Quote from the ICCMG agenda:

"As One Health is trendy, we had to invite Joakim Larsson to help us understanding how it could inspire clinical metagenomics"

D. G. Joakim Larsson, Professor in Environmental Pharmacology
Director, Centre for Antibiotic Resistance Research at University of Gothenburg (CARe)

Department of Infectious Diseases, Institute for Biomedicine
The Sahlgrenska Academy at the University of Gothenburg

E-mail: joakim.larsson@fysiologi.gu.se
Personal website: http://www.biomedicine.gu.se/joakimlarsson
CARe website: www.care.gu.se

Picture by M Kumar, Associated Press

On the environment's role in evolution, transmission and surveillance of antibiotic resistance

D. G. Joakim Larsson, Professor in Environmental Pharmacology
Director, Centre for Antibiotic Resistance Research at University of Gothenburg (CARe)

Department of Infectious Diseases, Institute for Biomedicine
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Picture by M Kumar, Associated Press

The environment's role in antibiotic resistance

- **Transmission route** for certain resistant bacteria (human/animal → environment → human/animal)

Larsson DGJ, Flach C-F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

Huijbers PMC, Flach C-F, Larsson DGJ. (2019). A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. Environ Int. 130:104880.

Bengtsson-Palme J, Kristiansson E, Larsson DGJ. (2018). Environmental factors influencing the development and spread of antibiotic resistance. FEMS Micro Rev. 1;42.

The environment's role in antibiotic resistance

- **Transmission route** for certain resistant bacteria (human/animal → environment → human/animal)

- **Source and evolutionary "arena"** for the emergence of new forms of resistance

Larsson DGJ, Flach C-F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

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Bengtsson-Palme J, Kristiansson E, Larsson DGJ. (2018). Environmental factors influencing the development and spread of antibiotic resistance. FEMS Micro Rev. 1;42.

The environment's role in antibiotic resistance

- **Transmission route** for certain resistant bacteria (human/animal → environment → human/animal)
- **Source and evolutionary "arena"** for the emergence of new forms of resistance
- **Possible indicator of the regional resistance situation**

Larsson DGJ, Flach C-F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

Huijbers PMC, Flach C-F, Larsson DGJ. (2019). A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. Environ Int. 130:104880.

Bengtsson-Palme J, Kristiansson E, Larsson DGJ. (2018). Environmental factors influencing the development and spread of antibiotic resistance. FEMS Micro Rev. 1;42.

1. Transmission risks

Environmental Science & Technology

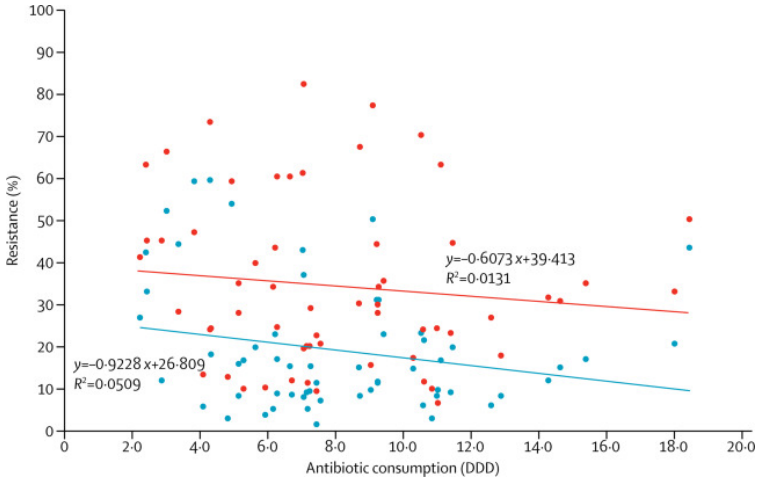
Viewpoint

pubs.acs.org/est

Underappreciated Role of Regionally Poor Water Quality on Globally Increasing Antibiotic Resistance

David W. Graham,*[,†] Peter Collignon,[‡] Julian Davies,[§] D. G. Joakim Larsson,[∥] and Jason Snape[⊥]

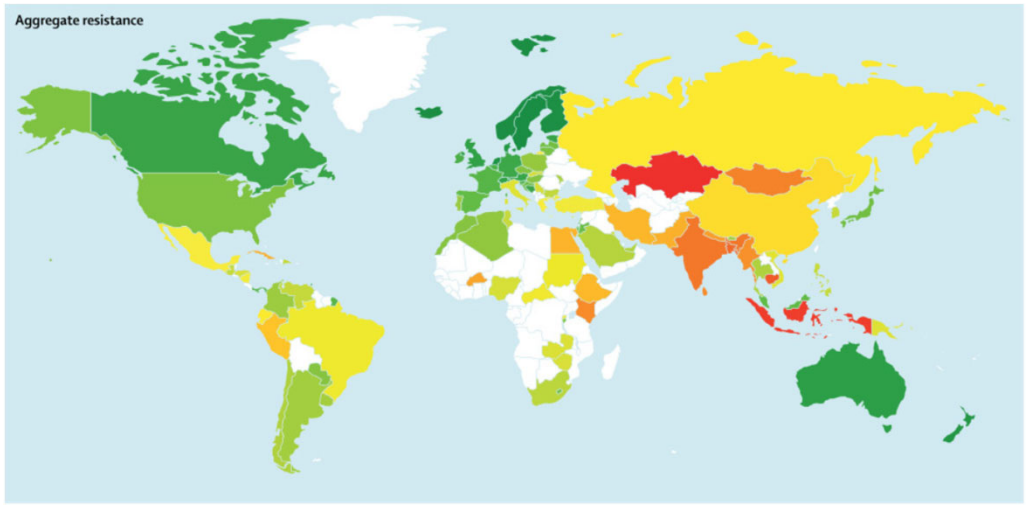
Globally, antibotic resistance is more strongly linked to lack of sanitation than to reported antibiotic use!



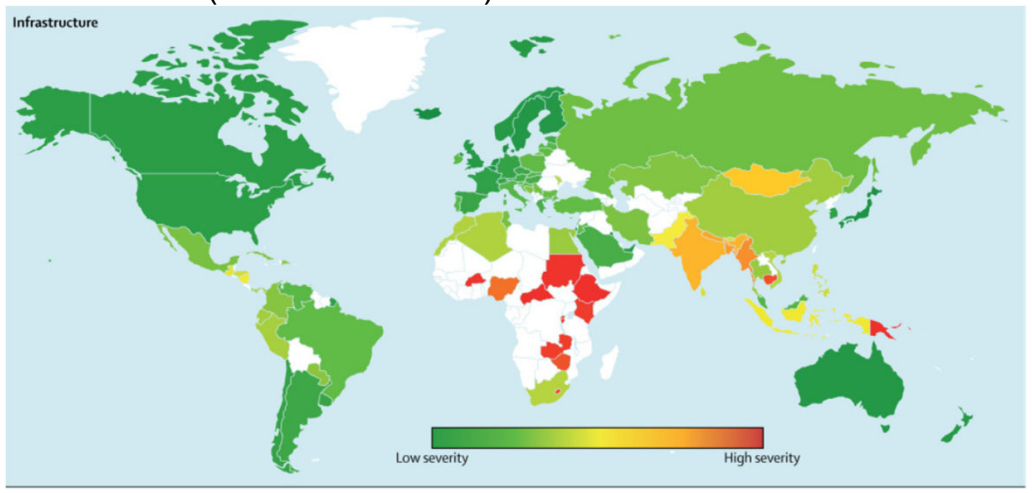
***Escherichia coli* resistance levels for fluoroquinolones and third-generation cephalosporins compared with antibiotic consumption**

Collignon P, Beggs JJ, Walsh TR, Gandra S, Laxminarayan R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. Lancet Planet Health. 2018 Sep;2(9):e398-e405

Aggregated resistance index

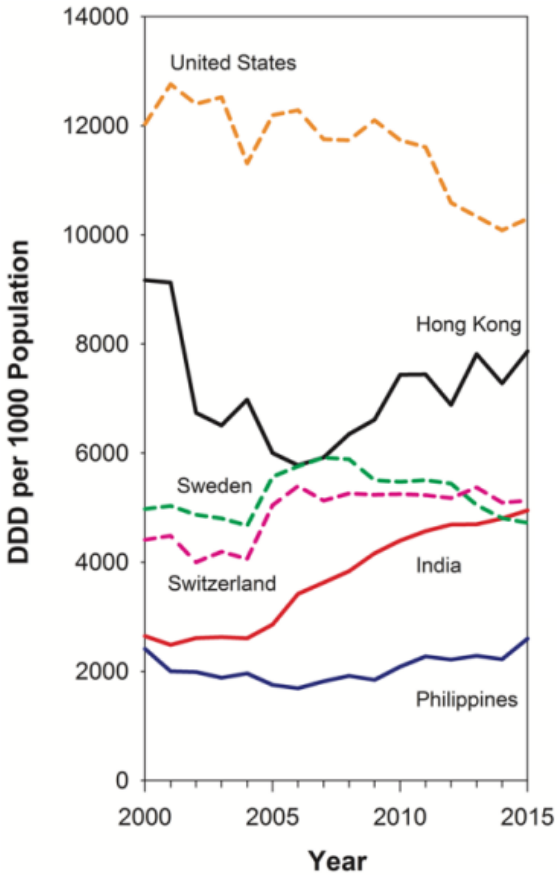


Infrastructure (sanitation control)



..but analyses of antibiotics in sewage influents suggest under-reported use in some regions

Reported antibiotic use



Measured antibiotic concentrations in influents (not adjusted for water-volume/capita)

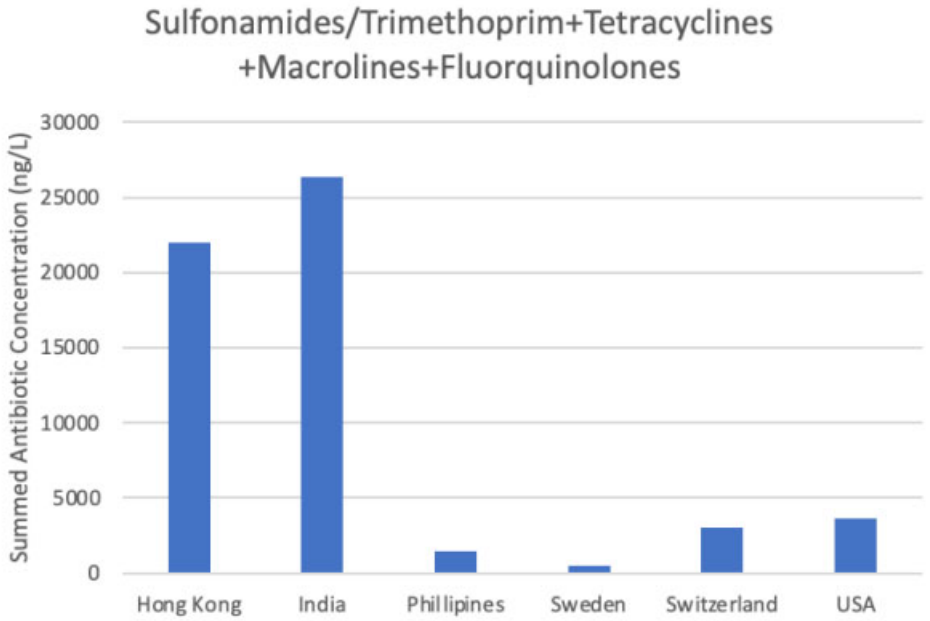


Figure S15. Average total antibiotic mass concentration in samples collected from Hong Kong, India, The Philippines, Sweden, Switzerland, and the US. Reported numbers reflect summed averages of data reported in **Table S5** for the antibiotics (= sulfonamides/trimethopim + tetracyclines + macrolides + fluoroquinolones) and one or more treatment plants at each location.

Maria Virginia Prieto Riquelme, Emily Garner, Suraj Gupta, Jake Metch, Ni Zhu, Matthew F. Blair, Gustavo Arango-Argoty, Ayella Maile-Moskowitz, An-dong Li, Carl-Fredrik Flach, Diana S. Aga, Indumathi M. Nambi, D. G. Joakim Larsson, Helmut Bürgmann, Tong Zhang, Amy Pruden, Peter J. Vikesland (2022). Demonstrating a Comprehensive Wastewater-Based Surveillance Approach That Differentiates Globally Sourced Resistomes. Environmental Science and Technology. https://doi.org/10.1021/acs.est.1c08673

Surfers are more likely to carry ESBL *E.coli*

Leonard et al. 2018. Environment International114: 326-333

Leonard et al. 2022. Current Opinion in Microbiology 65: 40-46.

…while sewage treatment plant workers across three European countries do not appear to be at increased risk

European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-021-04387-z

ORIGINAL ARTICLE

Check for updates

Carriage of ESBL-producing Enterobacterales in wastewater treatment plant workers and surrounding residents — the AWARE Study

Daloha Rodríguez-Molina[1,2,3] · Fanny Berglund[4,5] · Hetty Blaak[6] · Carl-Fredrik Flach[4,5] · Merel Kemper[6] · Luminita Marutescu[7,8] · Gratiela Pircalabioru Gradisteanu[7,8] · Marcela Popa[7,8] · Beate Spießberger[9,10,11] · Tobias Weinmann[1] · Laura Wengenroth[1] · Mariana Carmen Chifiriuc[7,8] · D. G. Joakim Larsson[4,5] · Dennis Nowak[1,12] · Katja Radon[1] · Ana Maria de Roda Husman[6] · Andreas Wieser[9,10,11] · Heike Schmitt[6]

International travel still appears to be (one of) the largest risk factors for ESBL *E. coli* carriage

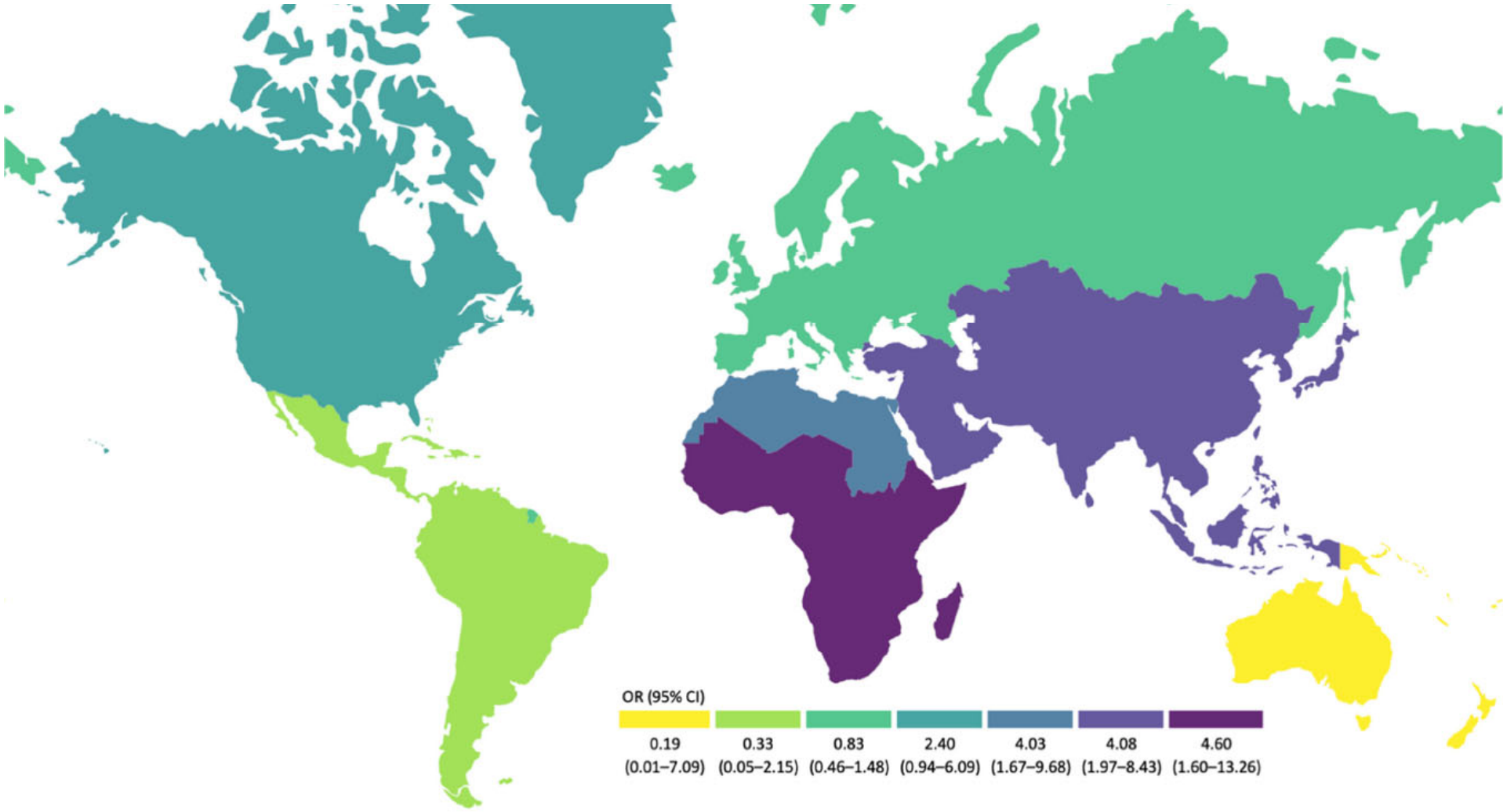
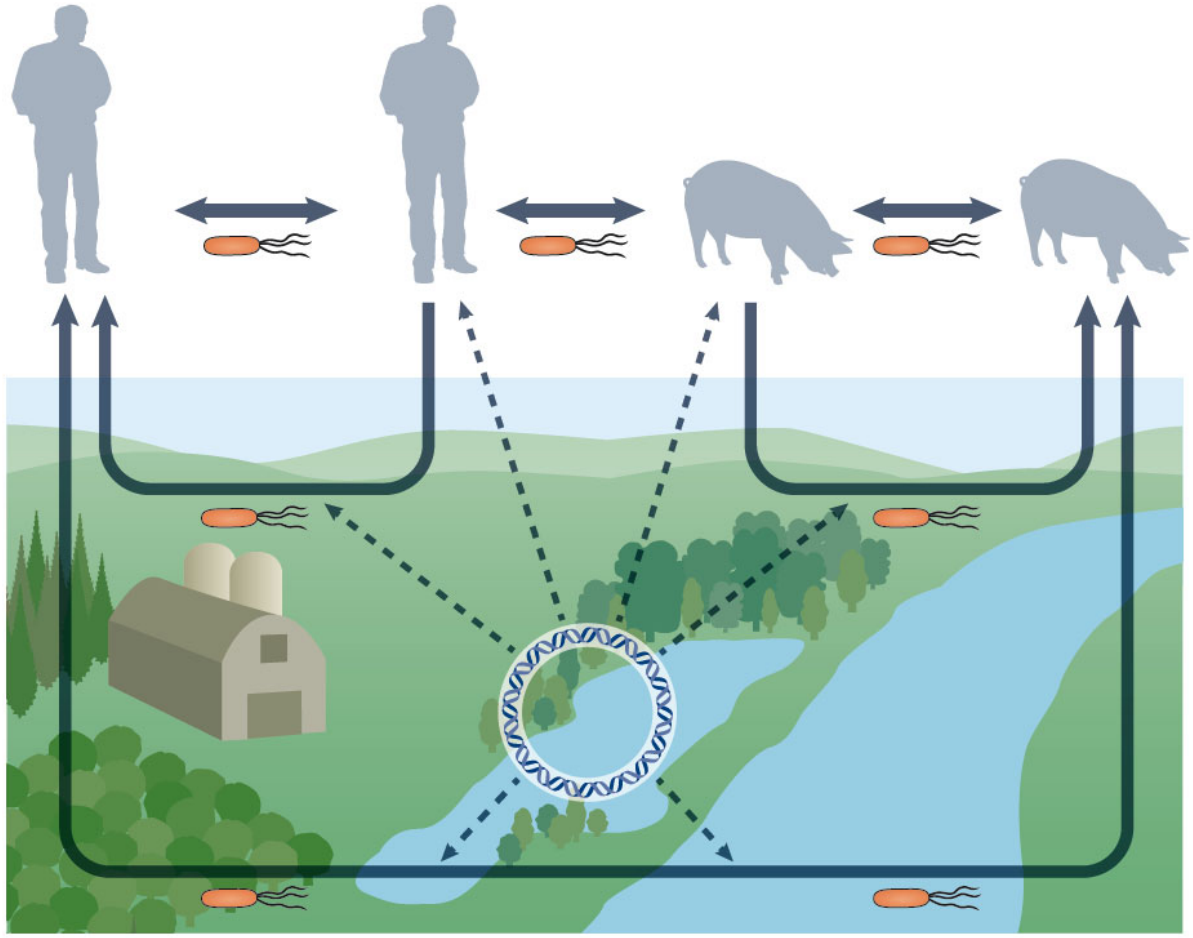


Figure 2. Travel areas as risk factors for ESBL-EC carriage (adjusted OR). Note: The European spot in South America corresponds to French Guiana.

Rodríguez-Molina D, Berglund F, Blaak H, Flach C-F, Kemper M, Marutescu L, Gradisteanu LP, Popa M, Spießberger B, Wengenroth L, Chifiriuc MC, Larsson DGJ, Nowak D, Radon K, de Roda Husman AM, Wieser A, Schmitt H. (2022). International travel as a risk factor for carriage of extended-spectrum β-lactamase-producing Escherichia coli in a large sample of European individuals - The AWARE Study. International Journal of Environmental Research and Public Health. 19:4758.

2. Evolution risks

Transmission of resistant bacteria

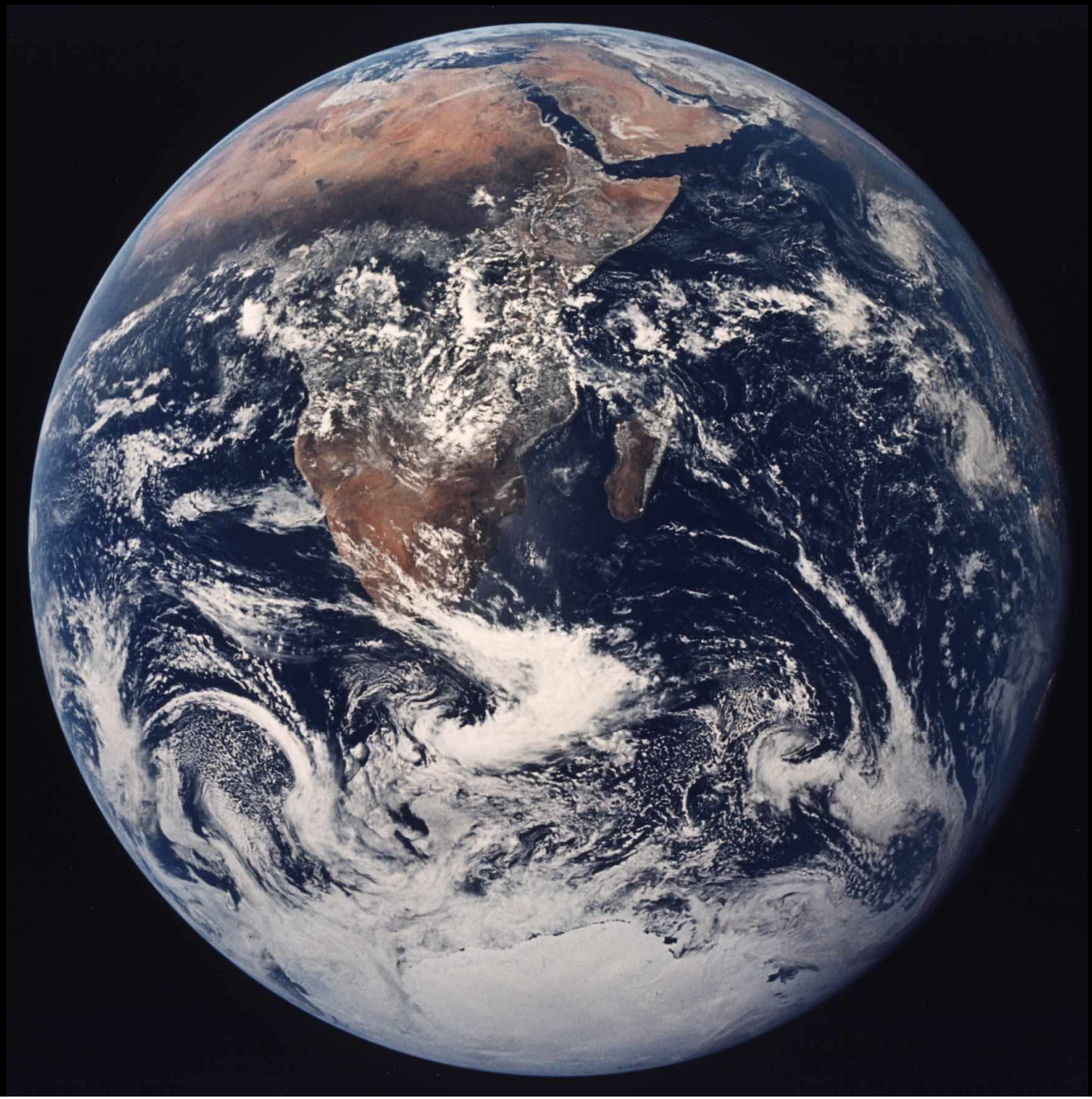


Transmission of pathogenic bacteria between humans, between animals or between humans and animals (either direct or via the environment):	Uptake of new resistance factors from the diverse environmental microbiota:
• Common	• Relatively rare
• Risks are in principle quantifiable and predictable	• More challenging to predict
• Consequences of each transmission event is limited	• Consequences of single transfer events may be vast
• Transmission rates can be reduced	• Irreversible

Emergence of resistance

Larsson DGJ, Flach C-F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

Microbial diversity of latent antibiotic resistance genes as a risk factor for the emergence of mobile resistance in pathogens



Exploring the latent resistome using hidden Markov models (fARGene) for different antibiotic classes

J Antimicrob Chemother 2021; 76: 117–123
doi:10.1093/jac/dkaa392 Advance Access publication 2 October 2020

Journal of Antimicrobial Chemotherapy

An updated phylogeny of the metallo-β-lactamases

Fanny Berglund [1,2], Anna Johnning[1,2,3], D. G. Joakim Larsson [2,4] and Erik Kristiansson[1,2*]

MICROBIAL GENOMICS

RESEARCH ARTICLE

Lund et al., Microbial Genomics 2022;8:000770
DOI 10.1099/mgen.0.000770

MICROBIOLOGY SOCIETY

OPEN DATA OPEN ACCESS

Large-scale characterization of the macrolide resistome reveals high diversity and several new pathogen-associated genes

David Lund[1,2], Nicolas Kieffer[2,3], Marcos Parras-Moltó[1,2], Stefan Ebmeyer[2,3], Fanny Berglund[2,3], Anna Johnning[1,2,4], D. G. Joakim Larsson[2,3] and Erik Kristiansson[1,2,*]

Berglund et al. Microbiome (2019) 7:52
https://doi.org/10.1186/s40168-019-0670-1

Microbiome

SOFTWARE Open Access

Identification and reconstruction of novel antibiotic resistance genes from metagenomes

Fanny Berglund[1,2], Tobias Österlund[1,2], Fredrik Boulund[3], Nachiket P. Marathe[2,4,5], D. G. Joakim Larsson[2,4] and Erik Kristiansson[1,2*]

MICROBIAL GENOMICS

RESEARCH ARTICLE

Berglund et al., Microbial Genomics 2020;6
DOI 10.1099/mgen.0.000455

MICROBIOLOGY SOCIETY

OPEN DATA OPEN ACCESS

Comprehensive screening of genomic and metagenomic data reveals a large diversity of tetracycline resistance genes

Fanny Berglund[1,2], Maria-Elisabeth Böhm[2,3], Anton Martinsson[1,2], Stefan Ebmeyer[2,3], Tobias Österlund[1,2], Anna Johnning[1,2,4], D. G. Joakim Larsson[2,3] and Erik Kristiansson[1,2,*]

Berglund et al. Microbiome (2017) 5:134
DOI 10.1186/s40168-017-0353-8

Microbiome

RESEARCH Open Access

Identification of 76 novel B1 metallo-β-lactamases through large-scale screening of genomic and metagenomic data

Fanny Berglund[1,2], Nachiket P. Marathe[2,3], Tobias Österlund[1,2], Johan Bengtsson-Palme[2,3], Stathis Kotsakis[2,3], Carl-Fredrik Flach[2,3], D G Joakim Larsson[2,3] and Erik Kristiansson[1,2*]

Boulund et al. BMC Genomics (2017) 18:682
DOI 10.1186/s12864-017-4064-0

BMC Genomics

RESEARCH ARTICLE Open Access

Computational discovery and functional validation of novel fluoroquinolone resistance genes in public metagenomic data sets

Fredrik Boulund[1,2], Fanny Berglund[1,2], Carl-Fredrik Flach[2,3], Johan Bengtsson-Palme[2,3], Nachiket P. Marathe[2,3], DG Joakim Larsson[2,3] and Erik Kristiansson[1,2*]

fARGene performs very well compared to other methods

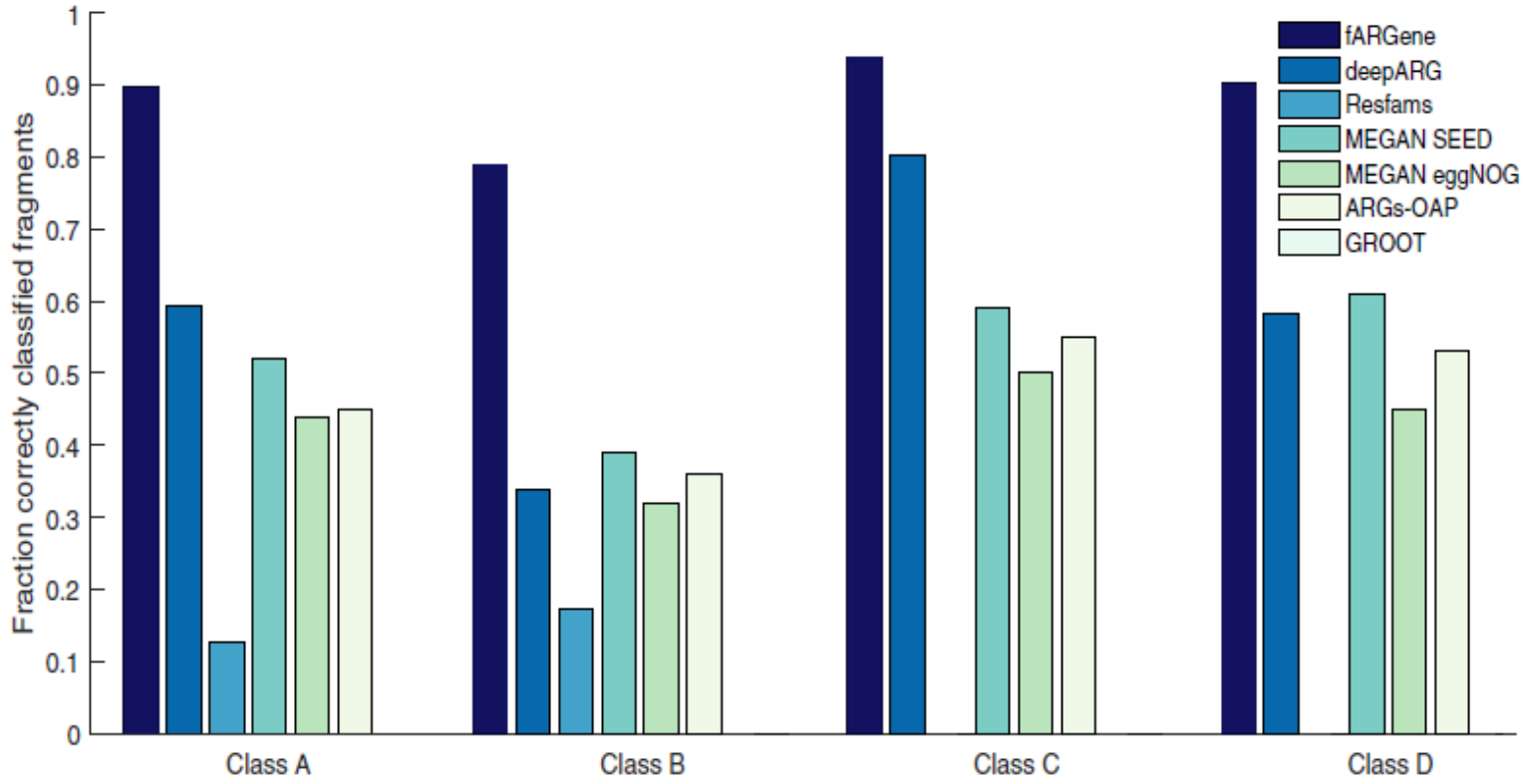


Fig. 4 The ability to correctly classify metagenomic fragments for fARGene and five competing methods. The performance of fARGene was consistently higher than all compared methods (in average, 87% compared to 55%, 7.5%, 52%, 42%, 46%, and 0%, for deepARG, Resfams, MEGAN SEED, MEGAN eggNOG, ARGs-OAP, and GROOT, respectively)

Berglund F, Österlund T, Boulund F, Marathe NP, Larsson DGJ, Kristiansson E. (2019). Identification and reconstruction of novel antibiotic resistance genes from metagenomes. Microbiome. 7:52.

Predicting novel aminoglycoside resistance genes using fARGene across bacterial genomes

Unpublished data removed from slide

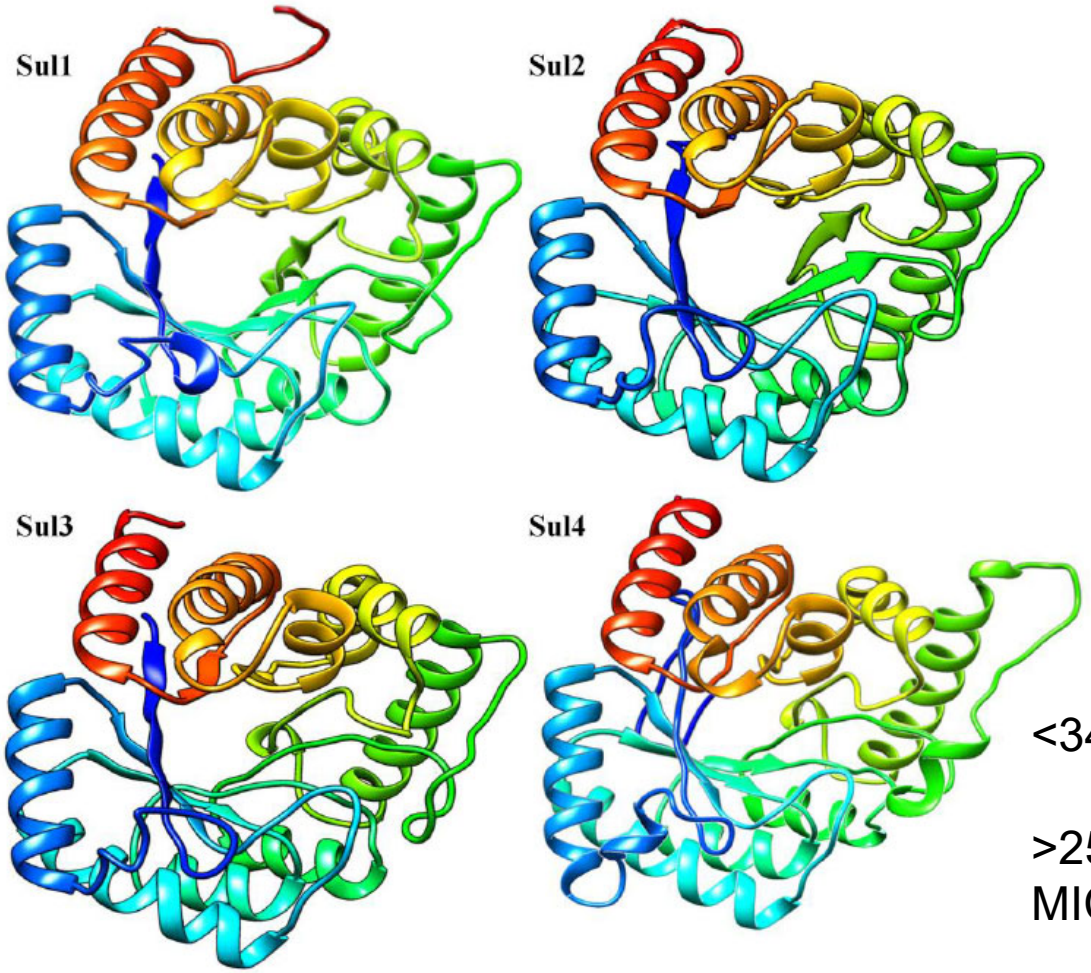
Lund D, Roelof Coertze R, Parras-Moltó M, Berglund F, Johnning A, Larsson DGJ, Kristiansson E. Detection of emerging aminoglycoside resistance genes in human pathogens. To be submitted soon.

Genetic context analysis reveales new aminoglycoside resistance genes of potential clinical relevance

Unpublished data removed from slide

Lund D, Roelof Coertze R, Parras-Moltó M, Berglund F, Johnning A, Larsson DGJ, Kristiansson E. Detection of emerging aminoglycoside resistance genes in human pathogens. To be submitted soon.

Exploring the latent mobile resistome by metagenomic sequencing of amplified integrons in polluted sediment from India led to the discovery of the fourth mobile sulfonamide resistance gene (*sul4*)

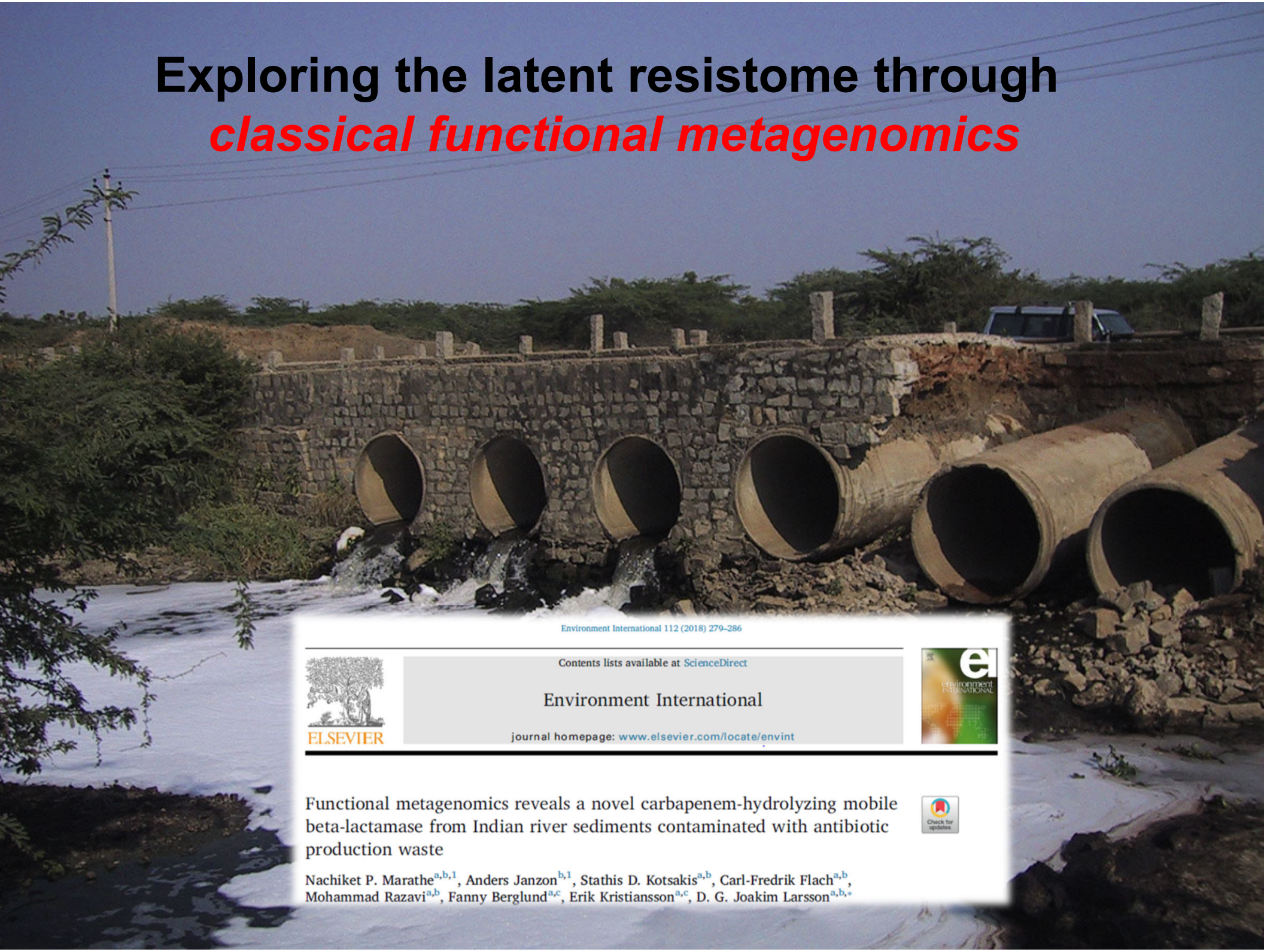


<34% aa-identity to Sul1-3

>256-fold increased MIC in *E. coli*

Razavi M, Marathe NP, Gillings MR, Flach C-F, Kristiansson E, Larsson DGJ. (2017). Discovery of the fourth mobile sulfonamide resistance gene. *Microbiome*. 5:160.

Exploring the latent resistome through *classical functional metagenomics*



Environment International 112 (2018) 279–286

Contents lists available at ScienceDirect

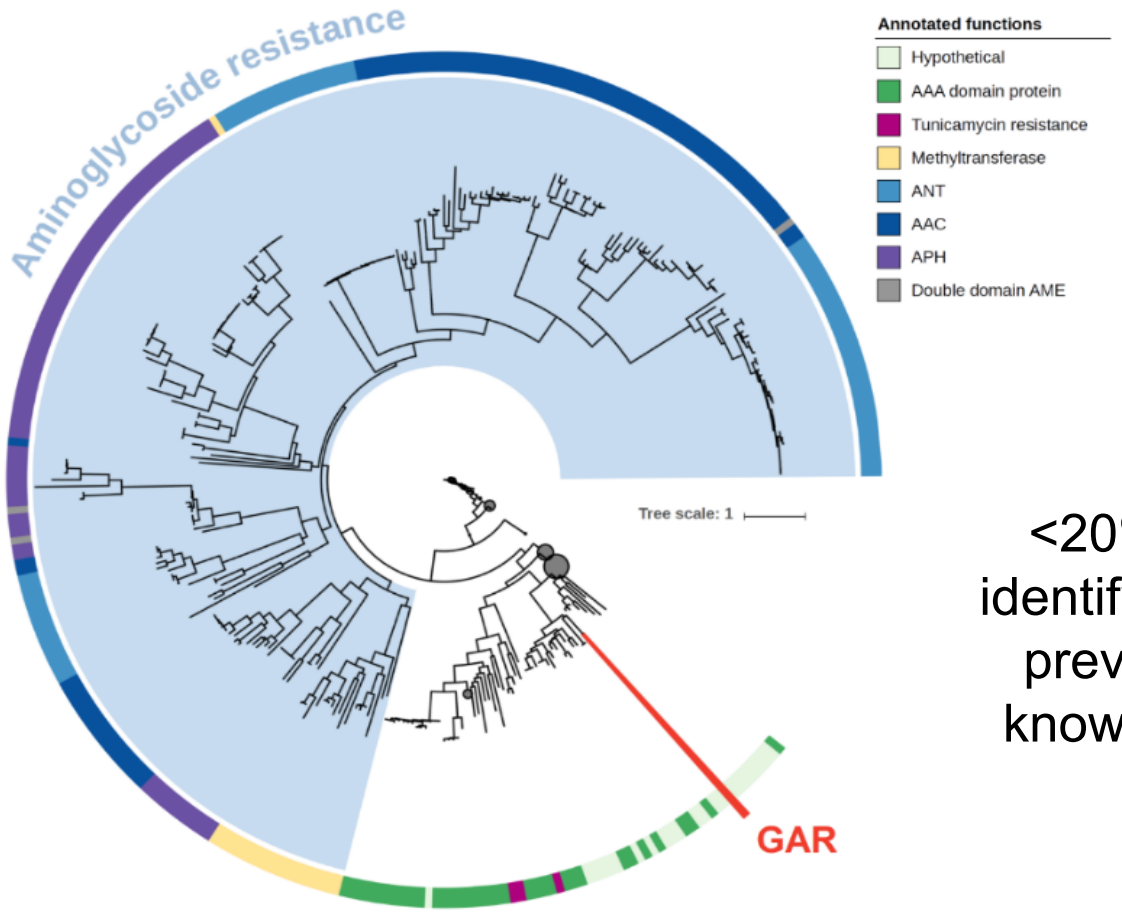
Environment International

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

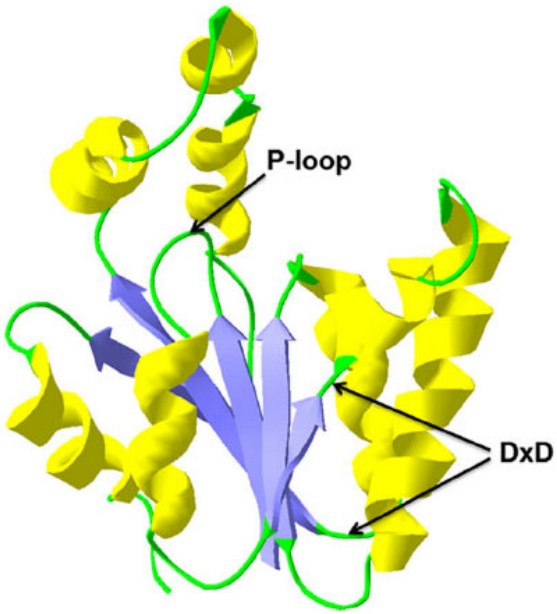
Functional metagenomics reveals a novel carbapenem-hydrolyzing mobile beta-lactamase from Indian river sediments contaminated with antibiotic production waste

Nachiket P. Marathe[a,b,1], Anders Janzon[b,1], Stathis D. Kotsakis[a,b], Carl-Fredrik Flach[a,b], Mohammad Razavi[a,b], Fanny Berglund[a,c], Erik Kristiansson[a,c], D. G. Joakim Larsson[a,b,*]

Discovery of novel integron-born aminoglycoside resistance gene that had escaped discovery in the clinic, using tailored functional metagenomics of wastewaters and sediments



<20% aa-identify to any previously known ARG



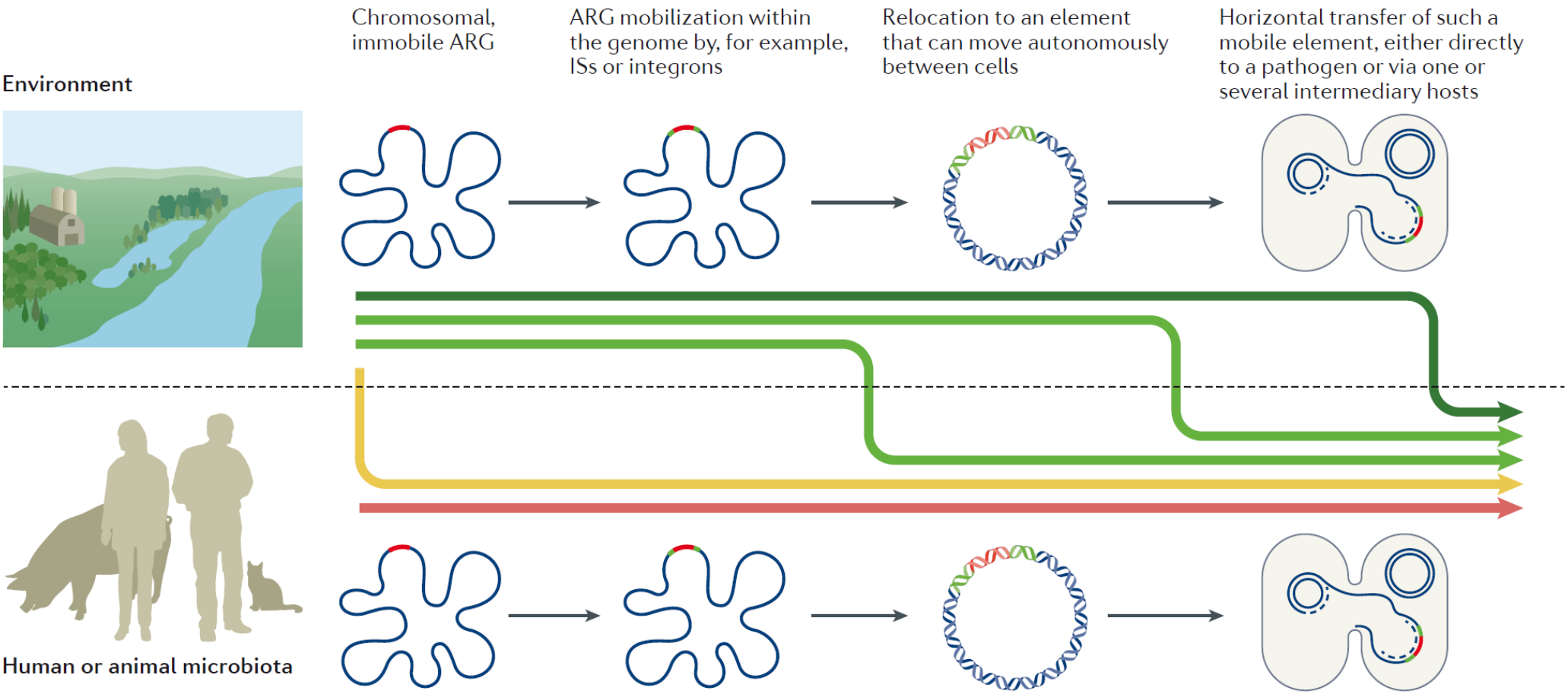
2760-fold increase of the MIC for gentamicin in *E. coli*

Present in clinical isolates of e.g. *Pseudomonas, Salmonella*

Böhm M-E, Razavi M, Marathe NP, Flach C-F, Larsson DGJ. (2020). Discovery of a novel integron-borne aminoglycoside resistance gene present in clinical pathogens by screening environmental bacterial communities. Microbiome. 8:41.

Where do ARGs become mobile?

Different scenarios for the emergence of resistance in pathogens with different levels of involvement of the external environment

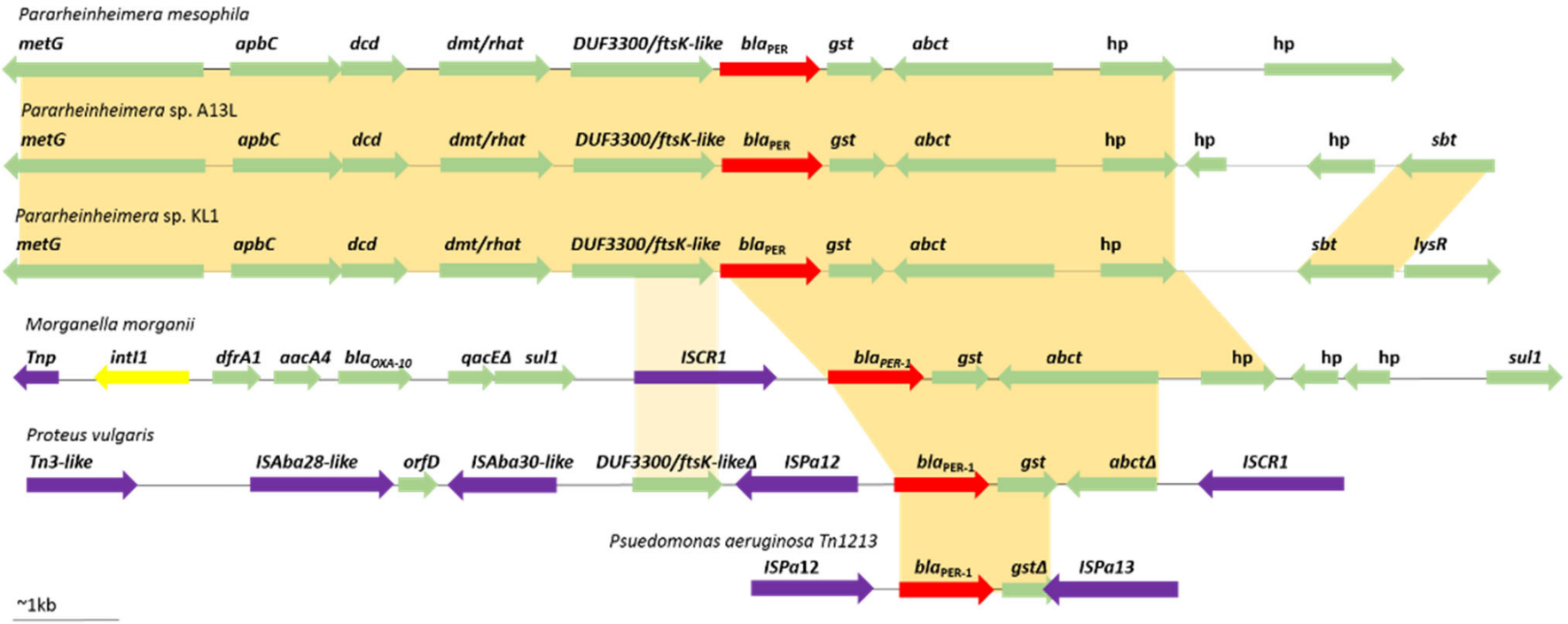


- The source for the immobile ARG is a common resident of the human or domestic animal microbiota. Mobilization and transfer to pathogens occurs entirely within humans or animals
- The source for the immobile ARG is a bacterium thriving in the external environment that sometimes enters the human or domestic animal microbiota. Mobilization and transfer to pathogen occurs entirely within humans or animals
- The source for the immobile ARG is a bacterium thriving in the external environment. The ARG is mobilized in the environment (to variable degrees), but its final transfer to pathogens occurs within humans or domestic animals
- The source for the immobile ARG is a bacterium thriving in the external environment. Mobilization and transfer to pathogen occurs entirely within the environment

Larsson DGJ, Flach C-F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

Can we draw conclusions on what environments were involved based on the ARGs where we know their origin?

Comparative genomics can reveal the taxonomic recent, chromosomal origin of mobilized ARGs



Ebmeyer S, Kristiansson E, Larsson DGJ. (2019). PER extended-spectrum beta-lactamases originate from *Pararheinheimera spp*. International Journal of Antimicrobial Agents. 53:158.

Ebmeyer S, Kristiansson E, Larsson DGJ. (2019). CMY-1/MOX-family AmpC beta-lactamases MOX-1, MOX-2 and MOX-9 were mobilized independently from three *Aeromonas* species. Journal of Antimicrobial Chemotherapy.74 (5) 1202-1206.

Ebmeyer S, Kristiansson E, Larsson DGJ (2019). The mobile FOX AmpC beta-lactamases originated in *Aeromonas allosaccharophila*. International Journal of Antimicrobial Agents 54 (6), 798-802.

Kieffer N, Ebmeyer S, Larsson DGJ (2020). The Class A Carbapenemases BKC-1 and GPC-1 Both Originate from the Bacterial Genus *Shinella*. Antimicrobial agents and chemotherapy 64 (12), e01263-20.

Kieffer N, Ebmeyer S, Larsson DGJ (2022). Evidence for *Pseudoxanthomonas mexicana* as the recent origin of the blaAIM-1 carbapenemase gene. International Journal of Antimicrobial Agents 59 (4), 106571.

Ebmeyer S, Coertze RD, Berglund F, Kristiansson E, Larsson DGJ (2022). GEnView: a gene-centric, phylogeny-based comparative genomics pipeline for bacterial genomes and plasmids. Bioinformatics 38 (6), 1727-1728.

Ebmeyer S, Kristiansson E, Larsson DGJ. (2021). A framework for identifying the recent origins of mobile antibiotic resistance genes. Communications Biology. 4:8.

Known recent origin species/genera for antibiotic resistance genes

Table 1 Evidence for proposed origins of specific ARGs.

Resistance determinant	Origin taxon	Antibiotic class	Co-mobilized genes	Nucleotide identity MGE/ origin	IS/ISCR on MGE	MGE in proposed origin	ARG loci in origin-related species	Conclusive evidence	Reference
APH(3')-IV	*Acinetobacter guillouiae*	Aminoglycosides	1	95-98%[a]	ISAba125, ISAba14	Absent	Yes	Yes	Yoon et al.[21]
AAC(6')-Ih	*Acinetobacter gyllenbergii*	Aminoglycosides	>1	98-≥99%[a]	ISAba23, ISAcsp5	Absent	Yes	Yes	Yoon et al.[60]
FOX	*Aeromonas caviae*	β-Lactams	1[a]	≤78%[a]	IS26, ISAs2, Tn3-like[a]	ISApu2Δ	(Yes)[b]	No	Fosse et al.[61]
FOX	*Aeromonas allosaccharophila*	β-Lactams	>1	95-98%	IS26, ISAs2, Tn3-like[a]	Absent	Yes	Yes	Ebmeyer et al.[62]
CMY-1/MOX-1	*Aeromonas sanarelli*	β-Lactams	>1	97-98%	ISCR1	Absent	Yes	Yes	Ebmeyer et al.[28]
MOX-2	*A. caviae*	β-Lactams	1	91-99%	ISKpn9	Absent	Yes	Yes	Ebmeyer et al.[28]
MOX-9	*Aeromonas media*	β-Lactams	138 bp upstream	98-99%	ISKpn9	Absent	Yes	Yes	Ebmeyer et al.[28]
CMY-2-like	*Citrobacter freundii*	β-Lactams	>1	≥98%	ISEcp1	Absent	Yes	Yes	Wu et al.[27]
DHA	*Morganella morganii*	β-Lactams	1	≥97%	Unknown/none detected[a]	Absent	Yes	Yes	Barnaud et al.[63]
ACT-1	*Enterobacter asburiae*	β-Lactams	1	95-96%	Unknown/none detected[a]	Absent	Yes	Yes	Rottman et al.[64], Reisbig et al.[30]
MIR-1	*Enterobacter cloacae*	β-Lactams	1	>99%[a]	ISPps1[a]	Absent	Yes	Yes	Conceicao et al.[31], Jacoby et al.[15]
ACC	*Hafnia alvei/paralvei*	β-Lactams	1	82->99%[a]	ISEcp1	Absent	Yes	Yes	Nadjar et al.[65]
SHV	*Klebsiella pneumoniae*	β-Lactams	>1	≥99%[a]	IS26, IS102	Absent	Yes	Yes	Ford et al.[23]
OXA-23	*Acinetobacter radioresistens*	β-Lactams	1	98->99%[a]	ISAba1, ISAba4	Absent	Yes	Yes	Poirel et al.[22]
OXA-48/181	*Shewanella xiamenensis*	β-Lactams	1	100%	ISEcp1	Absent	Yes	Yes	Potron et al.[66]
OXA-51-like	*Acinetobacter baumanii*	β-Lactams	>1	>99%[a]	ISAba1	ISAba1/absent	Yes	Yes	Chen et al.[33]
PER	*Pararheinheimera* spp.	β-Lactams	>1	78-96%	ISPa12,ISPa13, ISCR1	Absent	Yes	Yes	Ebmeyer et al.[8]
CTX-M-8/9/25	*Kluyvera georgina*	β-Lactams	1	99%	IS10, ISEcp1, ISCR1	Absent	Yes	Yes	Poirel et al.[25], Rodriguez et al.[67]
CTX-M-1,2,3,4,5,6,7	*Kluyvera ascorbata*	β-Lactams	1	100%	ISEcp1, ISCR1	Absent	Yes	Yes	Humeniuk et al.[68], Rodriguez et al.[69]
LMB-1	*Rheinheimera pacifica*	β-Lactams	1	99%[a]	IS6, IS91	Absent	(Yes)	(Yes)[b]	Lange et al.[70]
KPC	*Chromobacterium* spp.	β-Lactams	None identified	≤76%	Tn3-like (Tn4401)	Absent	(Yes)[b]	No	Gudeta et al.[71]
GPC-1	*Shinella* spp.	β-Lactams	None identified	89%	IS91, tnpA	Absent	Yes	(Yes)[b]	Kieffer et al.[42]
BKC-1	*Shinella* spp.	β-Lactams	None identified	87%	ISKpn23	Absent	Yes	(Yes)[b]	Kieffer et al.[42]
MCR-2	*Moraxella pluranimalium*	Colistin	1	96%[a]	IS1595	Absent	Yes	Yes	Poirel et al.[72], Kieffer et al.[73]
MCR-4	*Shewanella frigidimarina*	Colistin	None identified	100%	IS5	Tn5044	Not identified	No	Zhang et al.[74]
MCR-3	*Aeromonas* spp.[b]	Colistin	–[1]	85-95%	ISKpn3, ISAs17, TnAs2	Different IS at conserved locus	Yes	No	Yin et al.[75], Shen et al.[76], Khedher et al.[77]
MCR-8	*Stenotrophomonas*	Colistin	None identified	≤63%	IS903B, ISEcl1	Absent	/[b]	No	Khedher et al.[77]
MCR-9	*Buttiauxella* spp.	Colistin	1	82%	IS26	Absent	Yes	No	Kieffer et al.[78]
QnrB	*C. freundii*	Fluoroquinolones	>1	≥97%	ISCR1, ISEcp1, IS3000, IS6100, IS26	Absent	Yes	Yes	Jacoby et al.[19], Ribeiro et al.[79]
QnrA	*Shewanella algae*	Fluoroquinolones	>1	≥97%[a]	ISCR1	Absent	Yes	Yes	Poirel et al.[47]
QnrE	*Enterobacter* spp./*E. mori*	Fluoroquinolones	>1	83-≥99%[a]	ISEcp1	Absent	Yes	Yes	Albornoz et al.[20]/This article
QnrS	*Vibrio splendidus*	Fluoroquinolones	None identified	≤79%[a]	IS2[a]	Unknown	Yes	No	Cattoir et al.[80]
OqxAB	*K. pneumoniae*	Fluoroquinolones	1	97->99%[a]	IS26	Absent	Yes	Yes	Kim et al.[24]
FosA1	*E. cloacae/Enterobacter* spp.[b]	Fosfomycin	>1	88-99%[a]	Tn2921, IS4	Absent	Yes	Yes	Ito et al.[81]
FosA5/6	*K. pneumoniae*	Fosfomycin	>1	≥99%	IS10, IS1, IS26	Absent	Yes	Yes	Ma et al.[82]
FosA3/4	*Kluyvera georgiana*	Fosfomycin	>1	≥99%	IS26, ISEcp1	Absent	Yes	Yes	Rodriguez et al.[26], Ito et al.[48]
FosA8	*Leclercia adecarboxylata*	Fosfomycin	>1	≥99%	Unknown/none detected[a]	Absent	Yes	Yes	Poirel et al.[83]
TetX	*Sphingobacterium* spp.	Tetracyclin	None identified	≥99%	Tn6031	Different *mob* genes, integrases, transposases	Not identified	No	Ghosh et al.[84]

[a]Data added through this study.
[b]See information on respective resistance determinant in Supplementary Note 1 for details.

- **Almost all known origin species have been associated with infections**

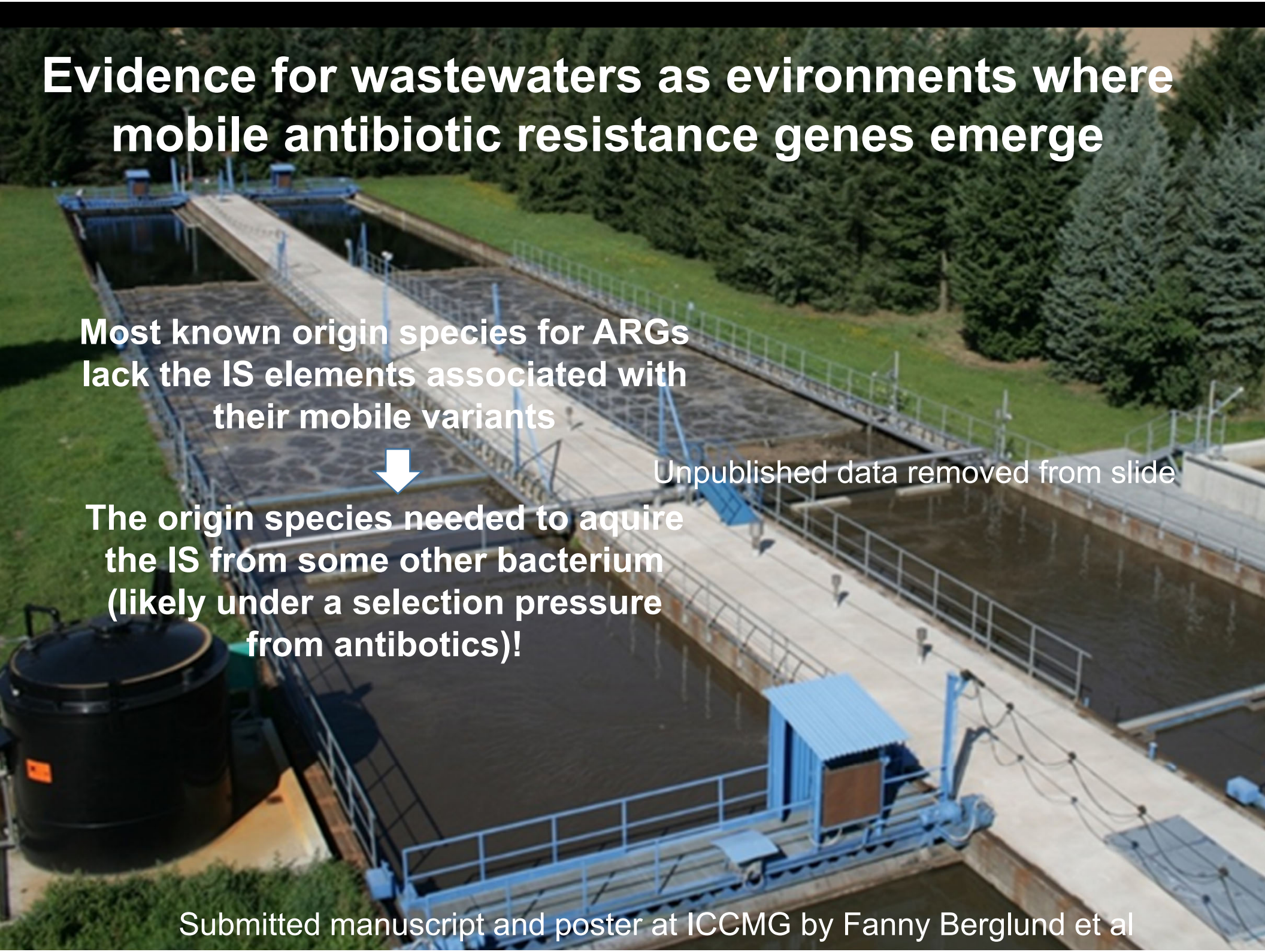
➢ Observation in line with mobilization/transfer primarily driven by exposure to antibiotics in humans/domestic anmals

- **We know the origin only for a very small fraction of all ARGs present in pathogens**

➢ Observation in line with a dominant role of the external environment in the evolution of resistance

Ebmeyer S, Kristiansson E, Larsson DGJ. (2021). A framework for identifying the recent origins of mobile antibiotic resistance genes. Communications Biology. 4:8.

Evidence for wastewaters as evironments where mobile antibiotic resistance genes emerge



Most known origin species for ARGs lack the IS elements associated with their mobile variants

The origin species needed to aquire the IS from some other bacterium (likely under a selection pressure from antibotics)!

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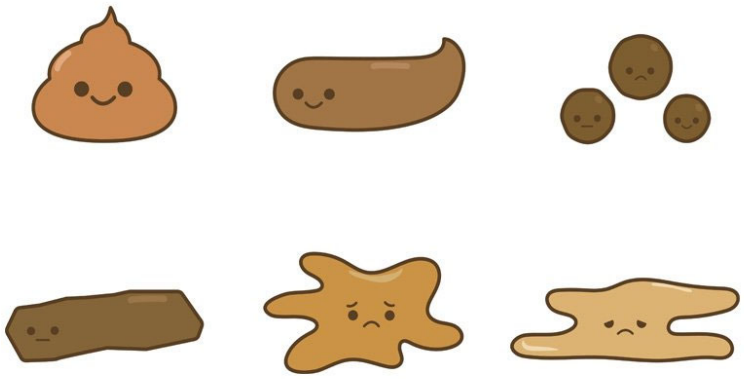
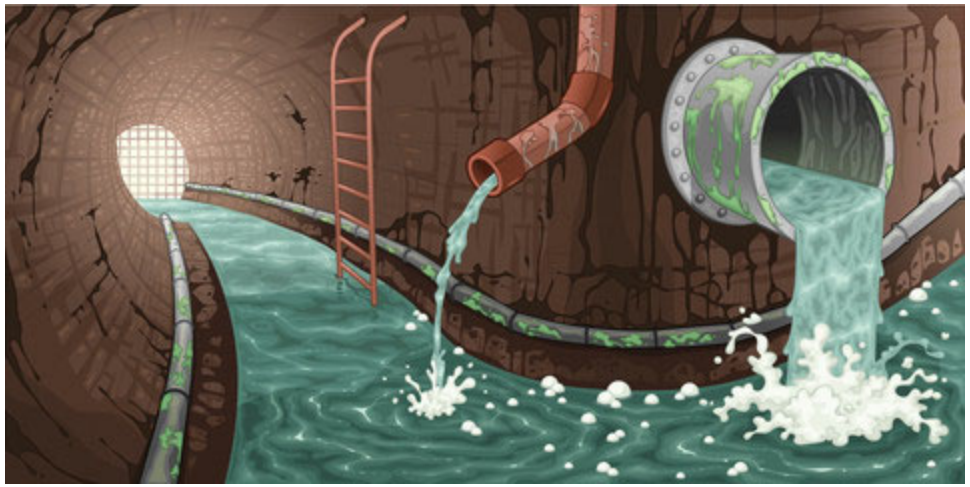
Submitted manuscript and poster at ICCMG by Fanny Berglund et al

Known origin species and corresponding IS-sequences are both much more abundant in sewage than in human stool, and they rarely co-exist in human stool!





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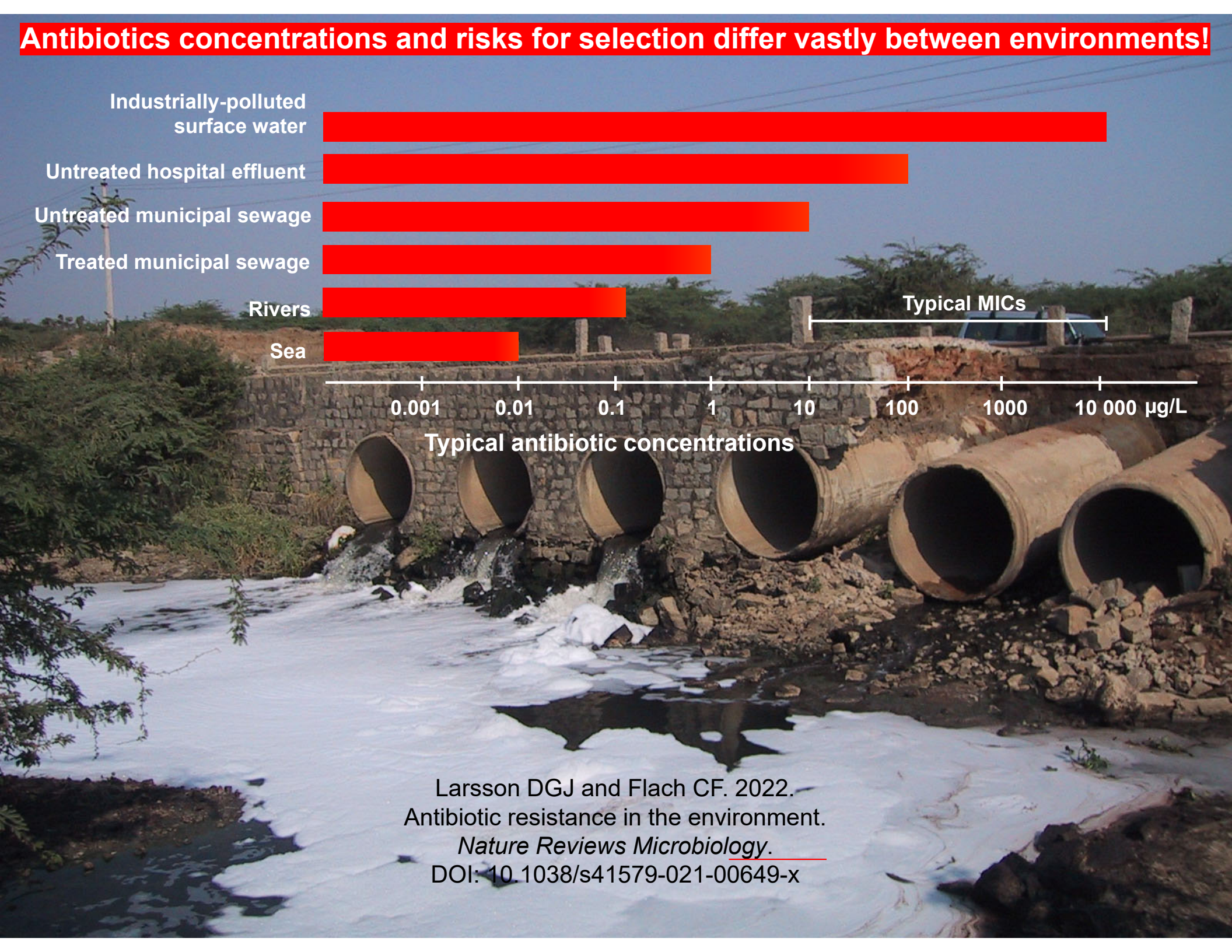
Berglund et al, submitted

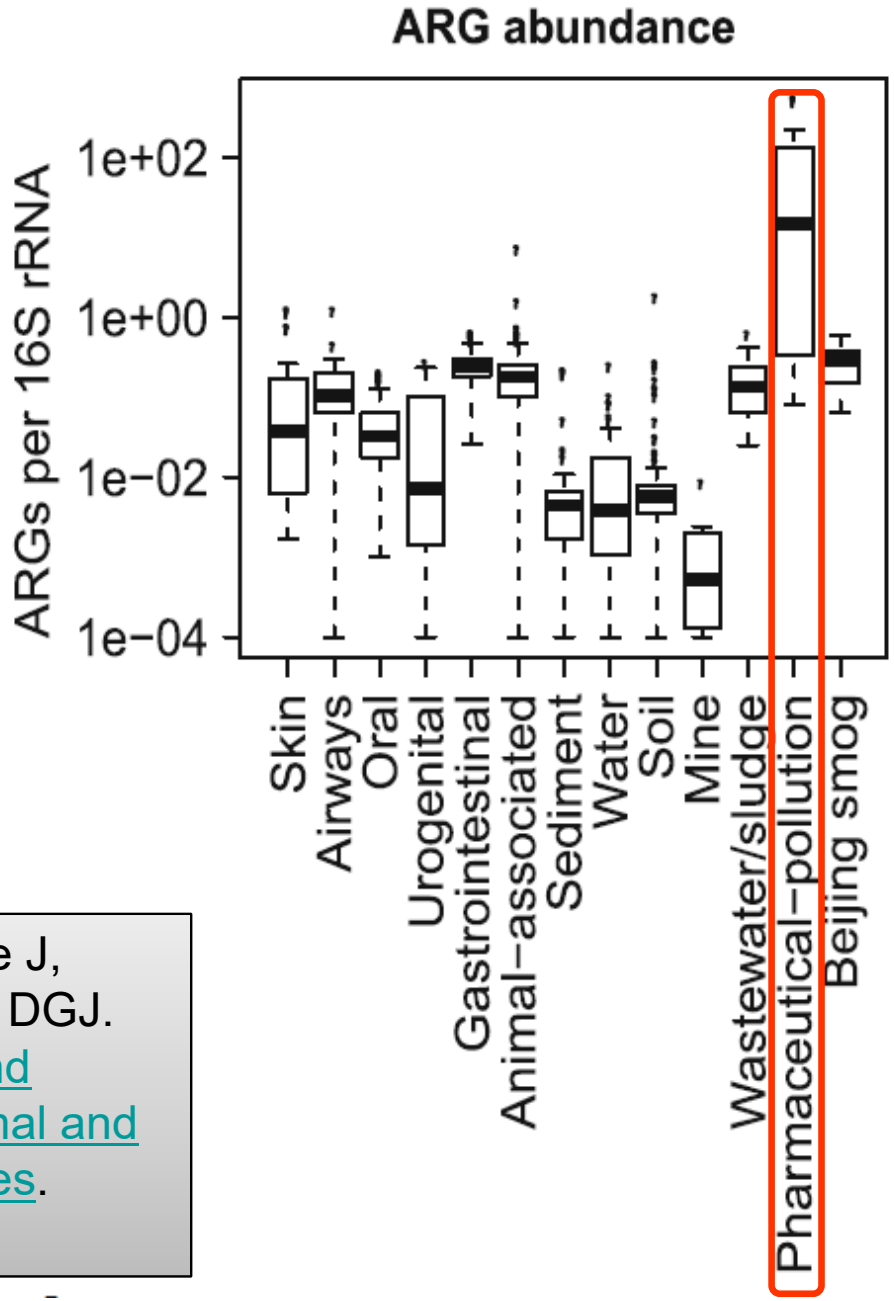
In what external environments are there selection pressures for resistant bacteria?

Antibiotics concentrations and risks for selection differ vastly between environments!



Larsson DGJ and Flach CF. 2022.
Antibiotic resistance in the environment.
Nature Reviews Microbiology.
DOI: 10.1038/s41579-021-00649-x

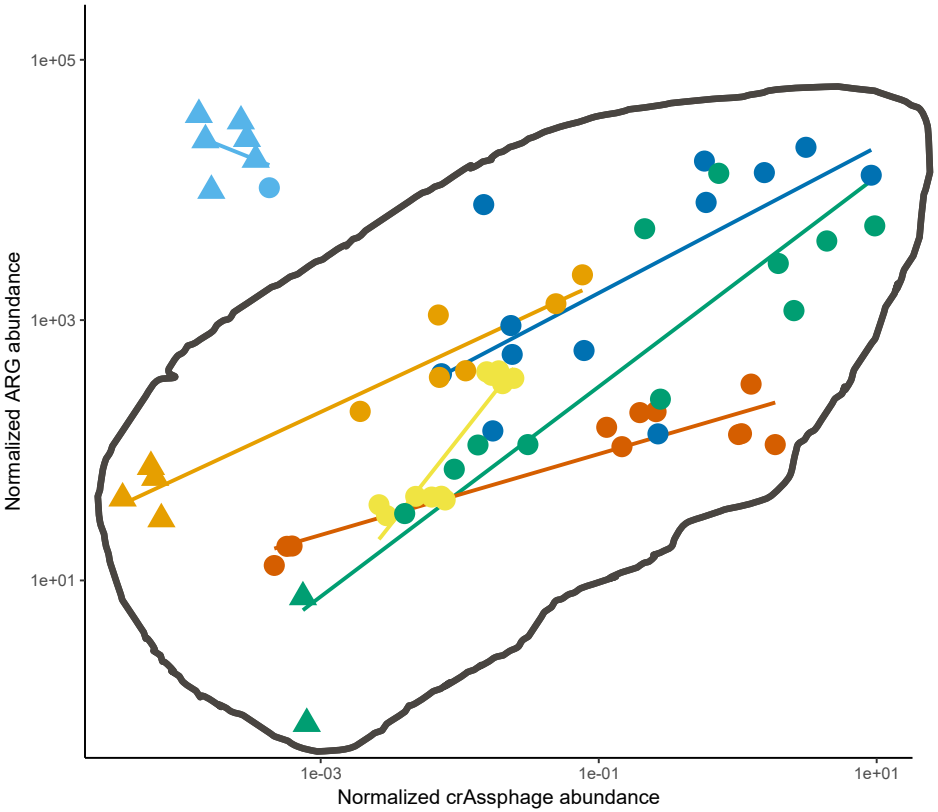
Environments polluted with waste from antibiotic manufacturing carry more antibiotic resistance genes than any other environment



N=864 metagenomes

Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DGJ. (2016). The structure and diversity of human, animal and environmental resistomes. Microbiome. 4:54.

Disentangling environmental transmission from environmental selection through combined analyses of ARGs and a fecal marker (crAssPhage)



crAssphage detection
- crAssphage detected
- crAssphage not detected

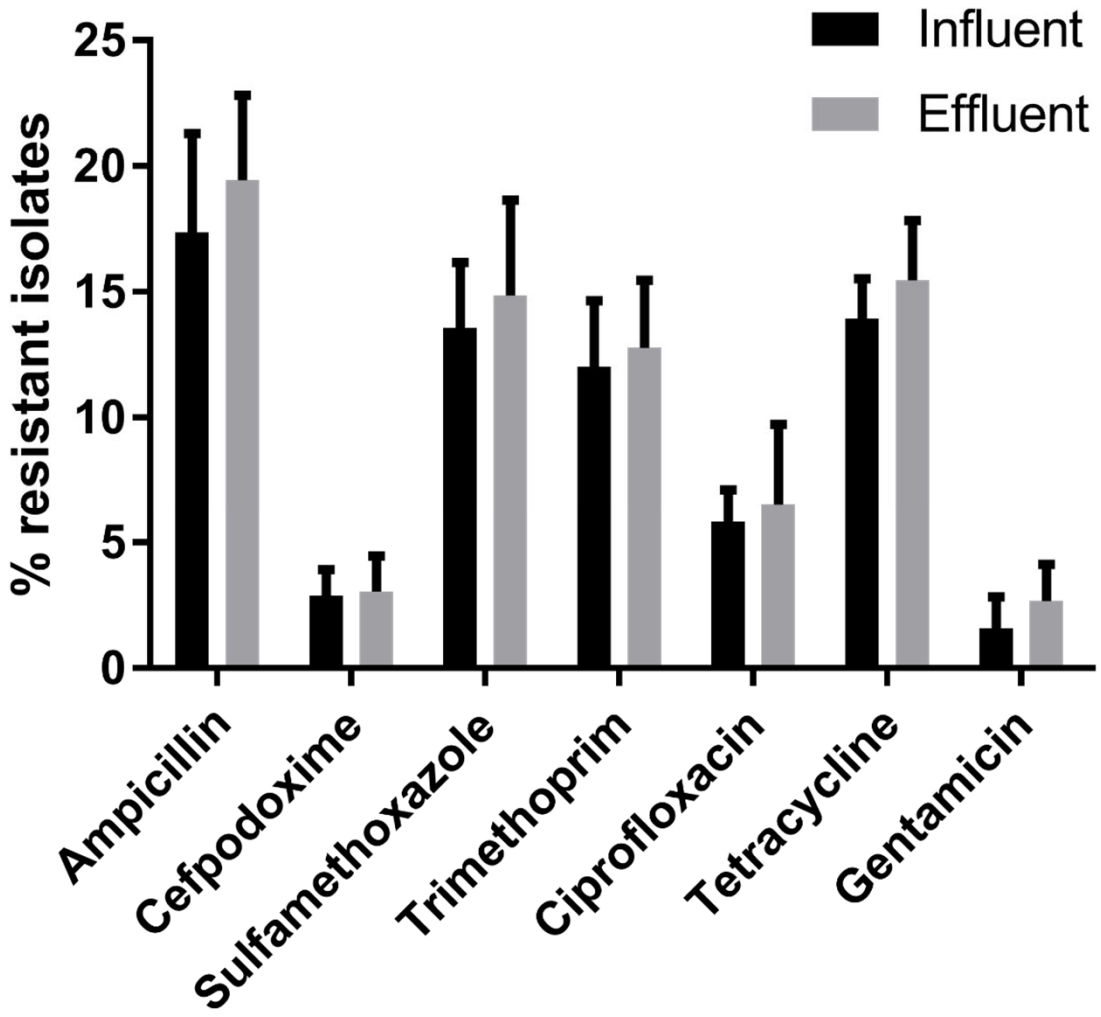
Study
- India
- India polluted
- Singapore
- Spain
- UK
- US

Karkman A., Pärnänen K. and Larsson DGJ. (2019). Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nature Commun. 80:10.

nature COMMUNICATIONS

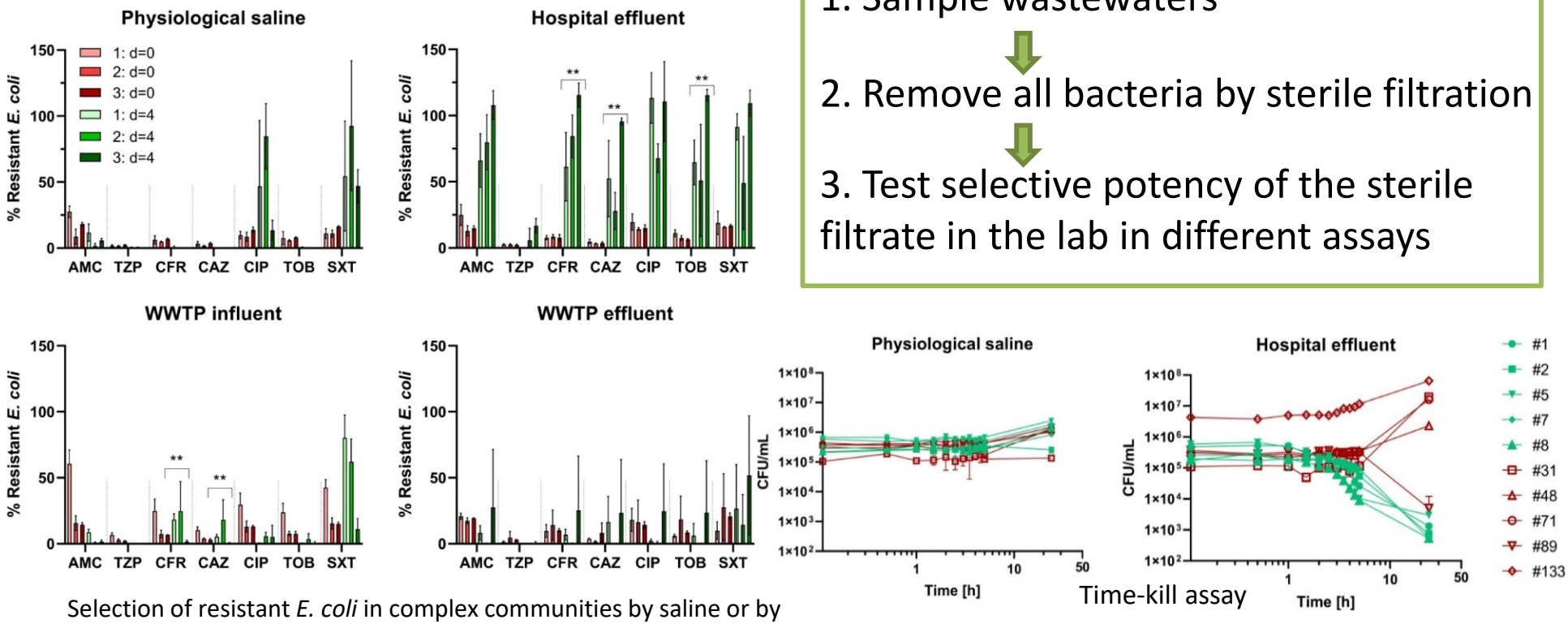
A comprehensive screening of *E. coli* isolates from Scandinavia's largest sewage treatment plant indicates no selection for antibiotic resistance

n=4028 isolates



Flach CF, Genheden M, Fick J, Larsson DGJ. (2018). Environ Sci Technol, 52 (19), pp 11419–11428.

..but Swedish hospital effluent strongly selects for multi-resistant *E. coli*!



1. Sample wastewaters
2. Remove all bacteria by sterile filtration
3. Test selective potency of the sterile filtrate in the lab in different assays

Selection of resistant *E. coli* in complex communities by saline or by different sterile-filtered waste-waters

Time-kill assay

green=sensitive strains; red=multi-resistant strains

Kraupner N, Hutinel M, Schumacher K, Gray DA, Genheden M, Fick J, Flach C-F, Larsson DGJ. (2021).
Evidence for selection of multi-resistant *E. coli* by hospital effluent.
Environment international. Vol. 150:106436. https://doi.org/10.1016/j.envint.2021.106436

The role of sub-MIC levels of antibiotics in the environment:

- **Plausible role in evolution of resistance**
- **Less likely to contribute to the transmission of already resistant, enteric pathogens**

OXFORD
FEDERATION OF EUROPEAN MICROBIOLOGICAL SOCIETIES FEMS JOURNALS investing in science

FEMS Microbiology Reviews, fux053, 42, 2018, 68–80

doi: 10.1093/femsre/fux053
Advance Access Publication Date: 24 October 2017
Review Article

REVIEW ARTICLE

Environmental factors influencing the development and spread of antibiotic resistance

Johan Bengtsson-Palme[1,2,*,†], Erik Kristiansson[1,3] and D. G. Joakim Larsson[1,2]

Environment International 86 (2016) 140–149

Contents lists available at ScienceDirect

Environment International

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

Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation

Johan Bengtsson-Palme, D.G. Joakim Larsson *

AMR Alliance Recommended PNECs for Risk Assessments

Active Pharmaceutical Ingredient	PNEC-ENV (µg/L)	PNEC-MIC (µg/L)	Lowest Value (µg/L)
Amikacin	N/A	16	16
Amoxicillin	Testing On-Going	0.25	0.25
Amphotericin B	N/A	0.02	0.02
Ampicillin	0.87	0.25	0.25
Anidulafungin	N/A	0.02	0.02
Avilamycin	N/A	8.0	8.0
Azithromycin	0.02	0.25	0.02
Aztreonam	N/A	0.50	0.50
Bacitracin	100	8.0	8.0

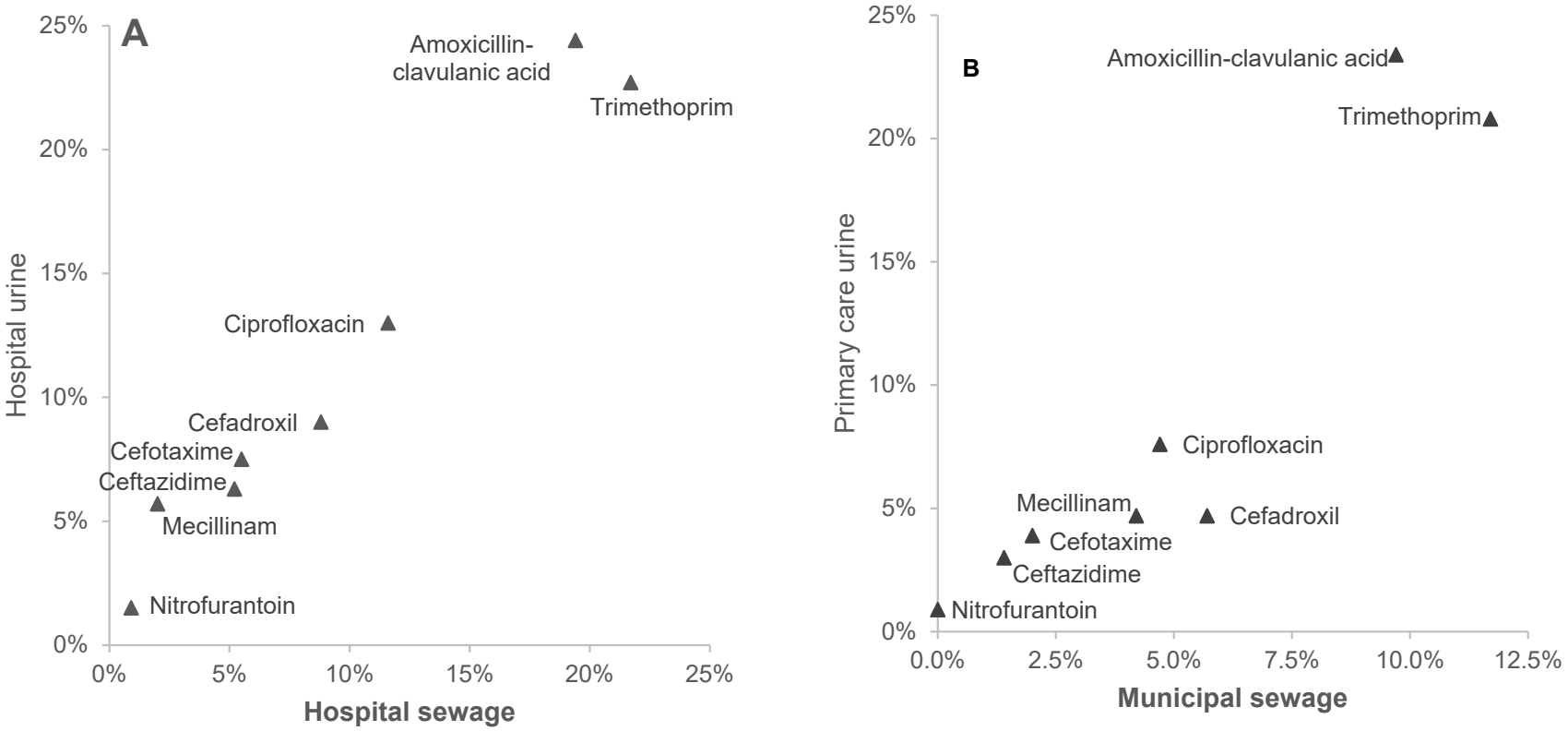
3. Reflection of the regional resistance situation

Untreated sewage -
contains pooled fecal bacteria from human populations

Sewage surveillance may:

- Reveal trends
- Discover new threats
- Evaluate effects of interventions
- Guide empiric therapy

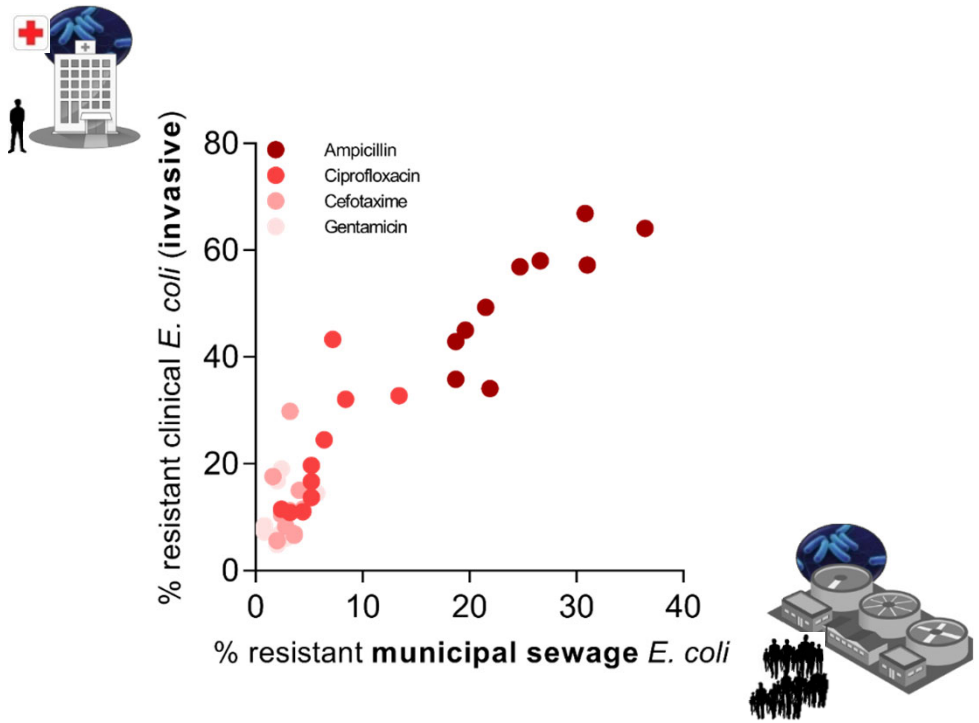
Comparing antibiotic resistance prevalence in clinical isolates of *E. coli* with sewage isolates



Mean resistance rates in E. coli isolated from **hospital (A)** or **municipal (B) sewage** samples compared to resistance rates in *E. coli* isolated from **urine f**rom patients at the same hospital or from primary care patients in the region served by the municipal treatment plant.

Hutinel M, Huijbers P, Fick J, Åhrén C, Larsson DGJ, Flach CF. 2019. Population-level surveillance of antibiotic resistance in Escherichia coli through sewage analysis. *Eurosurveillance, 24(37).*

Resistance rates in sewage *E. coli* correlate with resistance rates in the clinics across 10 countries



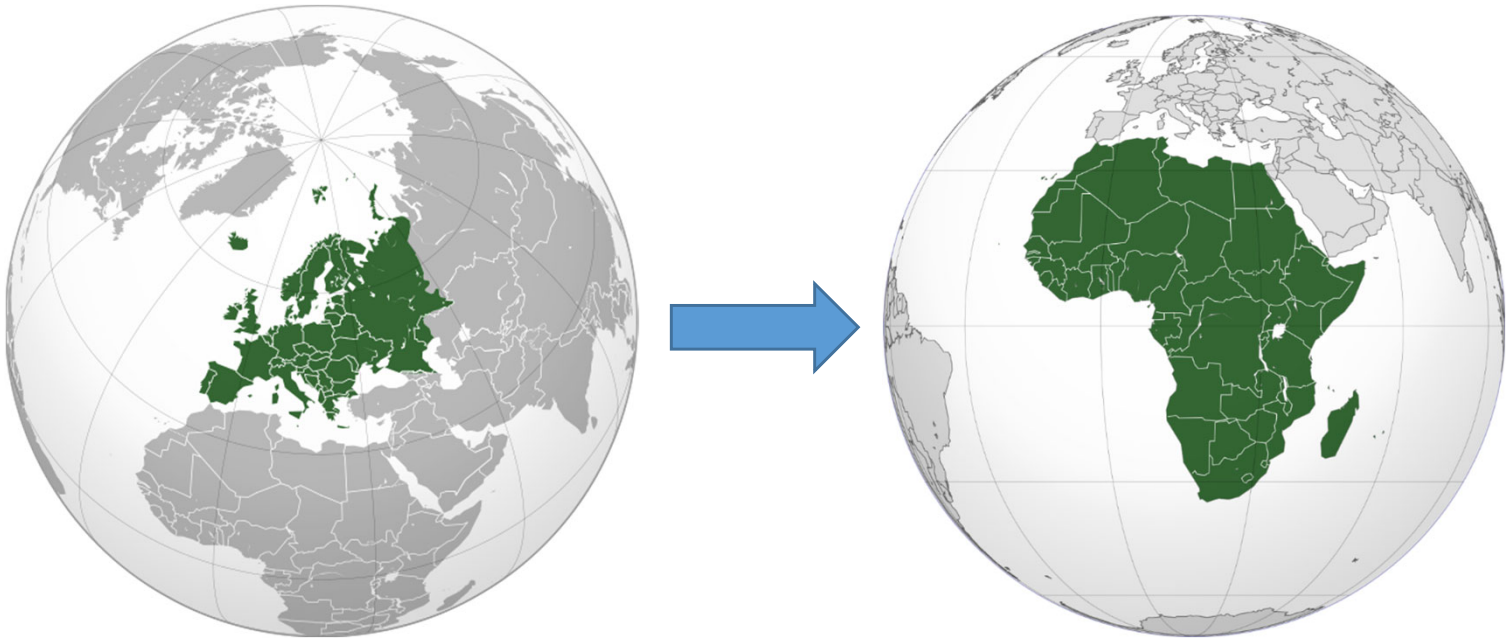
UNIVERSITY OF GOTHENBURG

Samples from **hospitalized patients** (invasive isolates) and **municipal sewage** in ten European countries

CARe

Huijbers P, Larsson DGJ, Flach CF. 2020. Surveillance of antibiotic resistance in human populations through urban wastewater. Environmental Pollution. 261:114200.

Greatest potential value in regions with limited or non-existing, systematic clinical surveillance



Flach CF et al, in prep

Analyses of resistance in sewage *E. coli* isolates from sub-Saharan African countries suggests high resistance to inexpensive antibiotics

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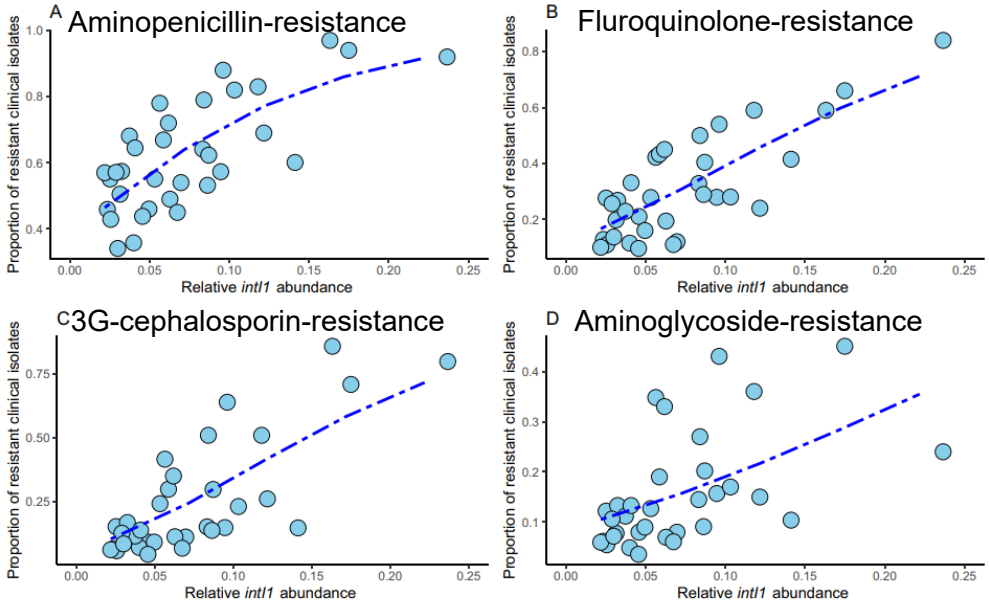
Flach CF et al, in prep

New project funded by the Wellcome trust will compare clinical and sewage surveillance data in three African countries (CF Flach is main PI)

A different approach: predicting clinical resistance prevalence from sewage metagenomic data (gene-based)



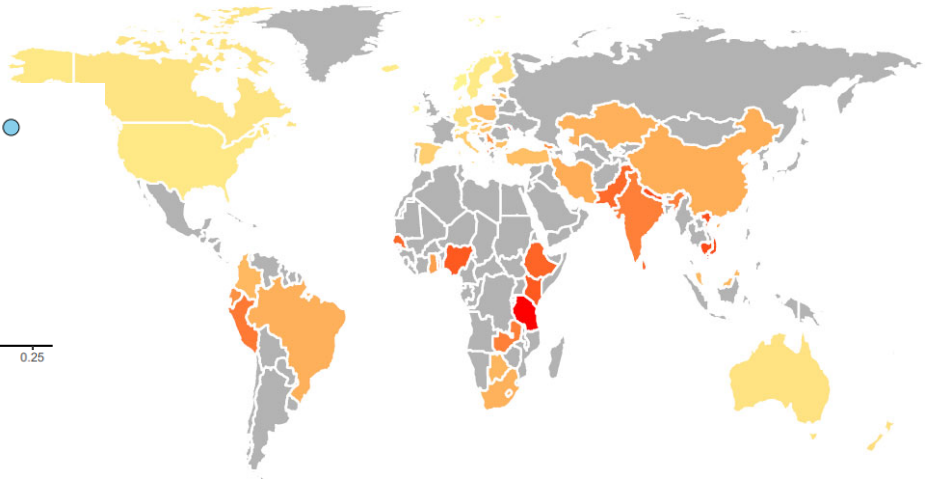


Fig. 2 *E. coli* clinical resistance models based on the *intI1* integrase gene. Proportion of resistant invasive *E. coli* clinical isolates to aminopenicillins **a**, fluoroquinolones **b**, third-generation cephalosporins **c**, and aminoglycosides **d** against *intI1* integrase gene abundance. The blue line shows the fitted clinical resistance from the beta regression model with *intI1* abundance as explanatory variable. Note that for some countries, data on clinical resistance was not available for all classes.

Karkman A, Berglund F, Flach C-F, Kristiansson E, Larsson DGJ. (2020). Predicting clinical resistance prevalence using sewage metagenomic data. Communications Biology. 3:711 https://doi.org/10.1038/s42003-020-01439-6

Analyses based on metagenomic data generated within the Global Sewage Project:

Hendriksen, R.S., Munk, P., Njage, P. *et al.* Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat Commun* **10,** 1124 (2019). https://doi.org/10.1038/s41467-019-08853-3

Genomic analysis of sewage from 101 countries reveals global landscape of antimicrobial resistance

Accepted in *Nature Communications* at 12:57 today!

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Munk P, Brinch C, Møller F, Petersen T, Hendriksen R, Seyfarth A, Kjeldgaard J, Svendsen C, van Bunnik B, Berglund F, Global Sewage Surveillance Consortium, Larsson DGJ, Koopmans M, Woolhouse M, Aarestrup F. (2022). Genomic analysis of sewage from 101 countries reveals global landscape of antimicrobial resistance. Nature Communications. Accepted for publication

Comparison of sewage-based resistance surveillance* (gene- or isolate-based) with traditional clinical resistance surveillance

Attribute	Sewage-based resistance surveillance (gene-based)	Sewage-based resistance surveillance (isolate-based)	Clinical resistance surveillance (isolate-based)
Potential bias comparing trends over time and space	Standardization of sampling easy, enables comparisons with limited bias	Standardization of sampling easy, enables comparisons with limited bias	Differences in sampling strategies often bias comparisons
Risk that the end points studied are influenced by a non-human bacterial population	High risk	Low to high risk depending on species	No risk
Reflects intestinal carriage or infections	Reflects carriage, but may correlate well with infection	Reflects carriage, but may correlate well with infection	Reflects infection or carriage depending on sample type
Reflects resistance in sick or healthy part of population	Reflects both, but to steer the focus, surveillance may target municipal or hospital sewage	Reflects both, but to steer the focus, surveillance may target municipal or hospital sewage	Reflects the resistance in people who are infected and seek care
Interpretation of numbers	Represents the average abundance of a selected gene or genes across the faecal microbiota	Represents the percentage of carriers times the average proportion of resistant strains within a species in the faecal microbiota of the carriers	Represents the percentage of infected individuals or the percentage of carriers depending on the sample type
Identification of resistance phenotypes	Predicts resistance phenotypes broadly from individual, acquired genes	Identifies resistance phenotypes	Identifies resistance phenotypes
Ability to link resistance to species	Difficult to link genes and thus predicted resistances to specific species	Links resistance to specific pathogen species	Links resistance to specific pathogen species

* Sewage surveillance with the specific objective to predict the resistance situation in humans

Larsson DGJ, Flach C-F. (2021). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

Comparison of sewage-based resistance surveillance* (gene- or isolate-based) with traditional clinical resistance surveillance

Attribute	Sewage-based resistance surveillance (gene-based)	Sewage-based resistance surveillance (isolate-based)	Clinical resistance surveillance (isolate-based)
Ability to identify multiresistance	Does not enable the identification of multiresistance patterns	Identifies multiresistance patterns	Identifies multiresistance patterns
Ability to identify rare types of resistance	Possible via targeted analyses (PCR)	Possible via selective culturing	Challenging
Provides patient-specific information	No	No	Yes
Ability to inform empirical treatment	Unlikely	Possibly, after evaluation	Informs empirical treatment
Prospect for acceptance in clinical community	Very different from current surveillance, major challenges	Different from current surveillance, but also bears similarities, challenging	The accepted standard among the clinical community
Ethical issues	No ethical issues with sampling	No ethical issues with sampling	Ethical issues may arise when carriers are identified
Cost	Inexpensive	Rather inexpensive	Expensive
Simplicity of sample collection and processing	Very simple sampling	Simple, but more elaborate sampling compared with gene-based sewage surveillance	Resource-demanding to process samples from many individual patients
Need for many samples	A single sample can (to some extent) reflect the resistance situation in an entire community	A single sample can (to some extent) reflect the resistance situation in an entire community	A large number of samples are needed to reflect the resistance situation
Need for calibration against clinical resistance prevalence	More calibration against clinical resistance needed	More calibration against clinical resistance needed	Considered 'gold standard' but suffers from, for example, sampling bias
Need for development of sampling protocol	One sampling protocol covers all enteric species (but without separation)	Efficient, specific sampling method evaluated for *Escherichia coli*, not yet for other species	Sampling method exists for almost all bacterial pathogens
Need for local health care infrastructure	No local health care infrastructure needed	No local health care infrastructure needed	Local health care infrastructure needed

* Sewage surveillance with the specific objective to predict the resistance situation in humans

Larsson DGJ, Flach C-F. (2021). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

As sewage contains fecal bacteria from a very large number of people, outbreaks of rare forms of resistance may be spotted early through selective culturing or qPCR

Water Research 200 (2021) 117261

Contents lists available at ScienceDirect

Water Research

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

Monitoring of hospital sewage shows both promise and limitations as an early-warning system for carbapenemase-producing Enterobacterales in a low-prevalence setting

Carl-Fredrik Flach[a,b,*], Marion Hutinel[a,b], Mohammad Razavi[a,b], Christina Åhrén[a,b,c], D.G. Joakim Larsson[a,b]

Conclusions

- The environment is a transmission route for already resistant pathogens – but how large proportion this route is responsible for is uncertain. Its relative contribution to other routes is likely large in regions with no or limited developed waste infrastructure. Consequences are incremental.

- Consequences of those rare evolutionary steps that lead to the emergence of resistance in pathogens may be very large and need more consideration.

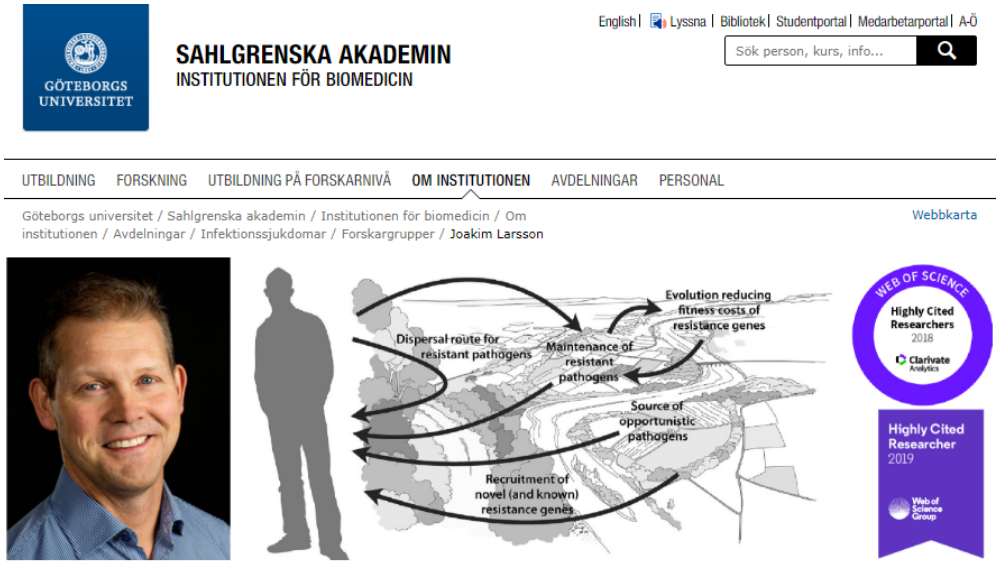
- The environment is likely an immense source for resistance genes that over time emerge in pathogens.

- We do not know where the different evolutionary steps towards the emergence of ARGs in pathogens takes place (discounting ”ancient” evolution of the ARGs themselves that indeed took place in the environment).

- Pollution with antibiotics, definitely from manufacturing and most likely also from use and excretion (particularly in hospital sewers), is a clear risk factor that needs urgent attention.

- Sewage surveillance is promising for identifying emerging resistance threats and possibly for predicting the regional, clinical resistance situation

Thanks for listening!



Vetenskapsrådet

VÄSTRA GÖTALANDSREGIONEN

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UNIVERSITAS GOTHOBURGENSIS 1891

UNIVERSITY OF GOTHENBURG

E-mail: joakim.larsson@fysiologi.gu.se
Personal website: https://gu.se/en/biomedicine/our-research/joakim-larsson-group
CARe website: http://gu.se/en/care