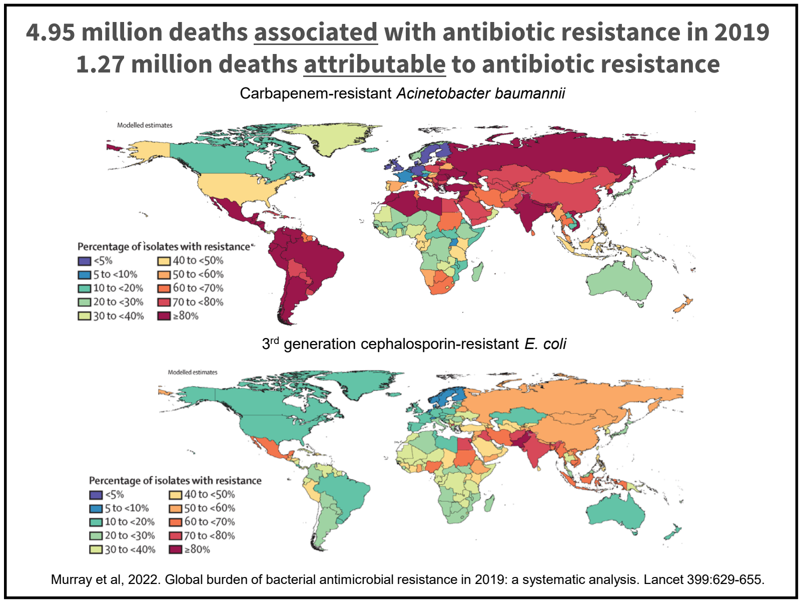
Resistensutmaningen kräver åtgärder långt utanför sjukvården

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



4.95 million deaths associated with antibiotic resistance in 2019
1.27 million deaths attributable to antibiotic resistance

Carbapenem-resistant *Acinetobacter baumannii*

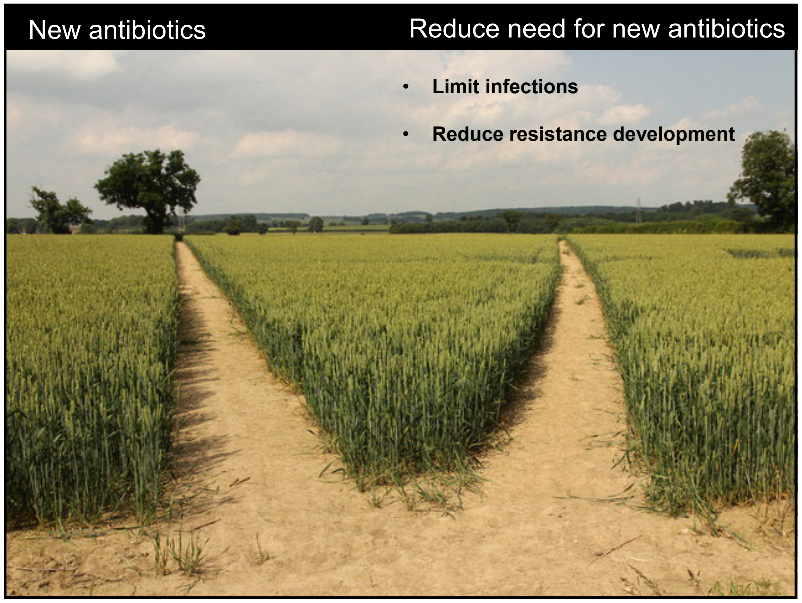
3rd generation cephalosporin-resistant *E. coli*

Murray et al, 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399:629-655.

The world is interconnected!

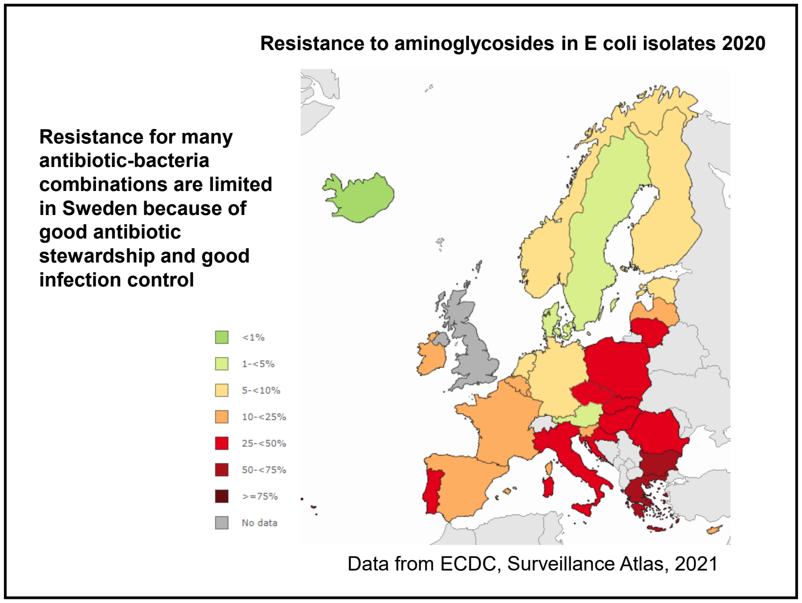
Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, Larsson DGJ, Johansson A. (2015) The human gut microbiome as a transporter of antibiotic resistance genes between continents. Antimicrobial Agents and Chemotherapy. doi: 10.1128/AAC.00933-15

Johnning A, Kristiansson E, Martin A, Marathe NP, Shouche YS, Johansson A, Larsson DGJ. (2015) Quinolone resistance mutations in the faecal microbiota of Swedish travellers to India. BMC Microbiology. 15:235.



New antibiotics | Reduce need for new antibiotics

- **Limit infections**
- **Reduce resistance development**



Resistance to aminoglycosides in E coli isolates 2020

Resistance for many antibiotic-bacteria combinations are limited in Sweden because of good antibiotic stewardship and good infection control

Data from ECDC, Surveillance Atlas, 2021

There is a need for a wide range of solutions, and thus competences from different disciplines

Joint Programming Initiative on Antimicrobial Resistance

Strategic Research and Innovation Agenda on Antimicrobial Resistance

jpiamr

Fhqwh#iru#Dqwlelrwlf#Uhvlvwdqfh#Uhvhdufk#dw#Xqlyhuvlw|#ri#Jrwkhqexuj

The Vision of CARe:

To limit mortality, morbidity and socioeconomic costs related to antibiotic resistance on a global scale through research

www.gu.se/en/care

+100 researchers representing six faculties and 18 departments, launched April 2016

Sahlgrenska Academy, Faculty of Science, Faculty of Business, Economics and Law, Faculty of Humanities, Faculty of Social Sciences, Faculty of Education

European Commission

A European One Health Action Plan against Antimicrobial Resistance (AMR)

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

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
- **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.

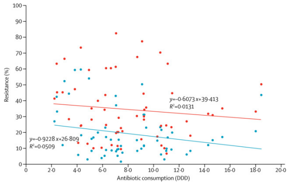
1. Transmission risks

Environmental Science & Technology
Viewpoint
pubs.acs.org/est

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

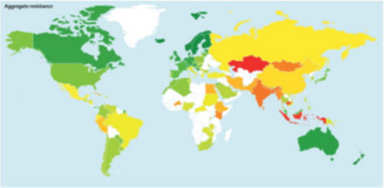
David W. Graham,*,† Peter Collignon,‡ Julian Davies,§ D. G. Joakim Larsson,‖ and Jason Snape⊥

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

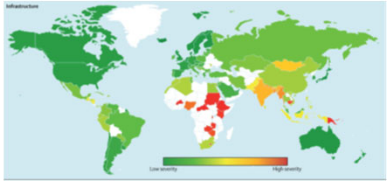


***Escherichia coli* resistance levels for fluoroquinolones and third-generation cephalosporins compared with antibiotic consumption**

Aggregated resistance index



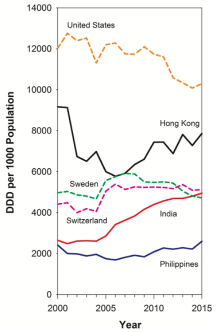
Infrastructure (sanitation control)



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

..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)

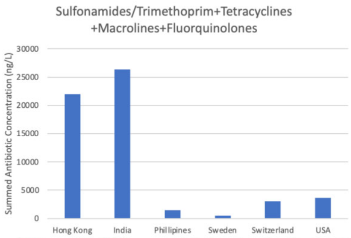


Figure S15. Average total antibiotic mass concentration in samples collected from Hong Kong, India, The Philippines, Sweden, Switzerland, and the US. Reported numbers reflect summed averages of data reported in **Table S5** for the antibiotics (= sulfonamides/trimethopim + tetracyclines + macrolides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

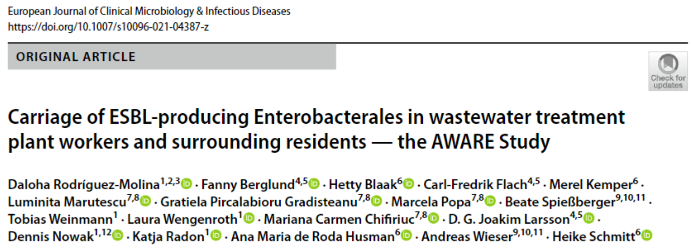
Maria Virginia Prieto Riquelme, Emily Garner, Suraj Gupta, Jake Metch, Ni Zhu, Matthew F. Blair, Gustavo Arango-Argoty, Ayella Maile-Moskowitz, An-dong Li, Carl-Fredrik Flach, Diana S. Aga, Indumathi M. Nambi, D. G. Joakim Larsson, Helmut Bürgmann, Tong Zhang, Amy Pruden, Peter J. Vikesland (2022). Demonstrating 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



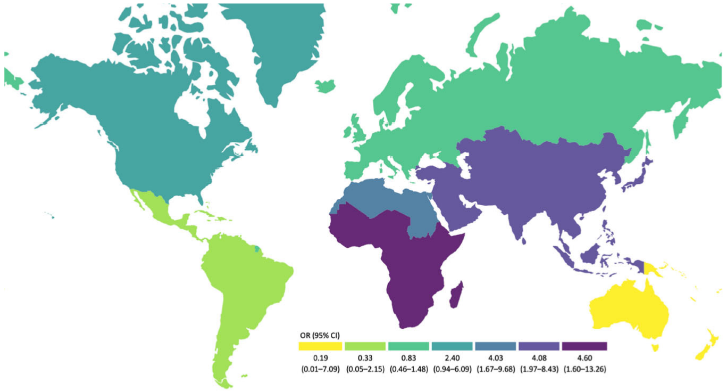
European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-021-04387-z

ORIGINAL ARTICLE

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

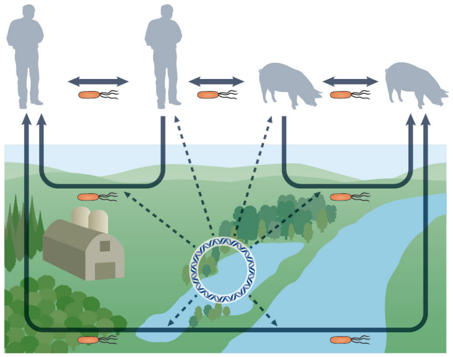


OR (95% CI)	🟨	🟩	🟩	🟦	🟦	🟪	🟪
OR	0.19	0.33	0.83	2.40	4.03	4.08	4.60
95% CI	(0.01–7.09)	(0.05–2.15)	(0.46–1.48)	(0.94–6.09)	(1.67–9.68)	(1.97–8.43)	(1.60–13.26)

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

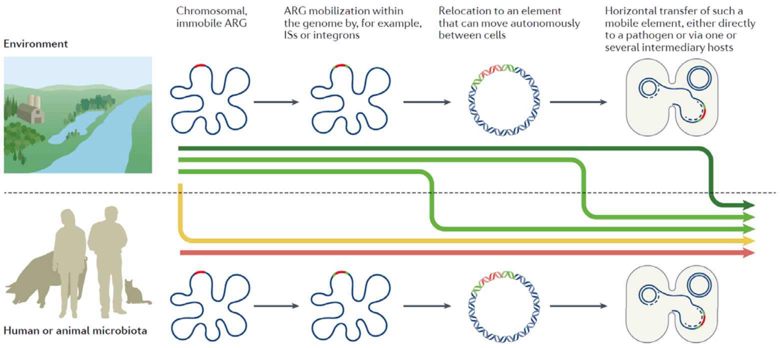
→ Transmission of pathogenic bacteria between humans, between animals or between humans and animals (either direct or via the environment):	- - → Uptake of new resistance factors from the diverse environmental microbiota:
• Common	• Relatively rare
• Risks are in principle quantifiable and predictable	• 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

Where do the critical mobilization and transfer events occur?

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?

Known recent origin species/genera for antibiotic resistance genes

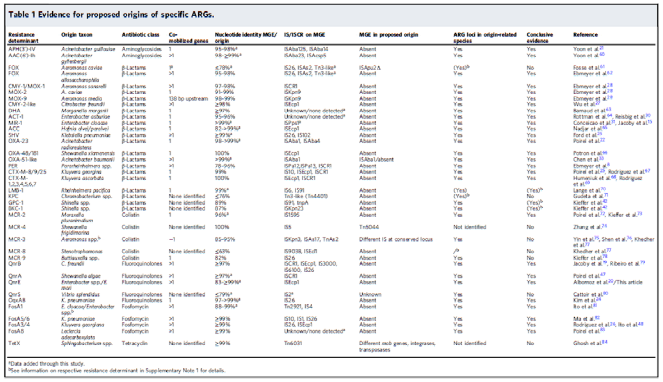


Table 1 Evidence for proposed origins of specific ARGs.

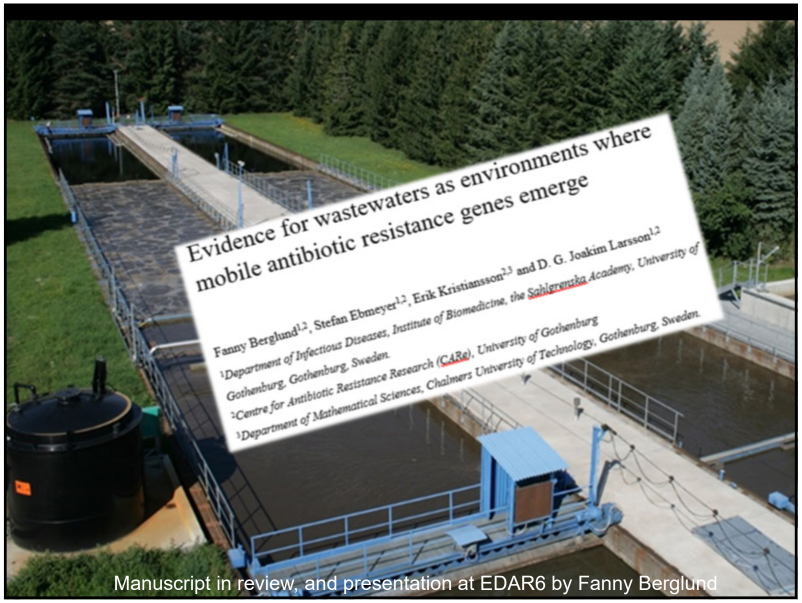
Resistance determinant	Origin taxon	Antibiotic class	Co-mobilized genes	Nucleotide identity MGE/origin	IS/ISCR on MGE	MGE in proposed origin	ARG loci in origin-related species	Conclusive evidence	Reference
APH(3′)-IV	*Acinetobacter guillouiae*	Aminoglycosides	1	95–98%[a]	ISAba125, ISAba14	Absent	Yes	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 sanarellii*	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 georgiana*	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 animals
- **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 environments where mobile antibiotic resistance genes emerge

Fanny Berglund[1,2], Stefan Ebmeyer[1,2], Erik Kristiansson[2,3] and D. G. Joakim Larsson[1,2]

[1]*Department of Infectious Diseases, Institute of Biomedicine, the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.*

[2]*Centre for Antibiotic Resistance Research (CARe), University of Gothenburg*

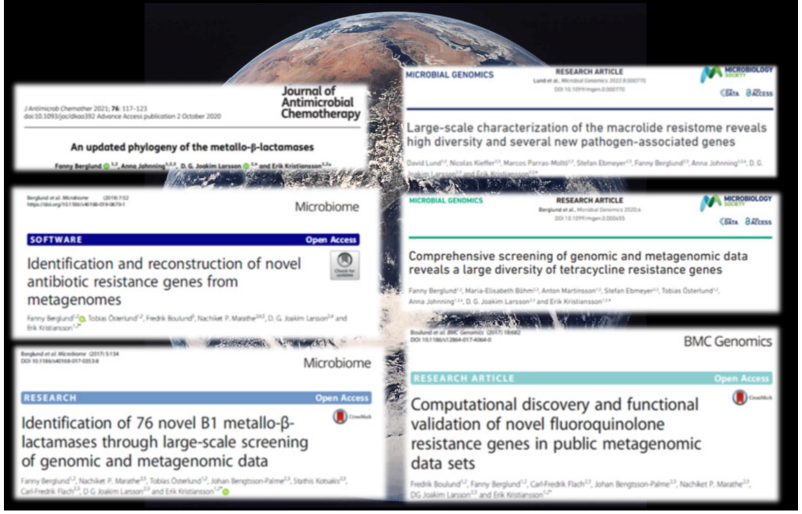
[3]*Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden.*

Manuscript in review, and presentation at EDAR6 by Fanny Berglund

Risk factors for the emergence of resistance in pathogens

Diversity of donors, reservoir size +

The external environment harbours many, many more resistance genes than those currently found in pathogens



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, Anna Johnning, D. G. Joakim Larsson and Erik Kristiansson

MICROBIAL GENOMICS

RESEARCH ARTICLE

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

David Lund, Nicolas Kieffer, Marcos Parras-Moltó, Stefan Ebmeyer, Fanny Berglund, Anna Johnning, D. G. Joakim Larsson and Erik Kristiansson

Microbiome

SOFTWARE — Open Access

Identification and reconstruction of novel antibiotic resistance genes from metagenomes

Fanny Berglund, Tobias Österlund, Fredrik Boulund, Nachiket P. Marathe, D. G. Joakim Larsson and Erik Kristiansson

MICROBIAL GENOMICS

RESEARCH ARTICLE

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

Fanny Berglund, Maria-Elisabeth Böhm, Anton Martinsson, Stefan Ebmeyer, Tobias Österlund, Anna Johnning, D. G. Joakim Larsson and Erik Kristiansson

Berglund et al. Microbiome (2017) 5:134

Microbiome

RESEARCH — Open Access

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

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

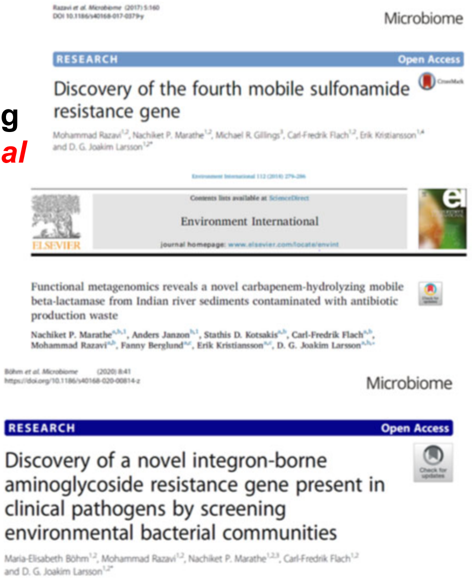
BMC Genomics

RESEARCH ARTICLE — Open Access

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

Fredrik Boulund, Fanny Berglund, Carl-Fredrik Flach, Johan Bengtsson-Palme, Nachiket P. Marathe, DG Joakim Larsson and Erik Kristiansson

Exploring the unknown and identifying emerging threats via ***functional metagenomics***



Razavi et al. Microbiome (2017) 5:160
DOI 10.1186/s40168-017-0379-y

Microbiome

RESEARCH — Open Access

Discovery of the fourth mobile sulfonamide resistance gene

Mohammad Razavi[1,2], Nachiket P. Marathe[1,2], Michael R. Gillings[3], Carl-Fredrik Flach[1,2], Erik Kristiansson[1,4] and D. G. Joakim Larsson[1,2*]

Environment International 112 (2018) 279–286

Contents lists available at ScienceDirect

Environment International

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

ELSEVIER

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

Nachiket P. Marathe, Anders Janzon, Stathis D. Kotsakis, Carl-Fredrik Flach, Mohammad Razavi, Fanny Berglund, Erik Kristiansson, D. G. Joakim Larsson

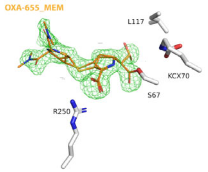
Böhm et al. Microbiome (2020) 8:41
https://doi.org/10.1186/s40168-020-00814-z

Microbiome

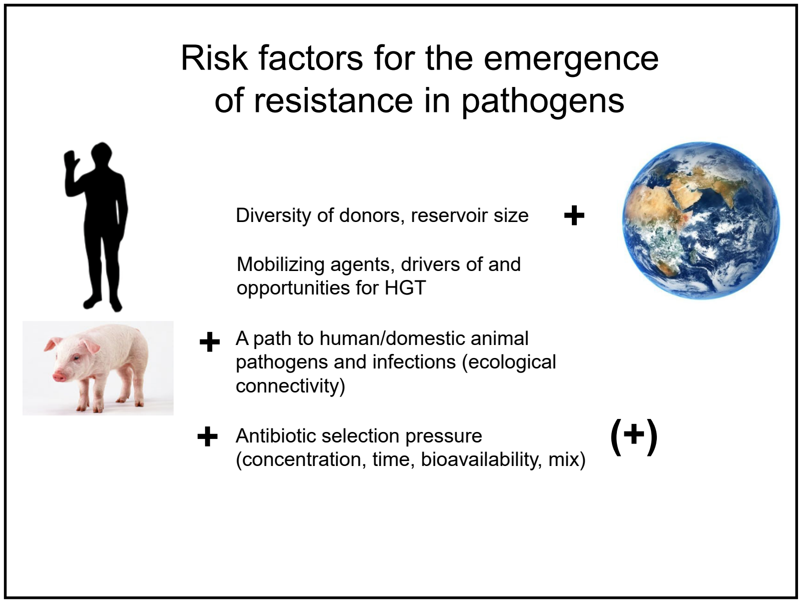
RESEARCH — Open Access

Discovery of a novel integron-borne aminoglycoside resistance gene present in clinical pathogens by screening environmental bacterial communities

Maria-Elisabeth Böhm[1,2], Mohammad Razavi[1,2], Nachiket P. Marathe[1,2,3], Carl-Fredrik Flach[1,2] and D. G. Joakim Larsson[1,2*]



Leiros H-K S, Thomassen A M, Samuelsen Ø , Flach C-F, Kotsakis S D, Larsson DGJ. 2020. Structural insight to the enhanced carbapenemase efficiency of OXA-655 compared to OXA-10.



Risk factors for the emergence of resistance in pathogens

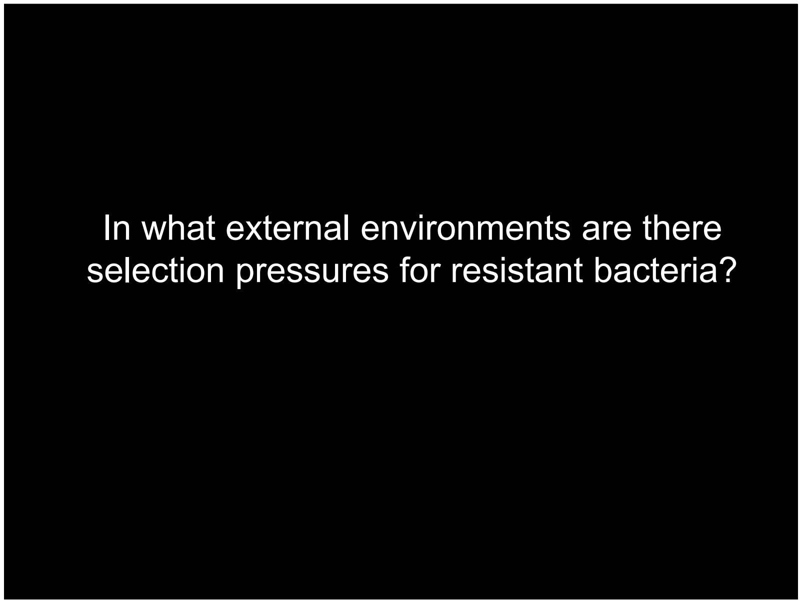
Diversity of donors, reservoir size ✚

Mobilizing agents, drivers of and opportunities for HGT

✚ A path to human/domestic animal pathogens and infections (ecological connectivity)

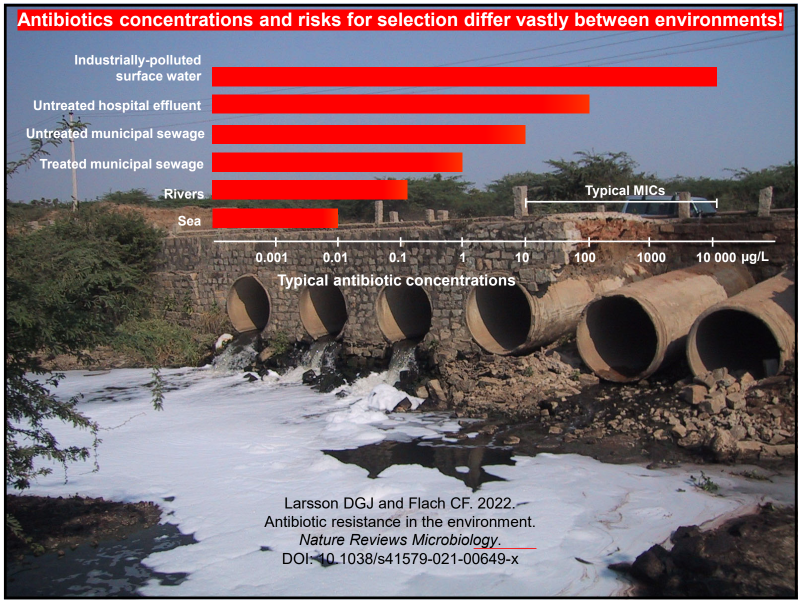
✚ Antibiotic selection pressure (concentration, time, bioavailability, mix) (✚)

29



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

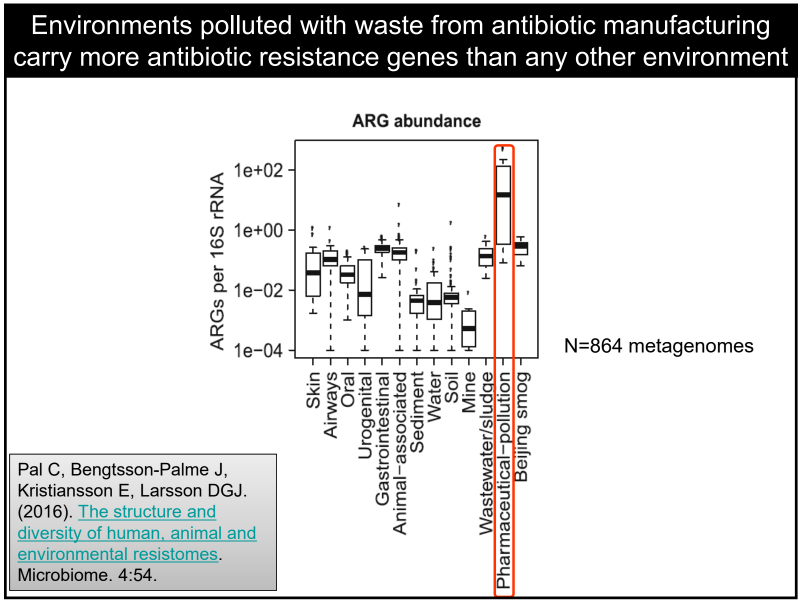
30



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

31

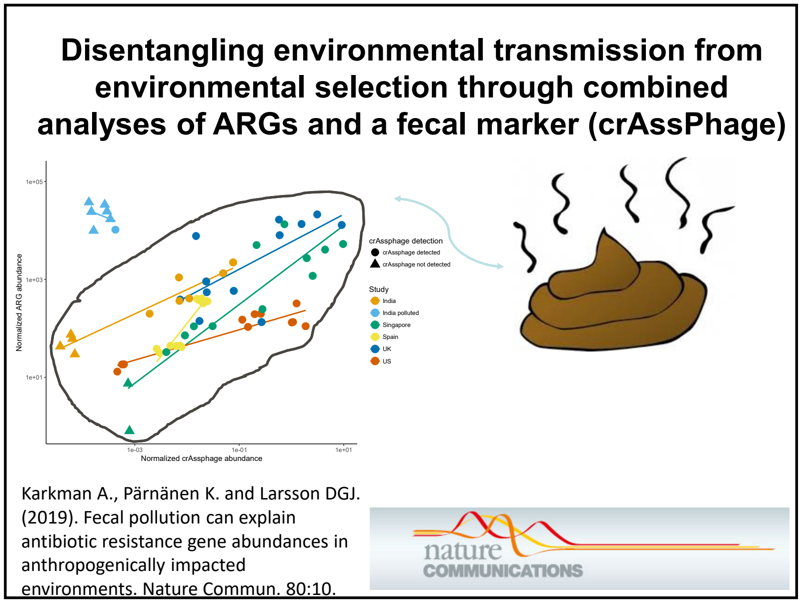


Environments polluted with waste from antibiotic manufacturing carry more antibiotic resistance genes than any other environment

N=864 metagenomes

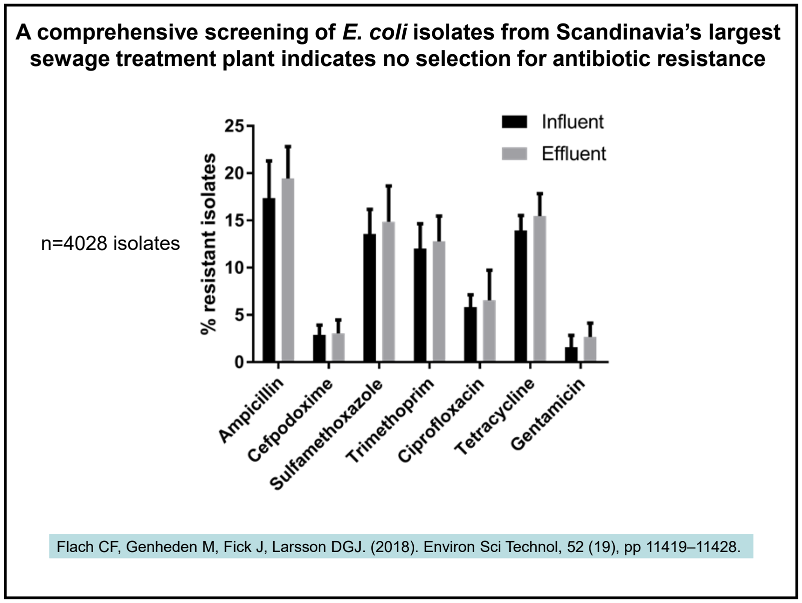
Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DGJ. (2016). The structure and diversity of human, animal and environmental resistomes. Microbiome. 4:54.

32



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

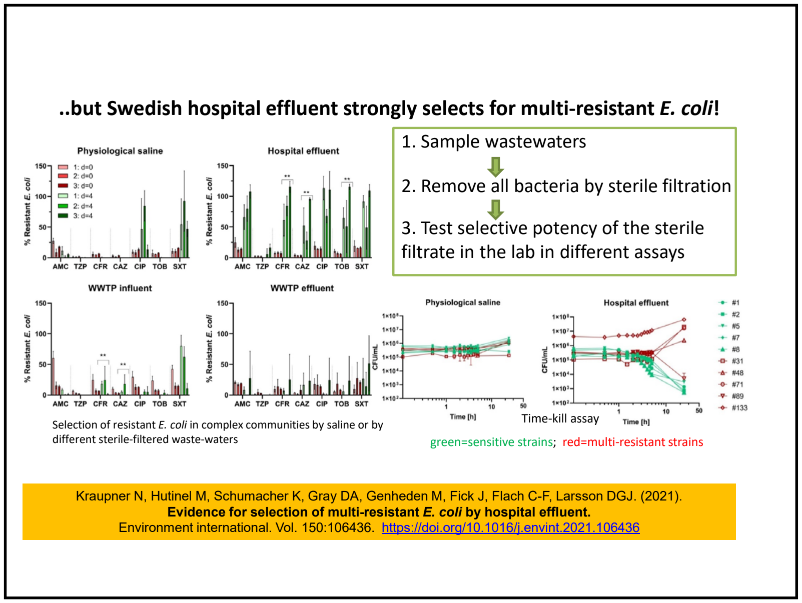
Karkman A., Pärnänen K. and Larsson DGJ. (2019). Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nature Commun. 80:10.



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*!

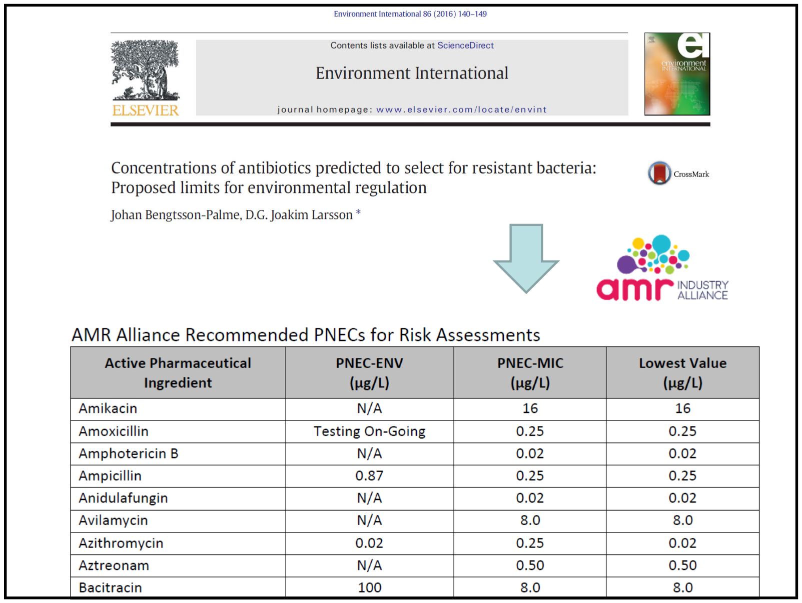
1. Sample wastewaters
2. Remove all bacteria by sterile filtration
3. Test selective potency of the sterile filtrate in the lab in different assays

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



Environment International 86 (2016) 140–149

Contents lists available at ScienceDirect

Environment International

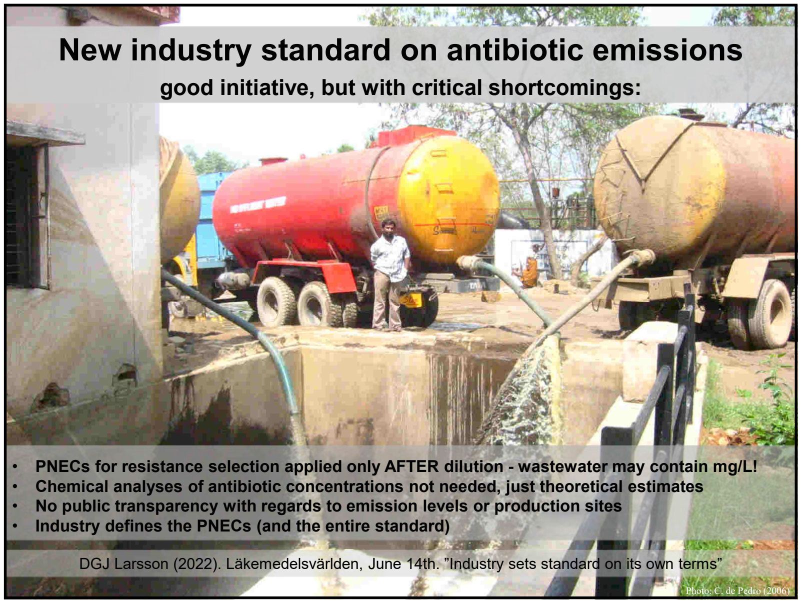
journal homepage: www.elsevier.com/locate/envint

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



New industry standard on antibiotic emissions

good initiative, but with critical shortcomings:

- **PNECs for resistance selection applied only AFTER dilution - wastewater may contain mg/L!**
- **Chemical analyses of antibiotic concentrations not needed, just theoretical estimates**
- **No public transparency with regards to emission levels or production sites**
- **Industry defines the PNECs (and the entire standard)**

DGJ Larsson (2022). Läkemedelsvärlden, June 14th. "Industry sets standard on its own terms"

Photo: C. de Pedro (2006)

3. Reflection of the regional resistance situation

Different objectives of environmental surveillance/monitoring/studies of AMR

Human and domestic animal health

- Transmission risks
- Evolution risks
- Resistance situation
- Use of antibiotics
- Ecological risks with antibiotic pollution (not AMR)

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

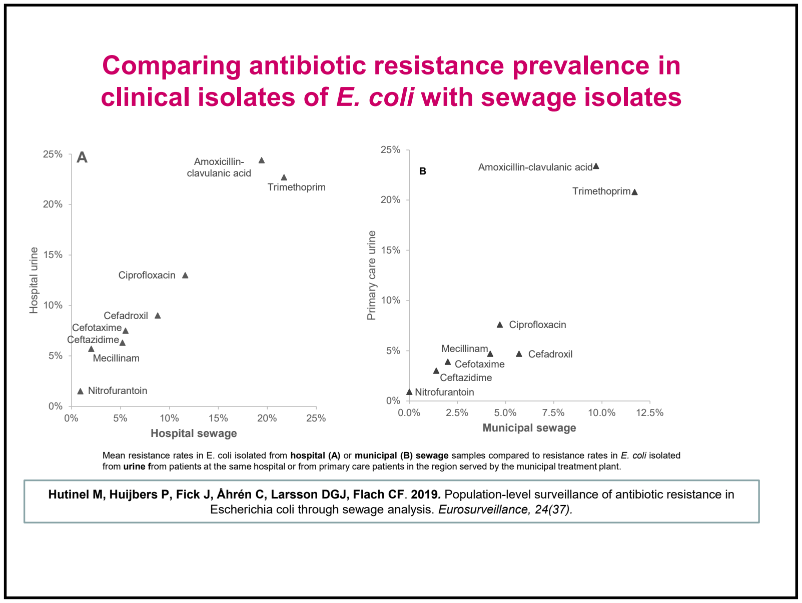
Larsson DGJ, Flach C-F. (2021). Antibiotic resistance in the environment. Nature Reviews Microbiology. DOI: 10.1038/s41579-021-00649-x

Untreated sewage -

contains pooled fecal bacteria from human populations

Sewage surveillance may:

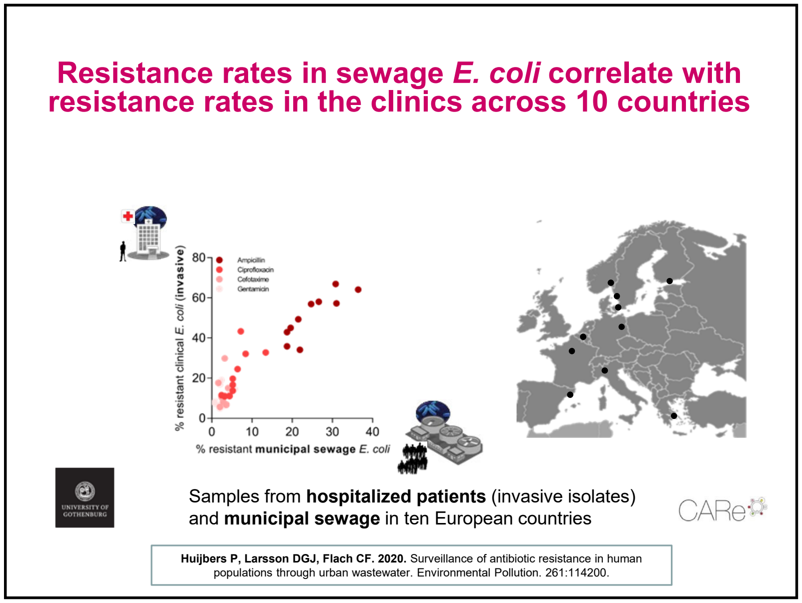
- Reveal trends
- Discover new threats
- Evaluate effects of interventions
- Guide empiric therapy



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.

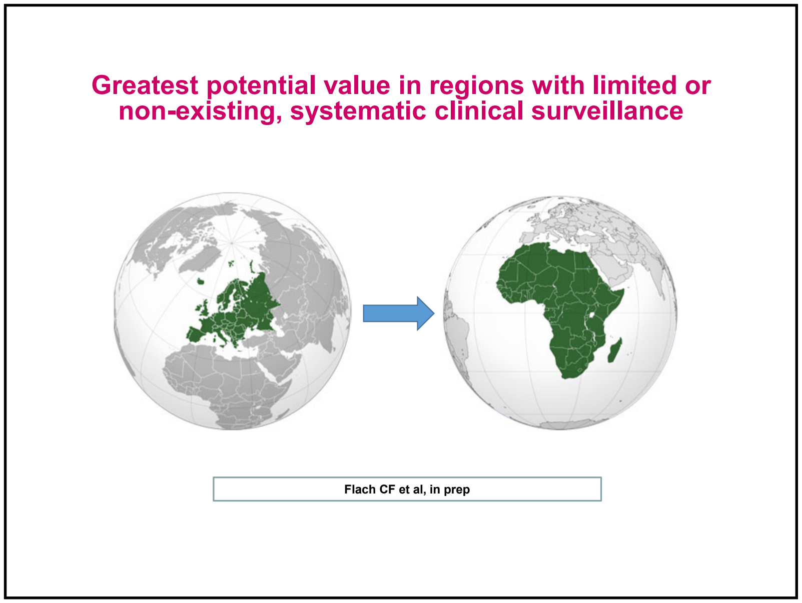
Hutinel M, Huijbers P, Fick J, Åhrén C, Larsson DGJ, Flach CF. **2019.** Population-level surveillance of antibiotic resistance in Escherichia coli through sewage analysis. *Eurosurveillance, 24(37).*



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

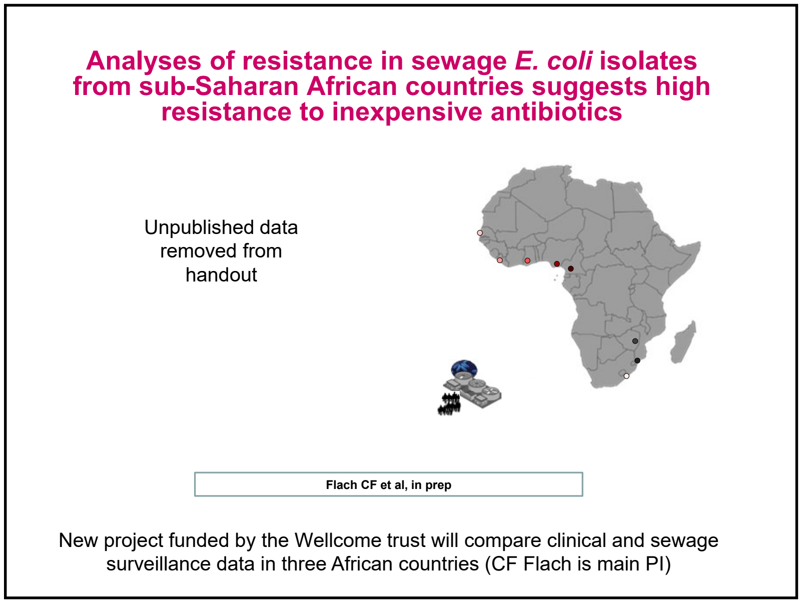
Samples from **hospitalized patients** (invasive isolates) and **municipal sewage** in ten European countries

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

Unpublished data removed from handout

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)

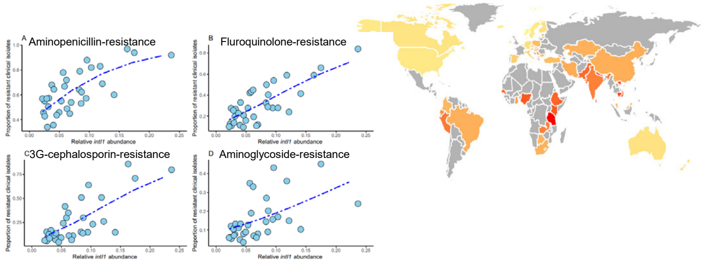


Fig. 2 *E. coli* clinical resistance models based on the *intI1* integrase gene. Proportion of resistant invasive *E. coli* clinical isolates to aminopenicillins **a**, fluoroquinolones **b**, third-generation cephalosporins **c**, and aminoglycosides **d** against *intI1* integrase gene abundance. The 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

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. (2022). 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. (2022). 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

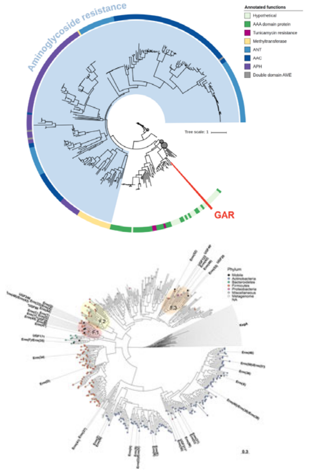
Water Research

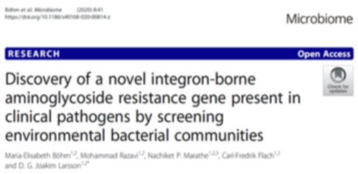
journal homepage: www.elsevier.com/locate/watres

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]

Using tailored functional metagenomics approaches, or predictive models, one can identify new resistance genes in pathogens (in sewage or other environments) that has escaped discovery in the clinic!




Böhm et al. Microbiome (2020) 8:41
https://doi.org/10.1186/s40168-020-00814-z

Microbiome

RESEARCH Open Access

Discovery of a novel integron-borne aminoglycoside resistance gene present in clinical pathogens by screening environmental bacterial communities

Maria-Elisabeth Böhm[1,2], Mohammad Razavi[1,2], Nachiket P. Marathe[1,2,3], Carl-Fredrik Flach[1,2] and D. G. Joakim Larsson[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[1,2,4*]

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

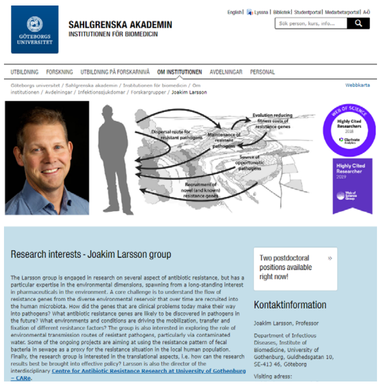

6TH INTERNATIONAL SYMPOSIUM ON THE ENVIRONMENTAL DIMENSION OF ANTIBIOTIC RESISTANCE – EDAR6

September 22– 27, 2022
WELCOME TO GOTHENBURG, SWEDEN

- ✓ Physical conference with online options
- ✓ Learn about the latest advances in the field
- ✓ Share your research through talks and posters
- ✓ Meet international colleagues and peers
- ✓ Enjoy Gothenburg and Sweden

Read more and register at:
https://www.gu.se/en/care/edar6-gothenburg-2022

Thanks for listening!



SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

Göteborgs universitet / Sahlgrenska akademin / Institutionen för biomedicin / Om institutionen / Avdelningar / Infektionssjukdomar / Forskargrupper / Joakim Larsson

Research interests - Joakim Larsson group

The Larsson group is engaged in research on several aspect of antibiotic resistance, but has a particular expertise in the environmental dimensions, spawning from a long-standing interest in pharmaceuticals in the environment. A core challenge is to understand the flow of resistance genes from the diverse environmental reservoir that over time are recruited into the human microbiota. How did the genes that are clinical problems today make their way into pathogens? What antibiotic resistance genes are likely to be discovered in pathogens in the future? What environments and conditions are driving the mobilization, transfer and fixation of different resistance factors? The group is also interested in exploring the role of environmental transmission routes of resistant pathogens, particularly via contaminated water. Some of the ongoing projects are aiming at using the resistance pattern of fecal bacteria in sewage as a proxy for the resistance situation in the local human population. Finally, the research group is interested in the translational aspects, i.e. how can the research results best be brought into effective policy? Larsson is also the director of the interdisciplinary Centre for Antibiotic Resistance Research at University of Gothenburg – CARe.

Two postdoctoral positions available right now!

Kontaktinformation

Joakim Larsson, Professor

Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, Guldhedsgatan 10, SE-413 46, Göteborg

Visiting adress:

CENTRE FOR ANTIBIOTIC RESISTANCE RESEARCH, CARe

University of Gothenburg / Centre for Antibiotic Resistance Research, CARe

Centre for Antibiotic Resistance Research, CARe

CARe - Centre for Antibiotic Resistance Research at University of Gothenburg – has a vision is to limit mortality, morbidity and socioeconomic costs related to antibiotic resistance on a global scale through research. CARe offers diverse expertise representing six faculties and a broad network of stakeholders within the health care sector and beyond to generate state-of-the-art science with the intention to support rapid revision of policies and their implementation.

A global challenge

"This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country"

"Without urgent, coordinated action by many stakeholders, the world is headed for a post-antibiotic era, in which common infections and minor injuries which have been treatable for decades can once again kill"

(World Health Organization 2014 regarding the global challenges with antibiotic resistance)

CARe Twitter News

Tweets by @CARe_GU

CARe @CARe_GU
Two postdoctoral positions in molecular microbiology and antibiotic resistance available within the research group led by Professor Joakim Larsson. The deadline for applying is the 10th of January, 2020. gu.se/english/about_...

Postdoctora...
gu.se

Vetenskapsrådet
VÄSTRA GÖTALANDSREGIONEN
jpiamr
formas
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