹)						
		bla _{TEM}	qnrS	bla _{CTX-M-32}	bla _{OXA-2}	bla _{OXA10}	bla _{GES}	
WWTP1 effluents		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
		BDL	BDL	BDL	BDL	BDL	BDL	BDL
WWTP2 effluents		4.6·10 ⁴ ±5.26·10 ⁴	DBNQ	DBNQ	BDL	BDL	BDL	BDL
		BDL	DBNQ	BDL	BDL	BDL	BDL	BDL
		DBNQ	DBNQ	BDL	BDL	BDL	BDL	BDL
WWTP2 reservoir		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
WWTP3 reservoir		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
		BDL	BDL	BDL	BDL	BDL	BDL	BDL
FW	DNQ- $\frac{2.1 \cdot 10^4}{\pm 9.5 \cdot 10^3}$	BDL	DBNQ	BDL	BDL	BDL	BDL	BDL

CROPS

Irrigation Water source	Crop type	ARGs (copies g matter ⁻¹)						
		bla _{TEM}	qnrS	bla _{CTX-M-32}	bla _{OXA-2}	bla _{OXA10}	bla _{GE}	
WWTP1 effluents		2.35 · 10 ⁴ ±1.1 · 10 ⁴	2.35 · 10 ⁴ ±1.09 · 10 ⁴	BDL	BDL	BDL	BDL	BDL
		BDL	BDL	BDL	BDL	BDL	BDL	BDL
WWTP2 effluents		4.17 · 10 ⁴ ±4.08 · 10 ⁴	BDL	BDL	BDL	BDL	BDL	BDL
		BDL	DBNQ	BDL	BDL	BDL	BDL	BDL
		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
WWTP2 reservoir		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
WWTP3 reservoir		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL
FW		DBNQ	BDL	BDL	BDL	BDL	BDL	BDL

DNQ- detectable but not quantifiable; BDL- below detection limit



Institute of Soil, Water & Environmental Sciences

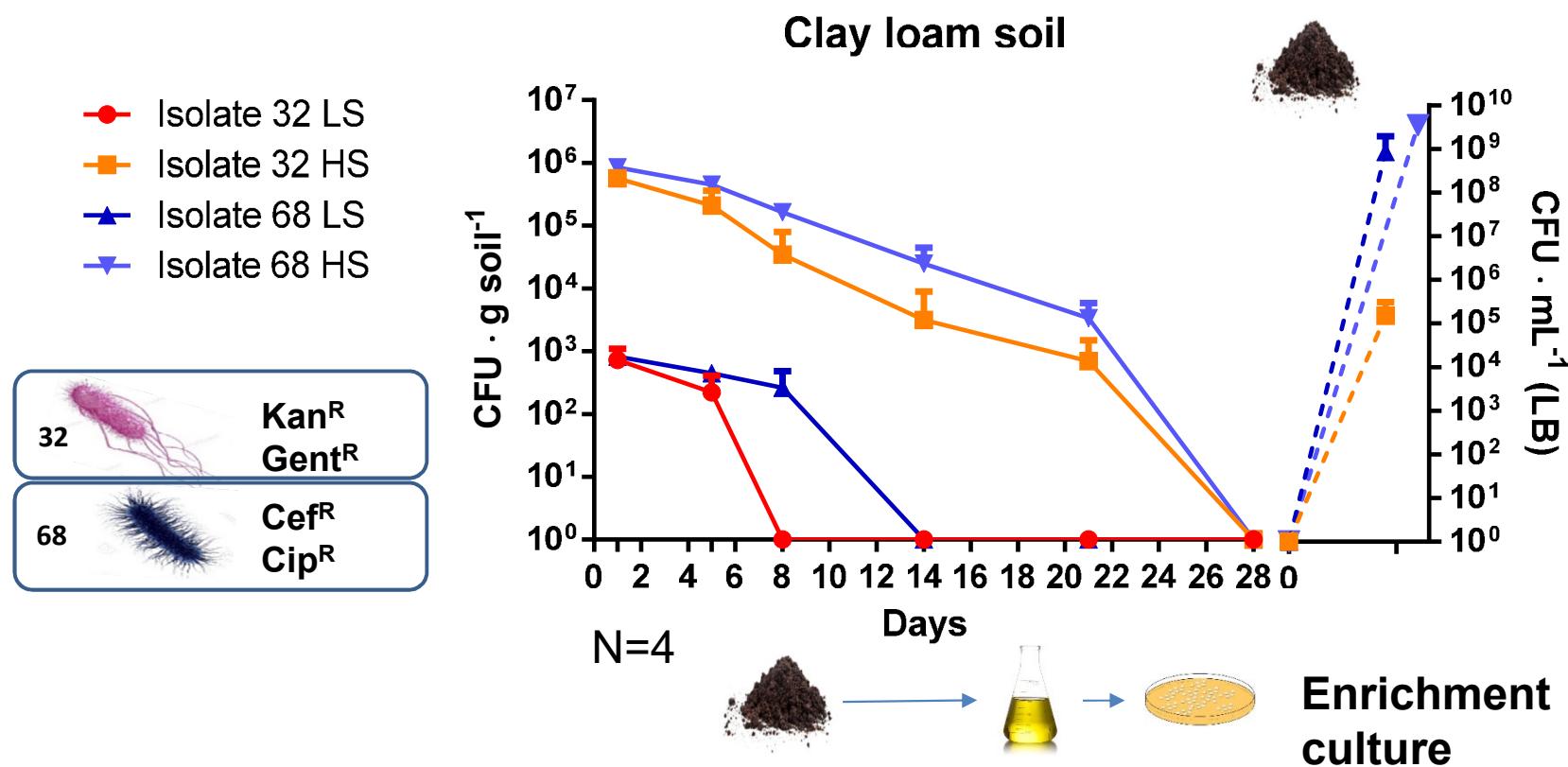
Courtesy of Eddie Cytryn

Marano et al., 2019 , *Water Research*

Ecological barriers: evidence of soil microbiome resilience

- ➔ TWW has negligible impact on soil ARB and ARG abundance”
Negreanu et al., 2012 Environmental Science and Technology.
- ➔ Soil is an ecological barrier that impedes dissemination of ARB and ARGs, Marano *et al.*, 2019, Water Research

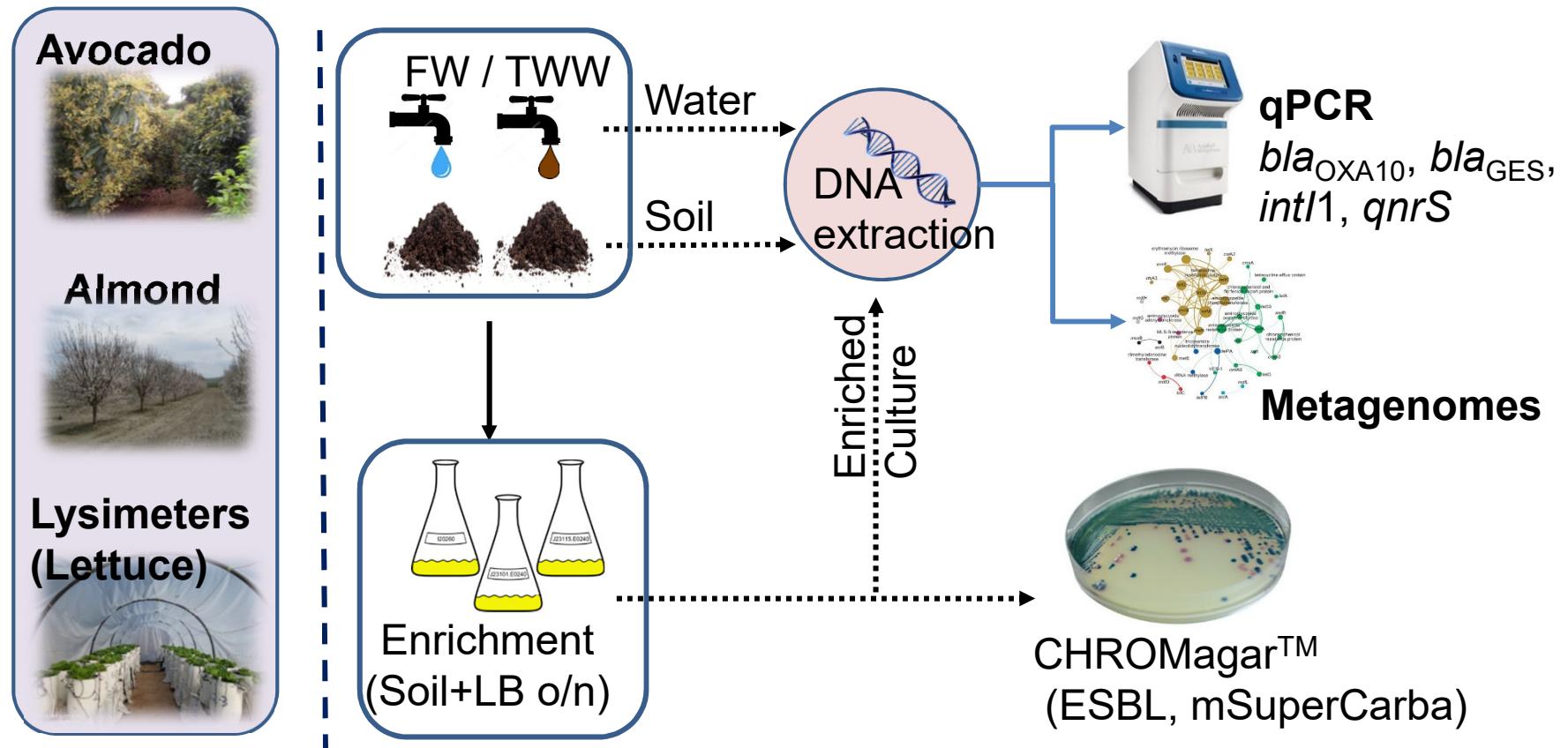
Persistence of MDR *E. coli* in soil



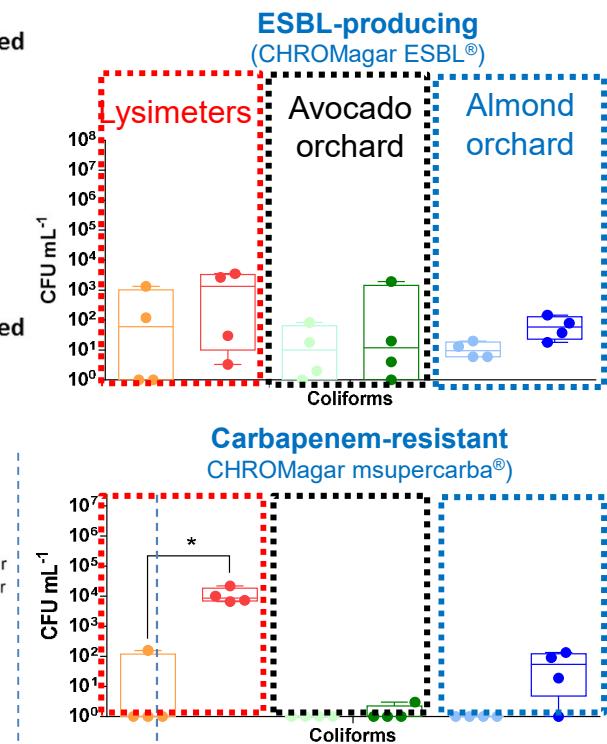
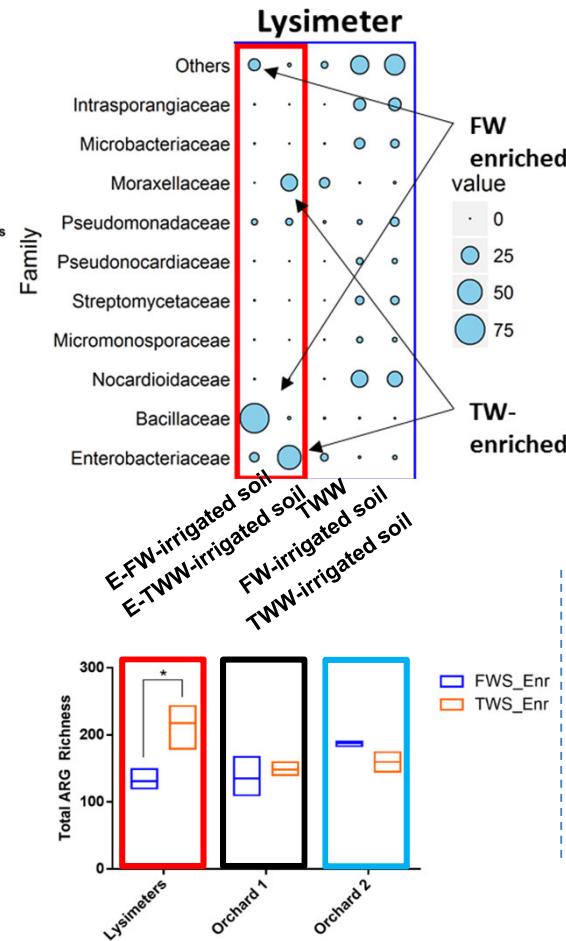
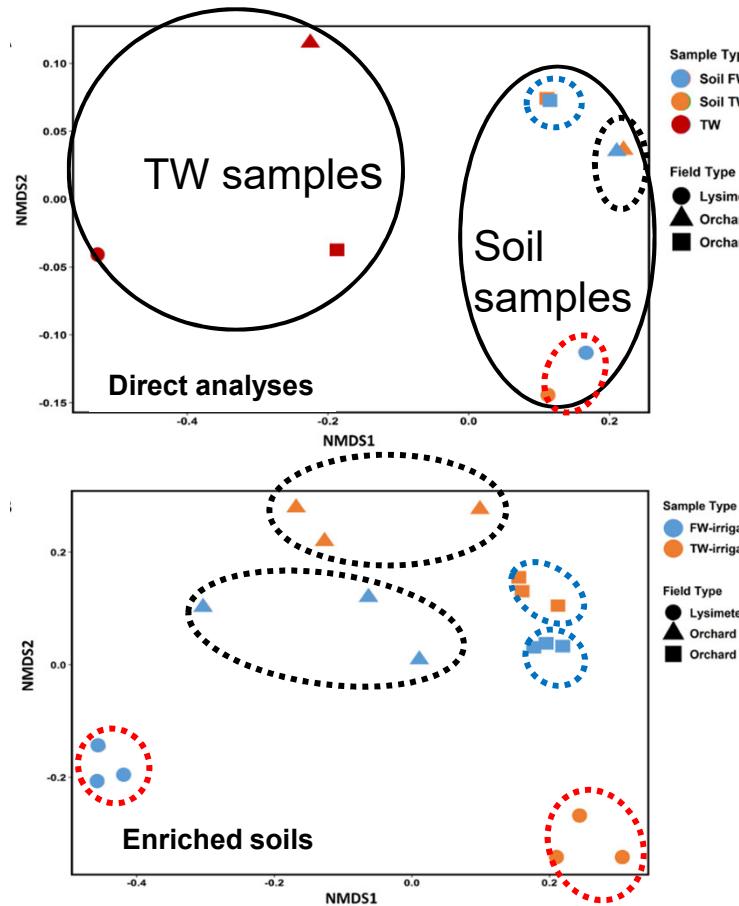
Courtesy of Eddie Cytryn

Marano et al., 2021, Environmental Science and Technology

Impact of irrigation water quality on the copiotrophic fraction of the soil microbiome



Impact of irrigation water quality on the copiotrophic fraction of the soil microbiome



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

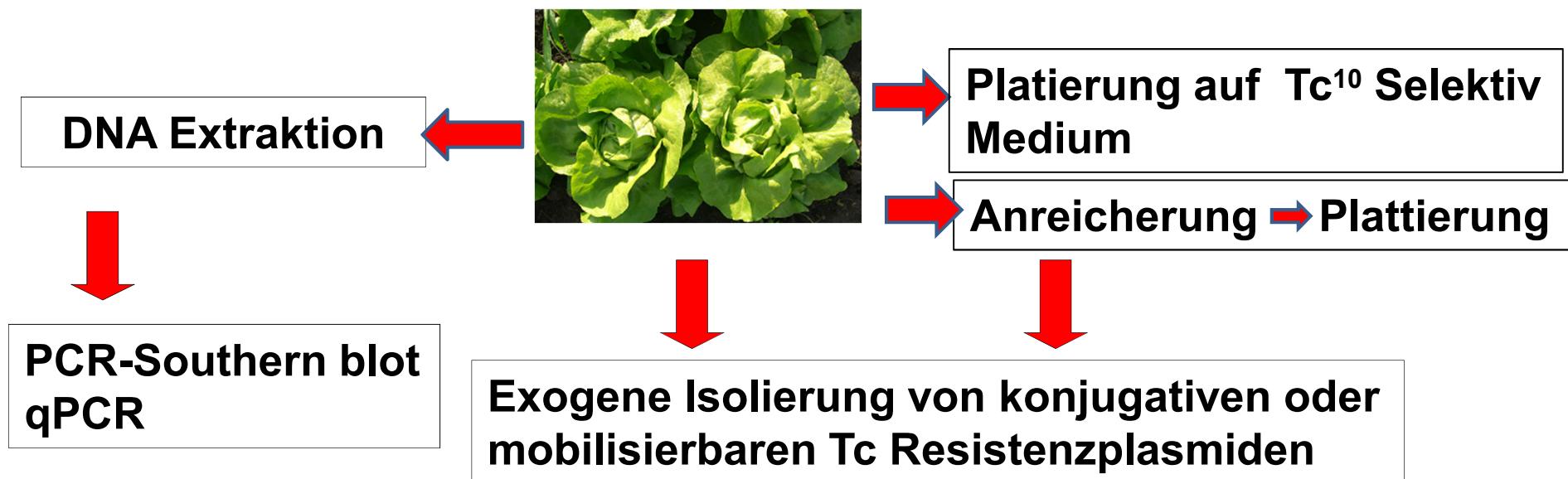
Courtesy of Eddie Cytryn

Take home messages

- Limits of detection need to be considered!
The fact that a particular ARB or ARG is not identified does not mean it is not present!
- Everything is not always everywhere:
Enrichment of environmental samples subjected to anthropogenic pressure can stimulate “*under the radar*” microbiomes and resistomes in both deterministic and stochastic manners
- Within a “*One Health*” perspective, targeting “*under the radar*” microbiomes and resistomes can be functional for risk assessment models

Übertragbare Antibiotikaresistenzplasmide in Pflanzen-assoziierten Bakterien

...open access: Blau et al., 2018, mBio
„The transferable resistome of produce“



Charakterisierung von Tetrazyklin-resistenten *E. coli* Isolaten von Koriander

<i>E. coli</i> isolates	ST	Inc groups ^a	<i>bla</i> genes	Resistance and integrase genes	Antibiotic resistance profile ^b
EK2.2	0	U ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul2, strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, CIP, OFX
EK2.19	0	FII ^l	<i>bla</i> _{TEM}	<i>intI1, tet(A), merRTΔP, sul2, aadA</i>	AM, AMX, TE, S, TMP, D, GM, KM
EK2.20	0	HI1 ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul2, sul3, aadA, qnrS</i>	AM, AMX, TE, S, TMP, SD, GM, OFX, C
EK2.21	0	X1 ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A)</i>	AM, AMX, TE, OFX
EK2.22	0	X1 ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, OFX
EK2.25	0	U ^h , X1 ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul2, strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, CIP, OFX
EK2.26	0	U ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul1, aadA, qacEΔ1, strA</i>	AM, AMX, TE, S, TMP, SD, CIP, NA, OFX
EK2.29^k	0	N ^g	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1}	<i>intI1, tet(A), sul1, strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, CRO, CTX, OFX, CIP
EK2.30	0	U ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul1, sul3, aadA, qnrS</i>	AM, AMX, TE, TMP, SD, C
EK3.33	0	X1 ^h	-	<i>intI1, sul1, strA</i>	TE, D
EK3.36	0	FIB ^l	<i>bla</i> _{TEM}	<i>intI1, tet(A), merRTΔP, sul1, aadA</i>	AM, AMX, TE, S, TMP, D, GM, KM
EK3.43^k	0	N ^g	<i>bla</i> _{CTX-M-1}	<i>tet(A), sul1, qnrS</i>	AM, AMX, TE, S, D, GM, CTX, OFX
EK3.44^k	7	N ^g	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1}	<i>intI1, tet(A), merRTΔP, sul1, strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, CRO, CTX, C, NA, CIP, OFX
EK5.16	7	FII ^l , I1 ^f	<i>bla</i> _{TEM}	<i>intI1, tet(A), aadA, qacEΔ1, qnrS</i>	AM, AMX, TE, S, TMP, D, OFX
EK5.19	7	X1 ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul2, strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, D, C
EK5.20	7	FII ^l , I1 ^f	<i>bla</i> _{TEM}	<i>intI1, tet(A), aadA, qacEΔ1, qnrS</i>	AM, AMX, TE, S, TMP, OFX
EK5.25	7	FII ^l	<i>bla</i> _{TEM}	<i>intI1, tet(A), qacEΔ1, qnrS</i>	AM, AMX, TE, TMP, OFX
EK5.28	7	FII ^l , I1 ^f	<i>bla</i> _{TEM}	<i>intI1, tet(A), aadA, qacEΔ1, qnrS</i>	AM, AMX, TE, S, TMP, CIP, OFX
EK5.30	7	FII ^l , FIB ^l , I1 ^f	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul1, aadA, qacEΔ1, qnrS</i>	AM, AMX, TE, TMP, SD, D, CIP, NA, OFX
EK5.32	7	X1 ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul1, sul2, strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, OFX, C
EK5.40	7	X1 ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A), sul2, strA, qnrS</i>	AM, AMX, TE, S, TMP, SD, C
EK7.6	7	U ^h	<i>bla</i> _{TEM}	<i>intI1, tet(A)</i>	AM, AMX, TE, TMP, D

Charakterisierung von repräsentativen Tetracyclin-resistenten *E. coli* Isolaten

Gemischter Salat

<i>E. coli</i> isolates ^a	ST	Inc groups ^b	<i>bla</i> genes	Resistance and integrase genes	Antibiotic resistance profile ^c
M1 ^E	0	ND	<i>bla</i> _{TEM}	<i>intI1</i> , <i>tet(A)</i> , <i>sul1</i> , <i>sul2</i> , <i>qacEΔ1</i> , <i>strA</i> , <i>merRTΔP</i>	AM, AMX, TE, S, TMP, SD, D
M19 ^E	0	ND	<i>bla</i> _{TEM}	<i>intI1</i> , <i>tet(A)</i> , <i>sul1</i> , <i>sul2</i> , <i>qacEΔ1</i> , <i>strA</i>	AM, AMX, TE, S, TMP, SD, D

Rucola

<i>E. coli</i> isolates ^a	ST	Inc groups ^b	<i>bla</i> genes	Resistance and integrase genes	Antibiotic resistance profile ^c
RE1 ^D	0	I ^{II}	-	-	TE, D
RE4 ^D	0	I ¹	-	<i>intI1</i> , <i>tet(A)</i> , <i>sul3</i> , <i>aadA</i> , <i>merRTΔP</i>	TE, S, TMP, SD, D, CIP, NA
RE9 ^D	0	I ¹	-	<i>intI1</i> , <i>tet(A)</i> , <i>sul3</i> , <i>aadA</i> , <i>merRTΔP</i>	TE, S, TMP, SD, D, CIP, NA
RE10 ^D	0	I ¹ ^f	-	<i>intI1</i> , <i>tet(A)</i> , <i>sul3</i> , <i>aadA</i> , <i>merRTΔP</i>	TE, S, TMP, SD, D, CIP, NA
RE11 ^E	0	I ¹ ^f	-	<i>intI1</i> , <i>tet(A)</i> , <i>sul3</i> , <i>aadA</i> , <i>merRTΔP</i>	TE, S, TMP, SD, D, CIP, NA
RE14 ^E	7	I ^{II}	-	-	TE, D
RE19 ^E	7	I ¹ ^f	-	<i>intI1</i> , <i>tet(A)</i> , <i>sul3</i> , <i>aadA</i> , <i>merRTΔP</i>	TE, S, TMP, SD, D, CIP, NA

^a: D: Direct plating; E: Enrichment.

^b: I: detected by RT-PCR and PBRT; f: detected by RT-PCR; g: detected by PCR; h: detected by PBRT.

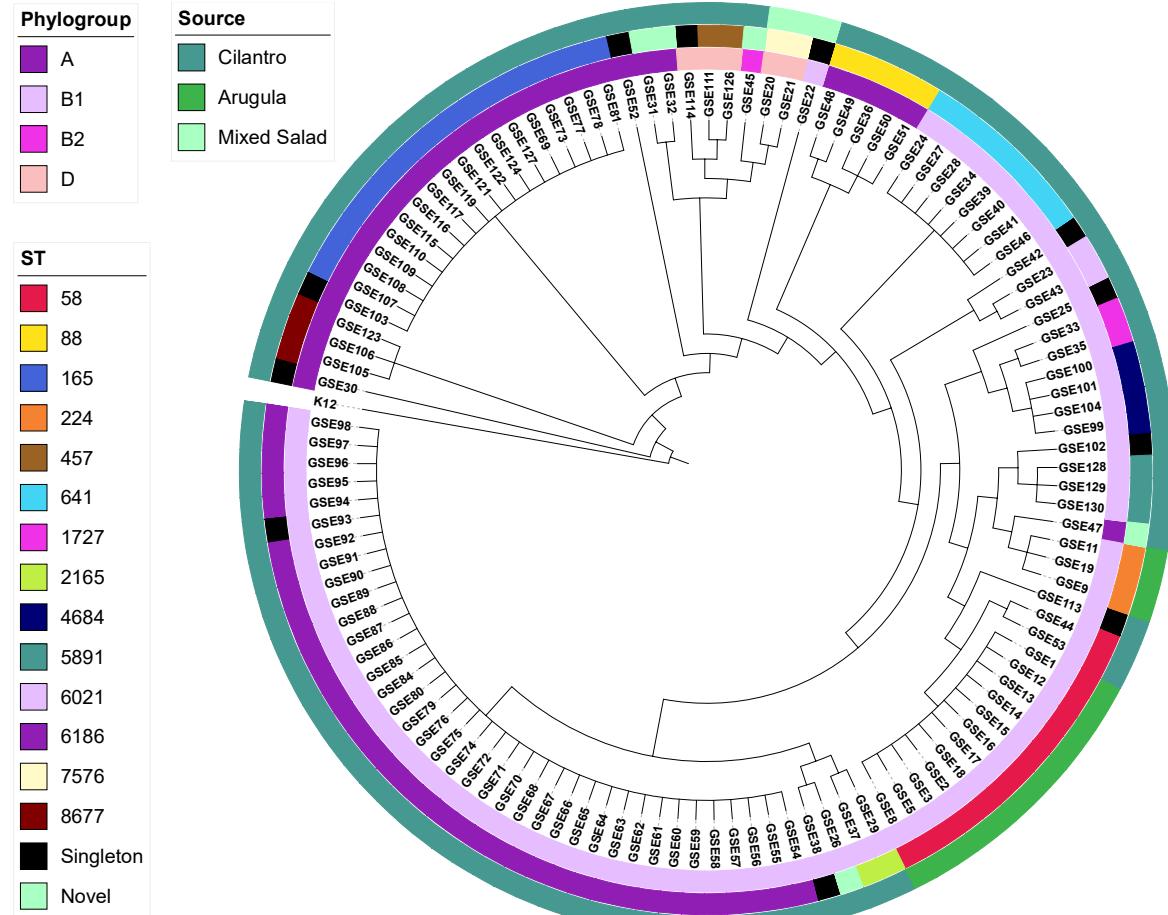
^c: C: Chloramphenicol; AM: Ampicillin; AMX: Amoxicillin; TE: Tetracycline; S: Streptomycin; TMP: Trimethoprim; SD: Sulfadiazine; CIP: Ciprofloxacin; OFX: Ofloxacin; D: Doxycycline; GM: Gentamicin; NA: Nalidixic acid; ND: not detected.

“Whole Genome Sequencing” (WGS) von 120 *E. coli* Isolaten von Frischeprodukten

Phylogruppe B1 dominiert
häufigsten STs:
6186, 165, 58, 641, 88

84/120 Stämmen trugen
Klasse 1 integrons (*intI1*)

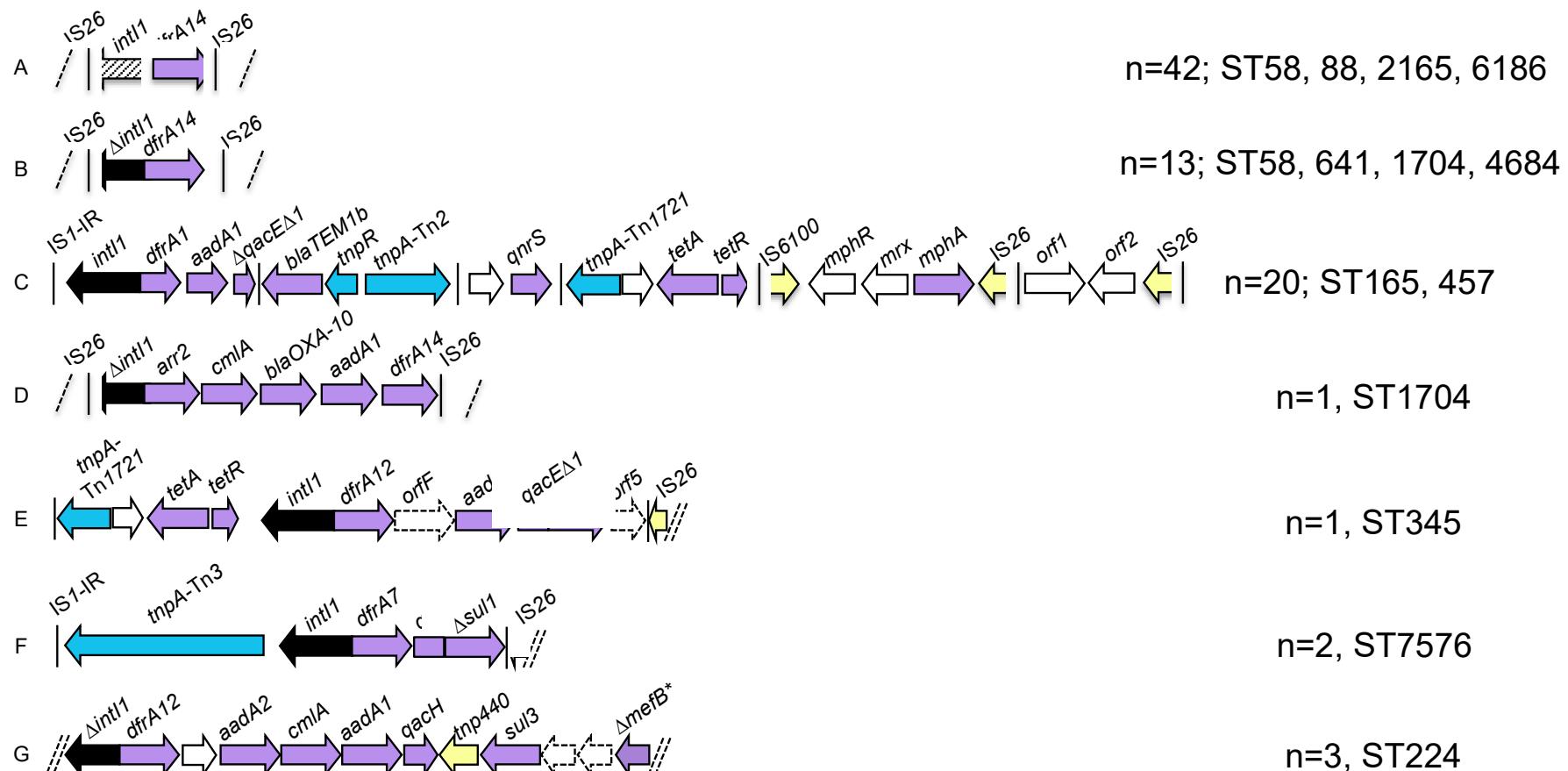
ST58: 2 von 14 strains trugen
ESBL gene *bla*_{CTX-M15}



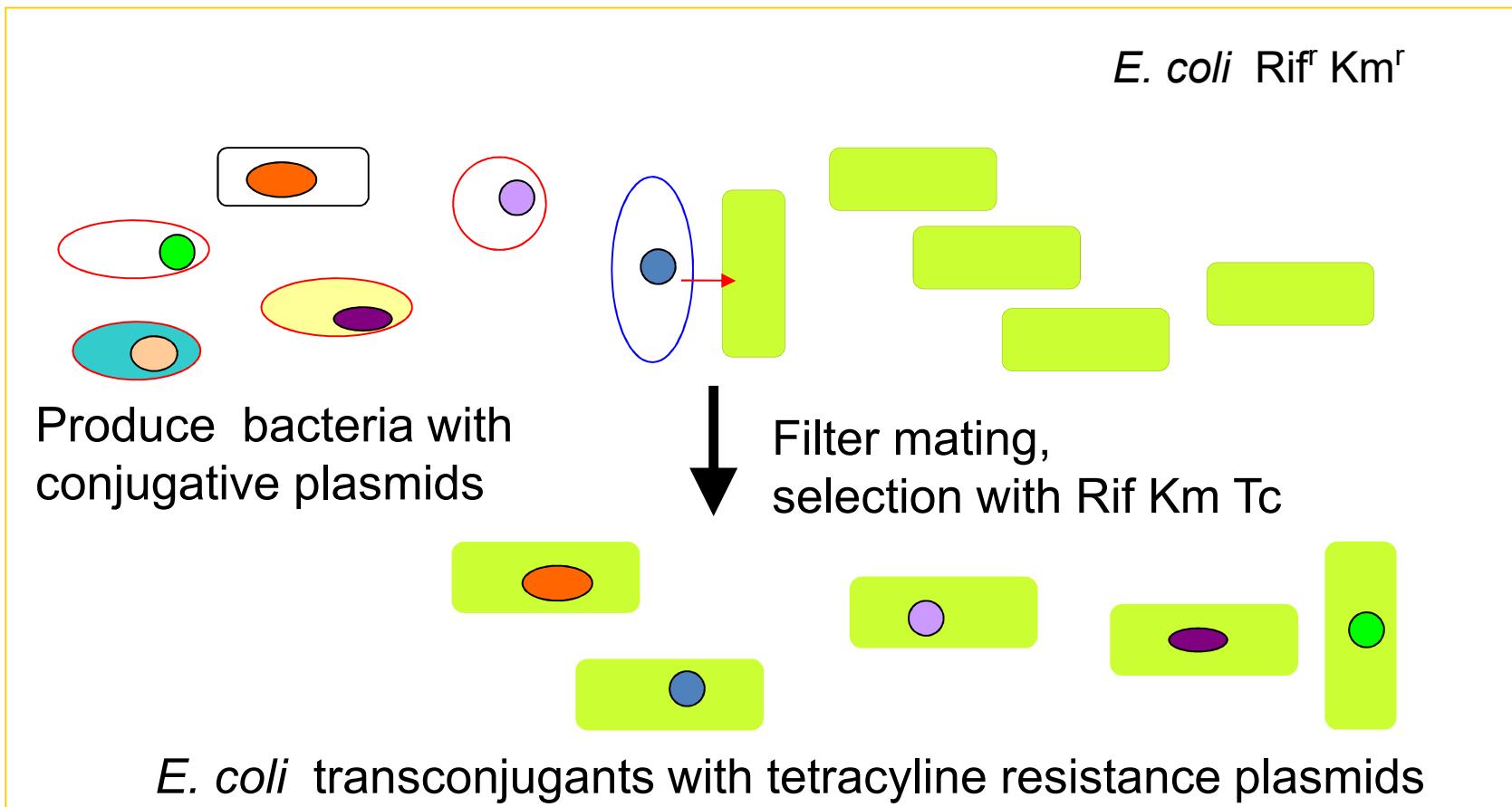
Reid, et al. 2020. Whole genome sequencing of *Escherichia coli* from store-bought produce.
Front. Microbiol. 10:3050. doi.org/10.3389/fmicb.2019.03050.

“Whole Genome Sequencing” (WGS) von 120 *E. coli* Isolaten von Frischeprodukten

- Viele Klasse 1 integrons waren auch mit IS Elementen wie IS26 und verschiedenen ARGs assoziiert.
- Die Detektion der gleichen Integrons in *E. coli*- Stämmen verschiedener STs deutet auf HGT hin



Übertragbare Resistom von Frischeprodukten



Charakterisierung von repräsentativen Tetracyclin-resistenten *E. coli* CV601 Transkonjuganten von Frischeprodukten

TET ^R <i>E. coli</i> CV601 transconjugants	Sample source ^a	Inc groups ^b	<i>bla</i> genes	Resistance, integrase genes and IS ^c	Antibiotic resistance profile ^d
pBC1.1	Ci	P-1β ^f	<i>bla</i> _{TEM}	<i>intI1, tet(A), merRTΔP, qacEΔ1, IS1071</i>	TE, AM, AMX, D
pBC1.3	Ci	P-1β ^f	-	<i>intI1, tet(A), merRTΔP, qacEΔ1, IS1071</i>	TE, AM, AMX, D
pBC1.9	Ci	P-1β ^f , FII ^l	<i>bla</i> _{TEM}	<i>intI1, tet(A), merRTΔP, qacEΔ1, IS1071</i>	TE, AM, AMX, D
pBC1.12	Ci	P-1β ^f , FII ^l	-	<i>intI1, tet(A), merRTΔP, strA, qacEΔ1, IS1071</i>	TE, AM, AMX, D, S
pBC2.1	Ci	FIB ^l	<i>bla</i> _{TEM}	<i>tet(A), sul1, qnrS</i>	TE, AM, AMX, D, CIP, NA, OFX, C
pBC2.2	Ci	FIB ^l	<i>bla</i> _{TEM}	<i>tet(A), qnrS</i>	TE, AM, AMX, D, CIP, OFX
pBC2.3	Ci	FIB ^l , I1 ^f	<i>bla</i> _{TEM}	<i>tet(A), qnrS</i>	TE, AM, AMX, D, CIP, OFX
pBC2.4	Ci	FIB ^l	<i>bla</i> _{TEM}	<i>tet(A), sul2, qnrS</i>	TE, AM, AMX, D, CIP, NA, OFX
pBC2.6	Ci	FIB ^l	<i>bla</i> _{TEM}	<i>tet(A), qnrS</i>	TE, AM, AMX, D, CIP, OFX, C
PBC2.8	Ci	FIB ^l	<i>bla</i> _{TEM}	<i>tet(A), qnrS</i>	TE, AM, AMX, D, CIP, NA, OFX
pBC2.11	Ci	FII ^l	<i>bla</i> _{TEM}	<i>tet(A), qnrS</i>	TE, AM, AMX, D, CIP, OFX
pBC2.15	Ci	I1 ^f	<i>bla</i> _{TEM}	<i>tet(A), qnrS</i>	TE, AM, AMX, D, CIP, OFX
pBMS1	MS	FII ^l	<i>bla</i> _{TEM}	<i>intI1, tet(Q), sul1, strA, merRTΔP</i>	TE, AM, AMX, D, TMP, C, S, SD
pBMS4	MS	FII ^l	<i>bla</i> _{TEM}	<i>intI1, sul1, strA, merRTΔP,</i>	TE, AM, AMX, D, TMP, C, S, SD
pBA1	A	ND	<i>bla</i> _{TEM}	<i>tet(A)</i>	TE, AM, AMX, D, TMP, C, CIP
<i>E. coli</i> CV601 (R)	-	-	-	-	-

^a: Ci: Cilantro; MS: Mixed salad; A: Arugula.

^b: l: detected by RT-PCR and PBRT; f: detected by RT-PCR; ^c: IS, Insertion Sequence.

^d: C: Chloramphenicol; AM: Ampicillin; AMX: Amoxicillin; TE: Tetracycline; CIP: Ciprofloxacin; OFX: Ofloxacin; D: Doxycycline; NA: Nalidixic acid; TMP: Trimethoprim; SD: Sulfadiazine; ND: not detected.

Nachweis von IncF, I1, I2 Plasmiden, *intI1* und *tet(A)* in TC-DNA extrahiert aus Frischeprodukten direct und nach Anreicherung

Produce	DNA	IncF		Incl1		Incl2		<i>intI1</i>		<i>tet(A)</i>		
		qPCR	Blot	qPCR	Blot	qPCR	Blot	qPCR	Blot	qPCR	Blot	
Mixed salad	Direct extraction	0	-	-	-	-	-	+++	+	(+++)	-	(++) ^{1*}
		7	-	-	-	-	-	+++	+	(+++)	-	(+++) ^{2*}
	Enrichment	0	+	(+++)	+	(++)	+	+++	+	(+++)	+	(++) ^{2*}
		7	+	(+++)	+	(++)	+	+++	+	(+++)	+	+++
Arugula	Direct extraction	0	-	-	-	-	-	(++) ^{3*}	+	(++)	-	(+++) ^{2*}
		7	-	-	-	-	-	(++) ^{1*}	+	(++)	-	(+++) ^{1*}
	Enrichment	0	+	(+++)	-	-	+	+++	+	(++)	+	+++
		7	+	(+++)	-	-	+	+++	+	(++)	+	+++
Cilantro	Direct extraction	0	-	-	-	-	-	(+) ^{1*}	(++)	(+) ^{1*}	(+++) ^{3*}	
		7	(+) ^{1*}	(++) ^{2*}	-	-	-	(+) ^{3*}	(+++)	(+) ^{2*}	(+++) ^{3*}	
	Enrichment	0	(+) ^{2*}	(++) ^{2*}	(+) ^{1*}	(++) ^{2*}	-	(+) ^{3*}	(+++)	(+) ^{2*}	(+++) ^{3*}	
		7	(+) ^{2*}	(++) ^{2*}	(+) ^{2*}	(++) ^{2*}	-	(+) ^{3*}	(+++) ^{3*}	(+) ^{3*}	(+++) ^{3*}	

(*): Number of positive replicates, -: Not detected or no signal, +: positive (qPCR), (++): Medium signal; (+++): Strong signal.

Faktoren, die das Mikrobiom und das übertragbare Resistom von Pflanzen beeinflussen

Pflanzenart und –sorte

Das Substrat (Bodentyp)

Beregnungswasser- Qualität und Häufigkeit

Nutzung organischer Dünger

Lagerung und Verarbeitung nach der Ernte: induzierte Mikrobiom-Verschiebungen

Zusammenfassung

- Aufbereitetes Abwasser enthält eine Vielzahl von Mikroschadstoffen, multi-resistente Bakterien, ARGs und mobile genetische Elemente
In Gegenwart von Nährstoffen und Selektionsdruck führt dies zum Wachstum von resistenten Populationen und stimuliert horizontalen Genaustausch.
- Beitrag von aufbereitetem Abwasser zur erhöhten Abundanz von Bakterien mit **transferablen Resistenzgenen** im Boden und an Pflanzen wesentlich durch die enthaltenen **Mikroschadstoffe** beeinflusst: Notwendigkeit der Entwicklung bzw. Nutzung von Methoden zur effizienten Entfernung von Mikroschadstoffen
- Das Pflanzenmikrobiom kann ein Reservoir für transferable Resistenzgene für Bakterien im menschlichen Darm sein