Selection of antibiotic resistance in the environment

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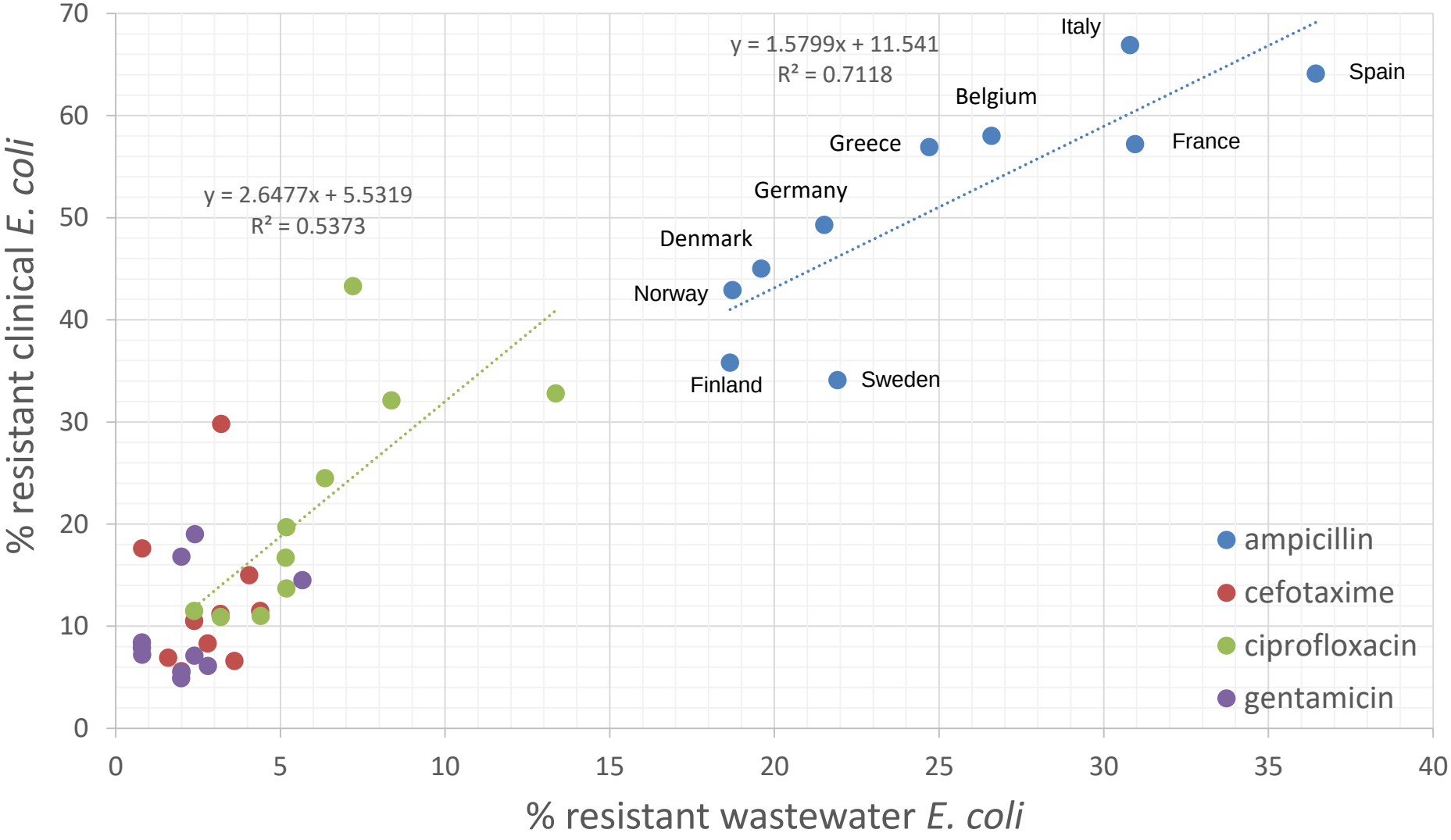
CARe website: www.care.gu.se

Picture by M Kumar, Associated Press

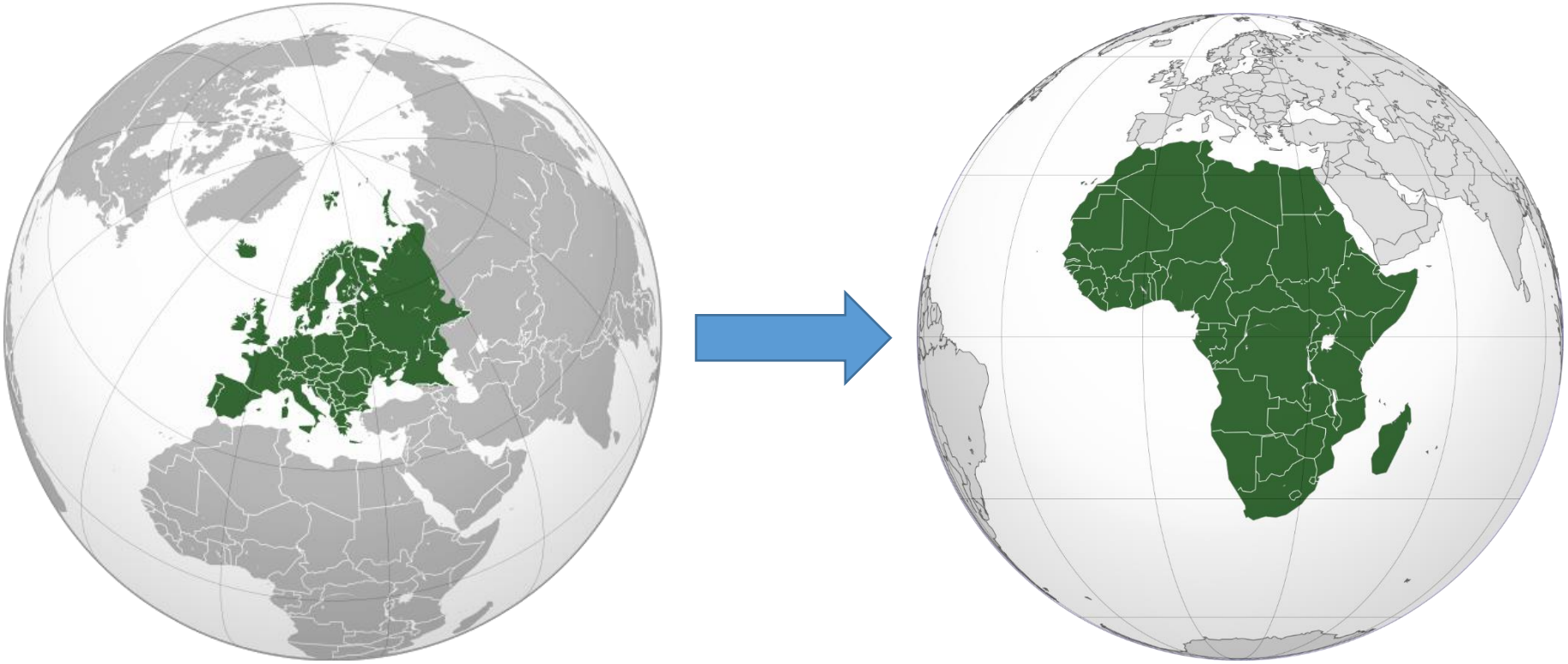
Three reasons to consider the environment

- **Possible indicator of the regional, clinical resistance situation**

Preliminary calibration of resistance in *E.coli* from untreated sewage in relation to reported resistance of clinical blood-stream infections in 10 European countries



Huijbers P, Larsson DGJ, Flach CF. In prep.



Flach CF. In prep.

Three reasons to consider the environment

- **Possible indicator of the regional, clinical resistance situation**

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

Environmental transmission of pathogens

Photo: Reuters/D Boylan

Environmental Science & Technology

Viewpoint

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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⊥

Three reasons to consider the environment

- **Possible indicator of the regional, clinical resistance situation**
- **Transmission route** for certain resistant bacteria (human/animal → environment → human/animal)
- **Evolutionary ”arena”** for the emergence of new forms of resistance (favoured by a selection pressure from antibiotics)

Antibiotic resistance in the environment is ancient

Nature (2011) **477**:457-61
Slide courtesy: Gerry Wright

Resistance i harmless bacteria

Resistance in pathogens

Could be a rare or one-time event - anywhere

”……we argue that risks are greatest in those cases in which the mobilized resistance gene has not yet been detected in pathogenic bacteria..”

CORRESPONDENCE

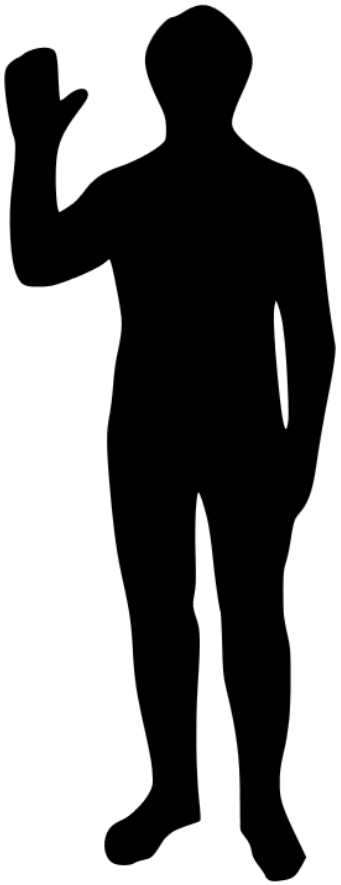
Online 27th April 2015

NATURE REVIEWS | MICROBIOLOGY

Antibiotic resistance genes in the environment: prioritizing risks

Johan Bengtsson-Palme and D. G. Joakim Larsson

Where do these critical mobilization and transfer events occur?



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*]

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*]

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,*]

Wide-spread but ”low” (often ng/L) environmental levels of antibiotics from animal and human feces and urine

OPEN ACCESS Freely available online PLoS PATHOGENS

Selection of Resistant Bacteria at Very Low Antibiotic Concentrations

Erik Gullberg[1¶], Sha Cao[1¶], Otto G. Berg[2], Carolina Ilbäck[1], Linus Sandegren[1], Diarmaid Hughes[1], Dan I. Andersson[1*]

1 Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, **2** Department of Molecular Evolution, Uppsala University, Uppsala, Sweden

Abstract

The widespread use of antibiotics is selecting for a variety of resistance mechanisms that seriously challenge our ability to treat bacterial infections. Resistant bacteria can be selected at the high concentrations of antibiotics used therapeutically, but what role the much lower antibiotic concentrations present in many environments plays in selection remains largely unclear. Here we show using highly sensitive competition experiments that selection of resistant bacteria occurs at extremely low antibiotic concentrations. Thus, for three clinically important antibiotics, drug concentrations up to several hundred-fold below the minimal inhibitory concentration of susceptible bacteria could enrich for resistant bacteria, even when present at a very low initial fraction. We also show that *de novo* mutants can be selected at sub-MIC concentrations of antibiotics, and we provide a mathematical model predicting how rapidly such mutants would take over in a susceptible population. These results add another dimension to the evolution of resistance and suggest that the low antibiotic concentrations found in many natural environments are important for enrichment and maintenance of resistance in bacterial populations.

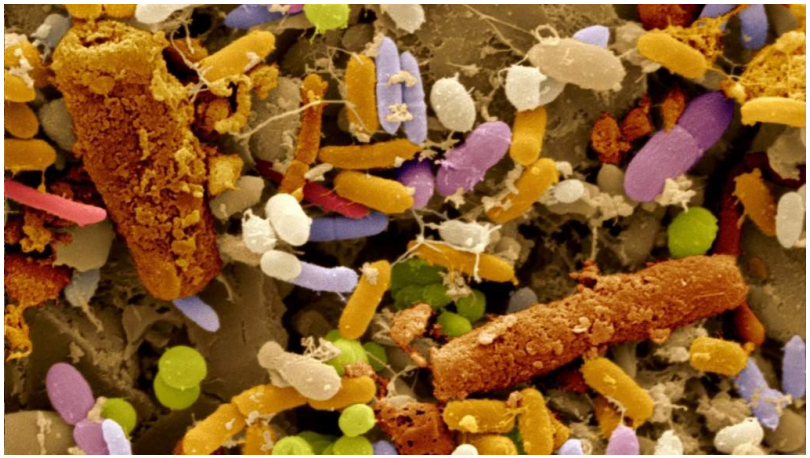
Citation: Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, et al. (2011) Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. PLoS Pathog 7(7): e1002158. doi:10.1371/journal.ppat.1002158

The most critical form of selection:

Within-species selection of acquired resistance

Single-species competition situation

Competition in complex, environmental communities



Endpoints that could indicate a selection pressure favouring bacterial strains with aquired resistance

- Many ”novel” ARGs found in a mobile context
- Changes in taxonomy
- Proportion of resistant bacteria
- Proportion of resistant bacteria within a species
- Relative abundance of resistance genes

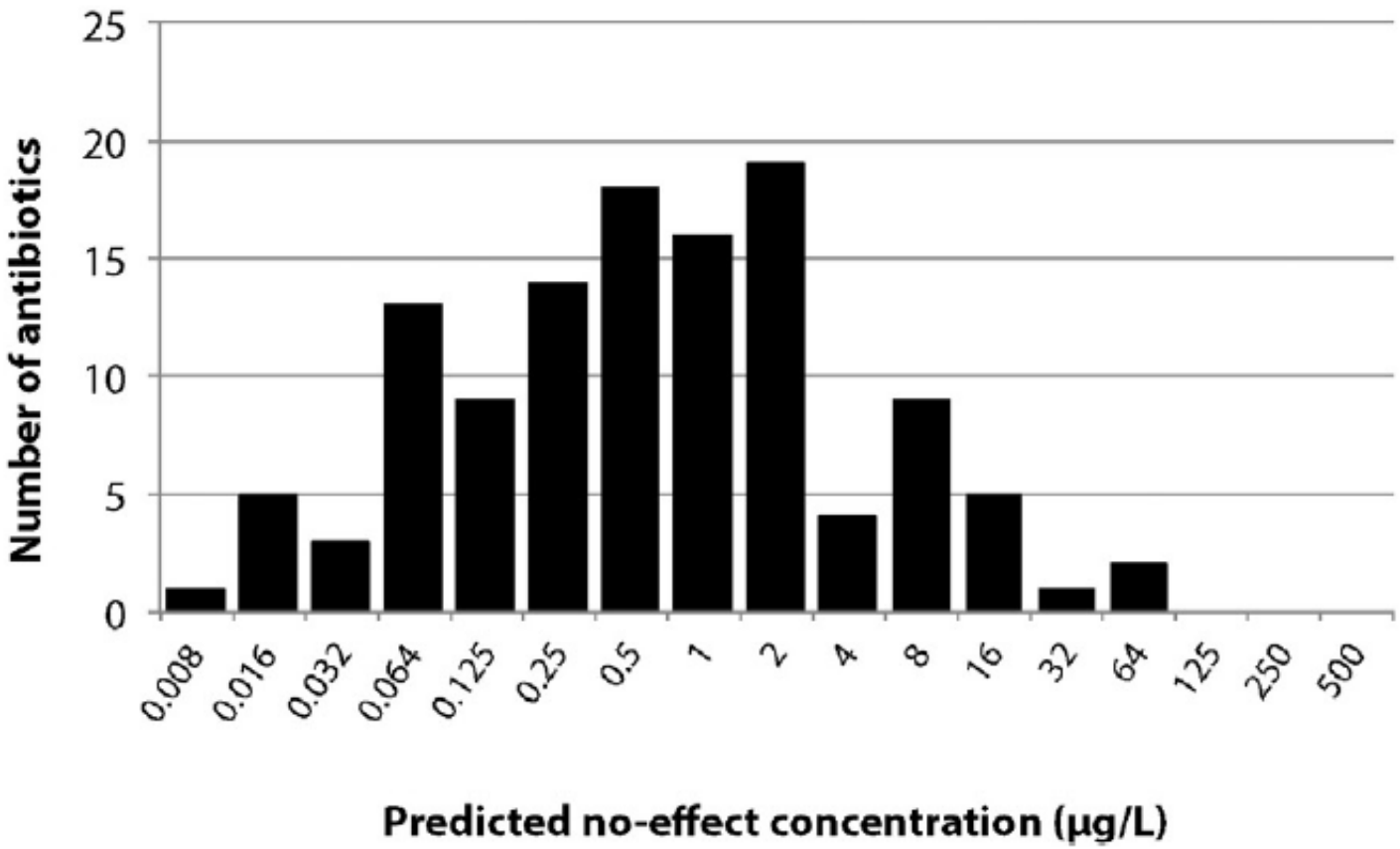
Correlations between antibiotics and resistance?

Very limited evidence for on site selection,
as they often have a common source

Is there evidence for selection of antibiotic resistance in sewage treatment plants?

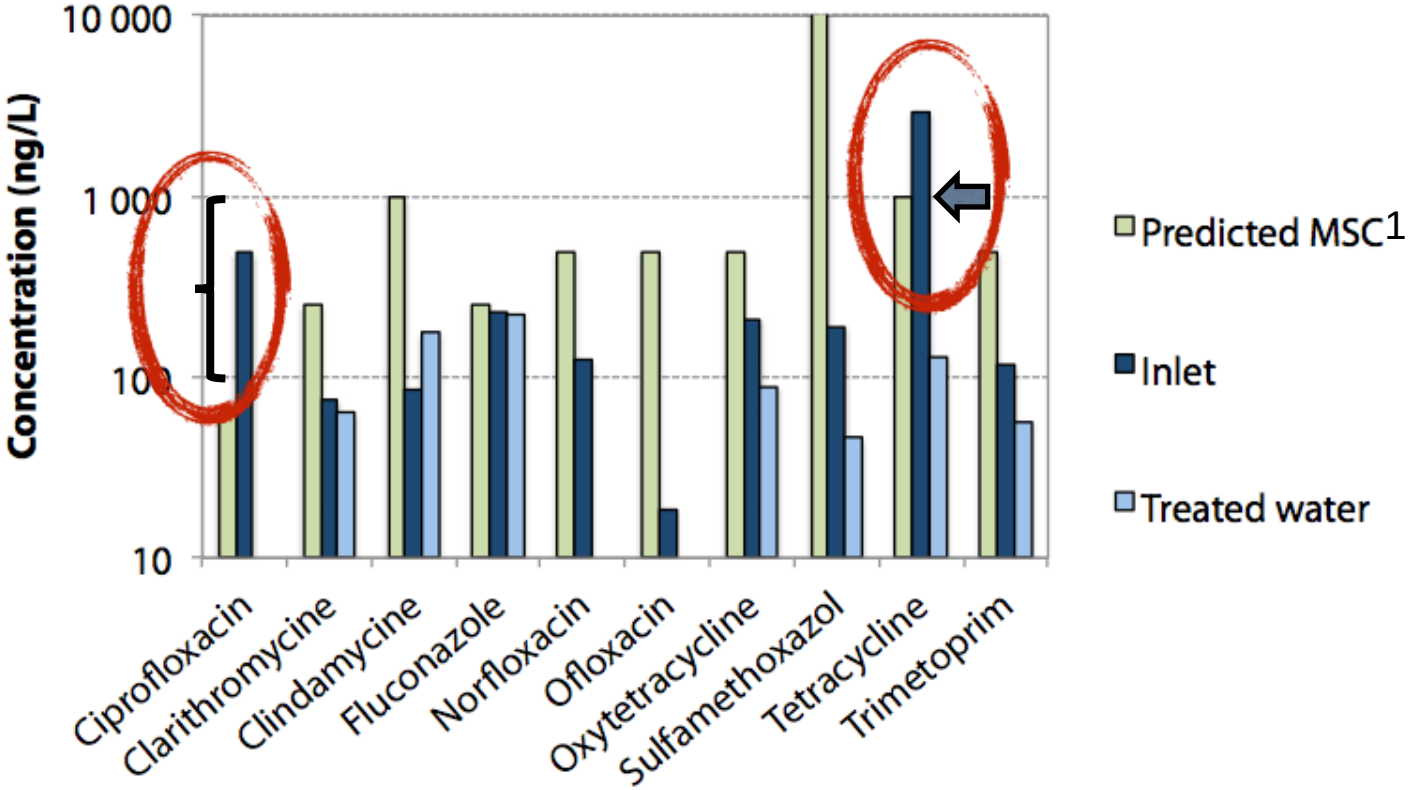
Predicted non-selective concentrations for 111 antibiotics

A concentration that completely inhibits growth (of certain strains) should also be able to select for resistance (at least under some circumstances)!



Bengtsson-Palme J, Larsson DGJ. (2016). Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International. 86:140-149.

Antibiotic concentrations in three Swedish STPs vs predicted Minimal Selective Concentrations



Lundström SV, Östman M, Bengtsson-Palme J, Rutgersson C, Thoudal M, Sircar T, Blanck H, Eriksson KM, Tysklind M, Flach CF, Larsson DGJ. (2016) Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. Sci Total Environ. 553:58

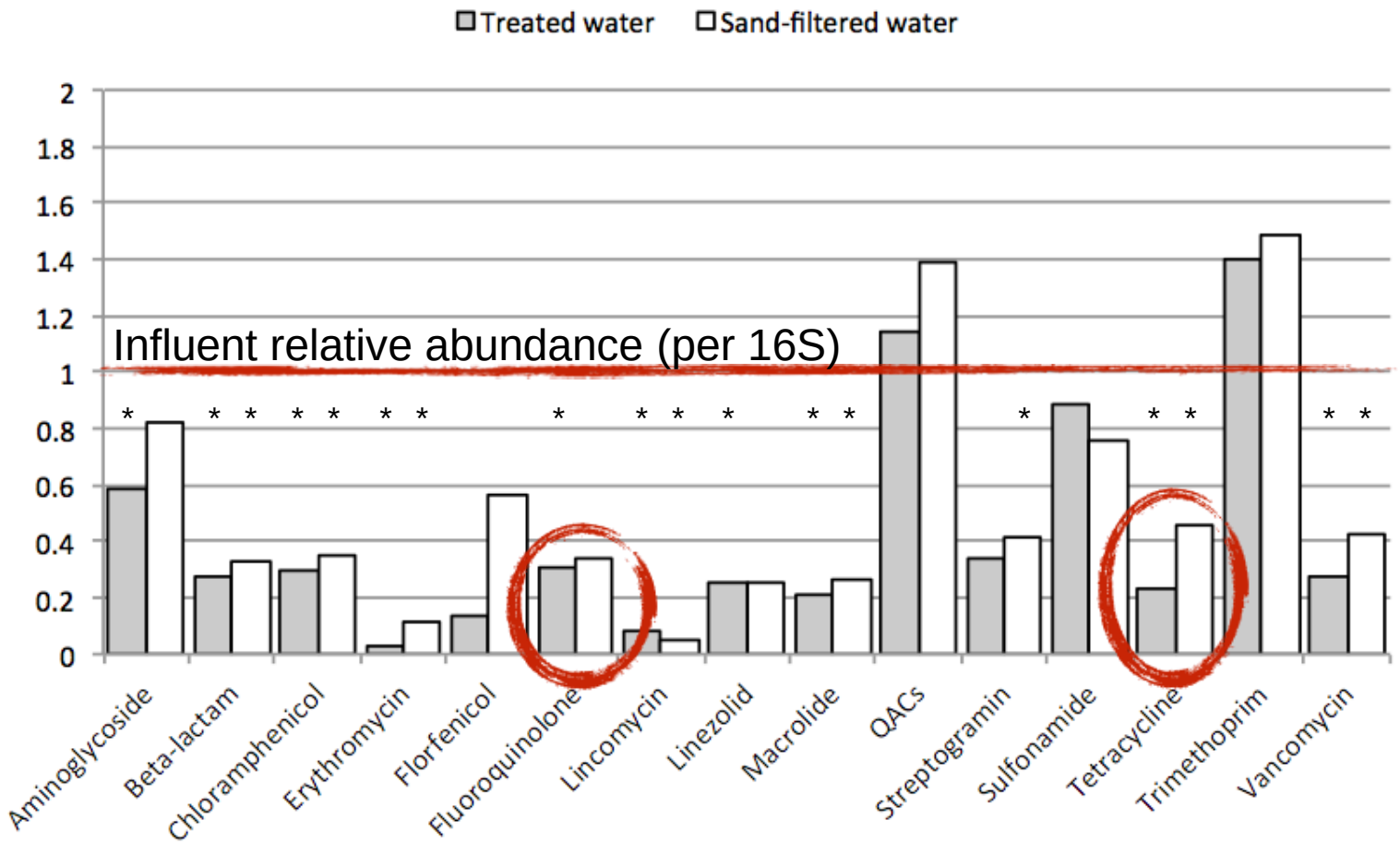
Kraupner N, Ebmayer S, Bengtsson-Palme J, Flach C-F, Larsson DGJ. Minimal selective concentration of ciprofloxacin for *E. coli* grown in complex aquatic bacterial biofilms. Revised version submitted.

Bengtsson-Palme et al (2016). Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Science of the Total Environment. 572:697–712

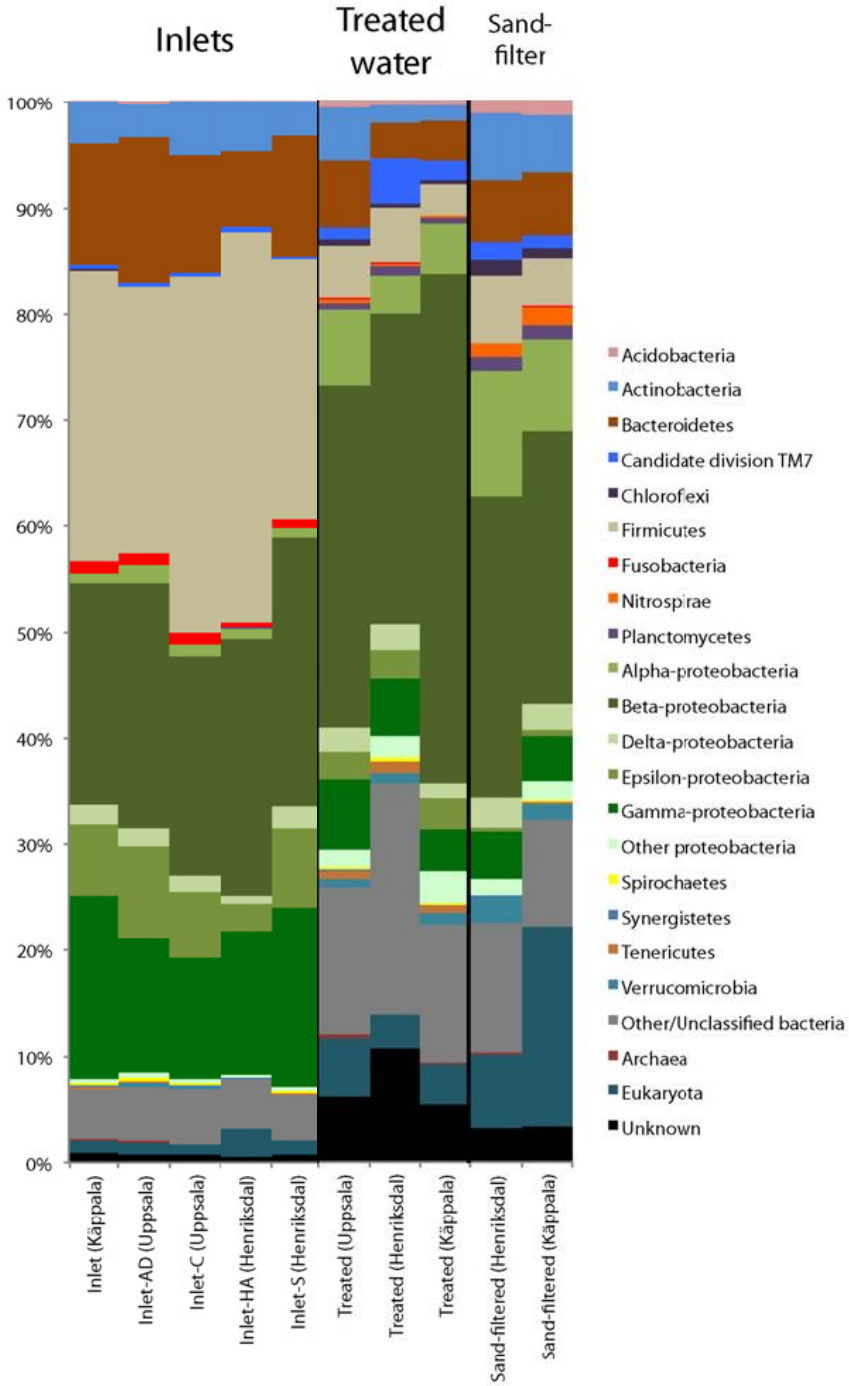
[1] Bengtsson-Palme J, Larsson DGJ. (2015). Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International. 86:140-149.

By comparing MSCs to measured concentrations of antibiotics we find some, but limited support for selection

Decreased relative resistance gene abundance after treatment



Bengtsson-Palme et al (2016). Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Science of the Total Environment. 572:697–712



Major taxonomic changes between influents and effluents

-

changes in relative resistance gene abundances expected as a consequence

ARG analyses provide no support for within species selection of antibiotic resistance in Swedish STPs

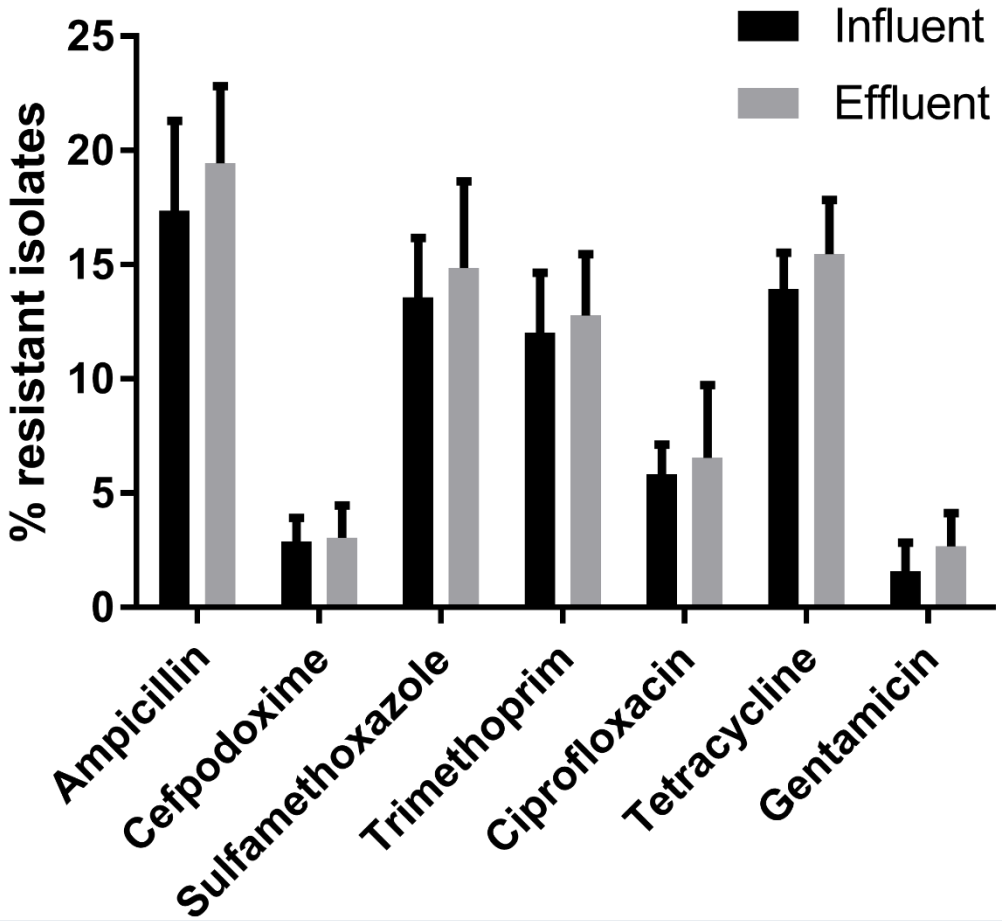
–

but this is by no means conclusive as results are confounded by taxonomic changes

Studying within-species selection of E. coli in Ryaverket in Gothenburg, Sweden - the largest STP in Scandinavia

Results from eight sampling campaigns 2015-2017 provide NO support for selection of resistant *E.coli*

n=4028 isolates



Flach C-F, Genheden M, Larsson DGJ. A comprehensive screening of E. coli from Scandinavia's largest wastewater treatment plant shows no selection of antibiotic resistance. In prep.

Reasons why sewage treatment plants could be ”hotspots” for the evolution of resistance in pathogens

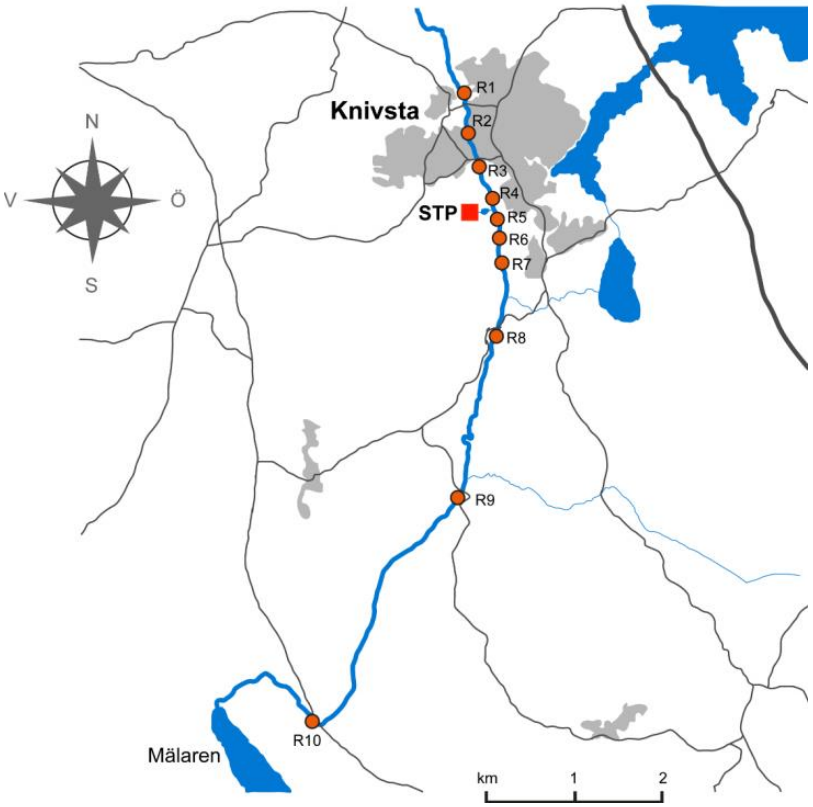
- Lots of pathogens
- High diversity of potential donors
- High bacterial density
- A selection pressure for antibiotic resistance

Reasons why sewage treatment plants could be ”hotspots” for the evolution of resistance in pathogens

- Lots of pathogens
- High diversity of potential donors
- High bacterial density
- (A selection pressure for antibiotic resistance)

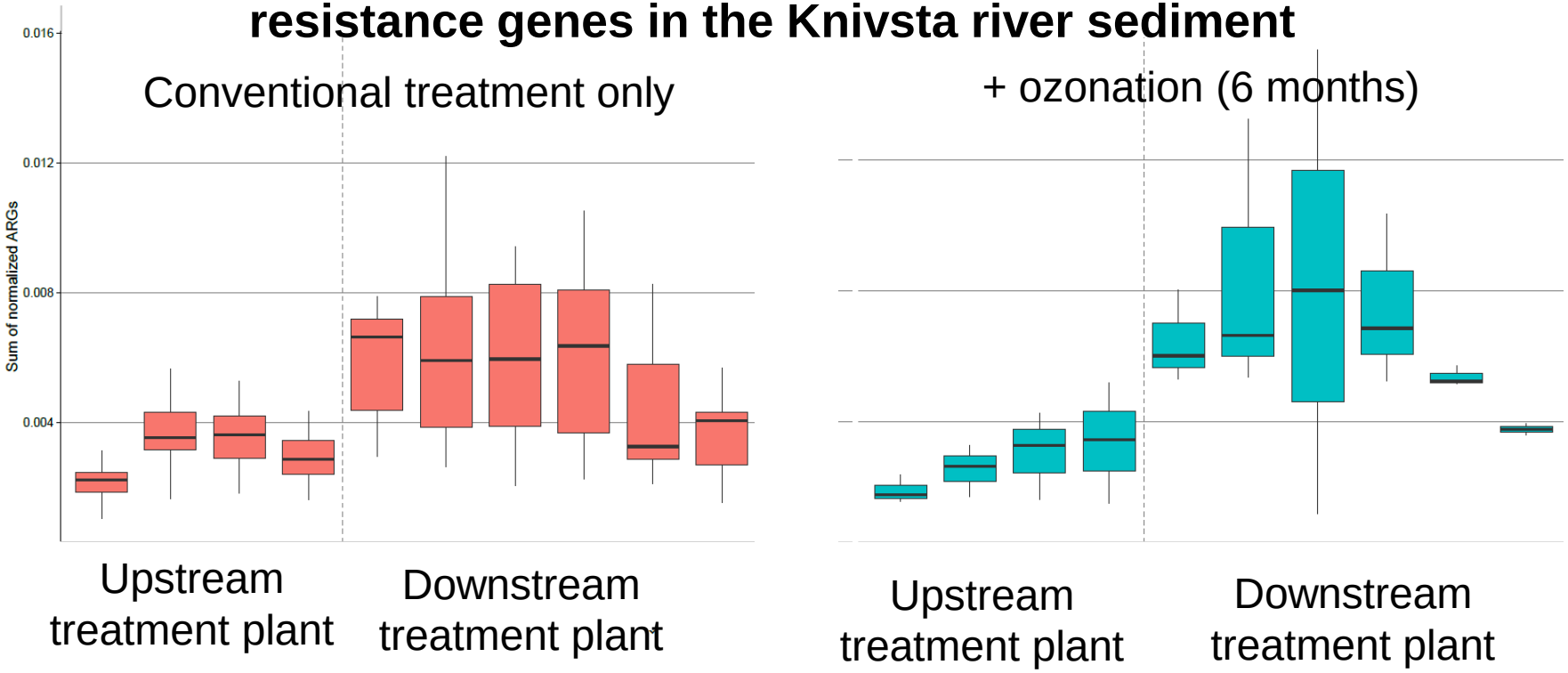
Full-scale field experiment:

Removing antibiotics the antibiotics by ozonation – what happens with resistance in the river?



Karkmann, Rutgersson et al, in prep

Effects of full-scale ozonation on Antibiotic resistance genes in the Knivsta river sediment



The ozonation reduced antibiotics in effluents and downstream sediment to non-detectable levels!

…but the presence of resistance genes in downstream sediment was not affected!

The concentrations of antibiotics present in effluent during conventional treatment did not seem to matter for the downstream presence of resistance genes!

Karkmann, Rutgersson et al, in prep

Antibiotic production sites and surrounding environments

Larsson DGJ, de Pedro C, Paxeus N. (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater. 148:751

Larsson DGJ. (2014) Pollution from drug manufacturing: review and perspectives. Philosophical Transactions of the Royal Society B, 369: 20130571.

Photo: C. de Pedro (2006)

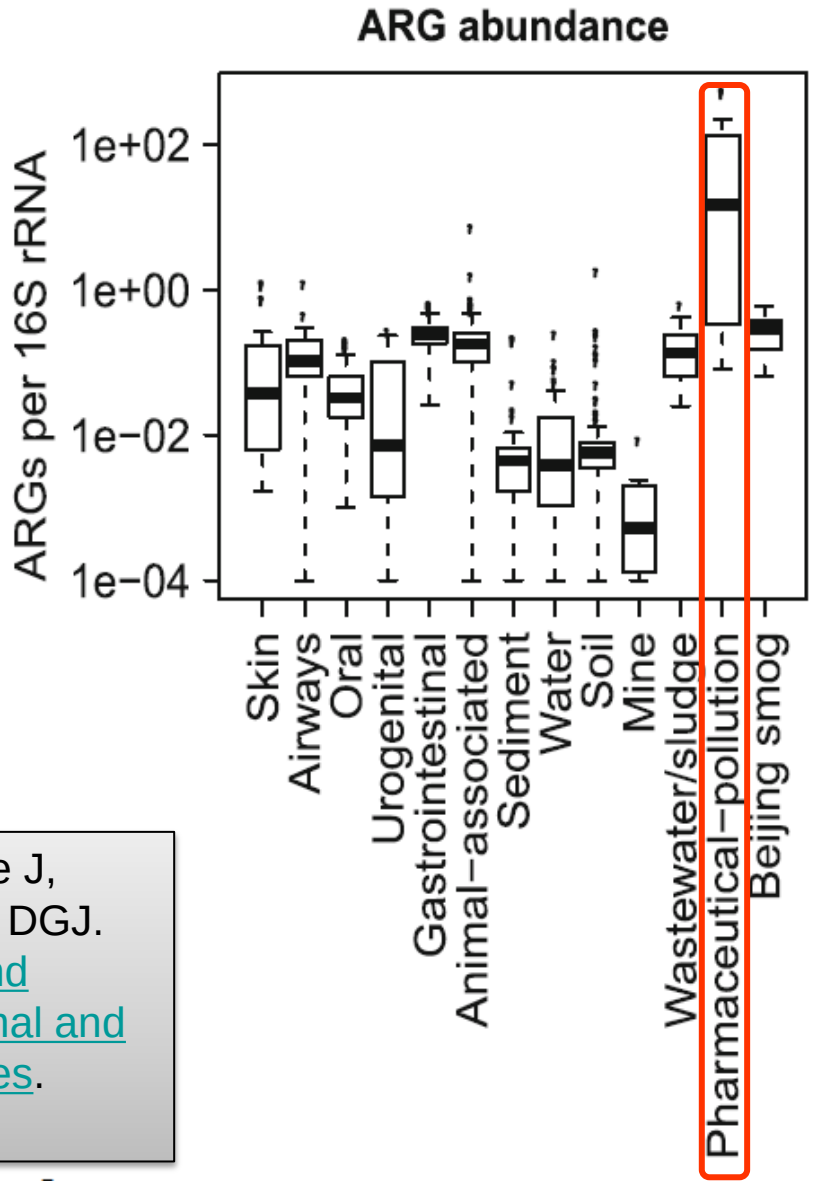
30 mg/L ciprofloxacin

30 ng/L ciprofloxacin

Surface, ground and drinking water highly contaminated with antibiotics and other drugs

J Fick, H Söderström, RH Lindberg, Chau DNP, M Tysklind, DGJ Larsson 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. Environmental Toxicology & Chemistry 28:2522–2527

No other environment carry as many antibiotic resistance genes



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.

Bacteria from these environments are very efficient in transferring novel resistance plasmids to E. coli

Flach CF, Johnning A, Nilsson I, Smalla K, Kristiansson E, Larsson DGJ. (2015) Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. Journal of Antimicrobial Chemotherapy 70:2709-2717.

Picture by M Kumar, Associated Press

Larsson DGJ. (2014) Pollution from drug manufacturing: review and perspectives. Philos Trans R Soc Lond B Biol Sci 369:2013057

Transmission is global - resistance is everyones concern

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.

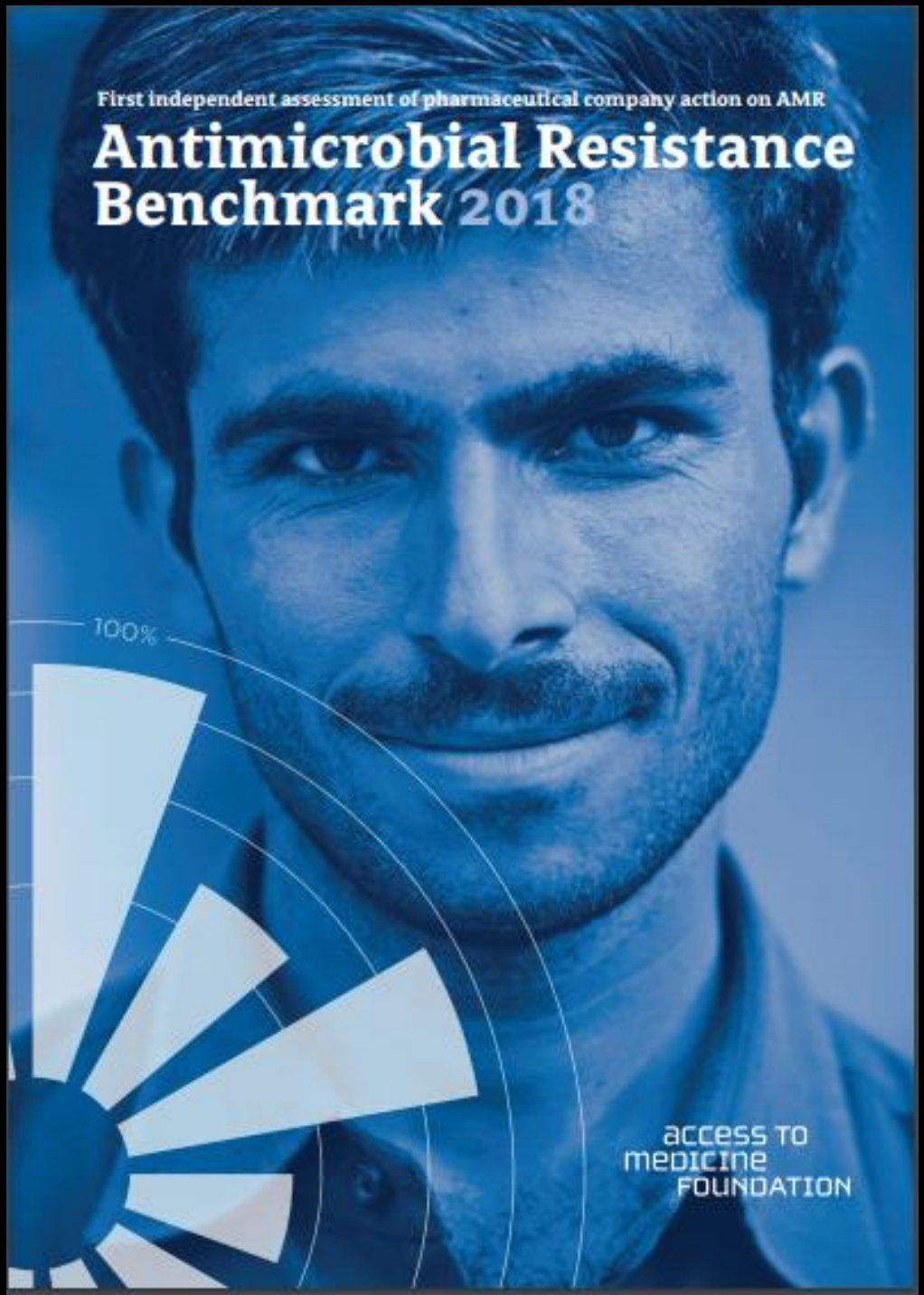
These factories produce the drugs we all use!

***Bengtsson-Palme J, Gunnarsson L, Larsson DGJ**. 2018. Can branding and price of pharmaceuticals guide informed choices towards improved pollution control during manufacturing?*
Journal of Cleaner Production. 171:137-146.

***Larsson DGJ and Fick J.** 2009. Transparency throughout the production chain – a way to reduce pollution from the manufacturing of* pharmaceuticals*. Regulatory Toxicology and Pharmacology. 53:161-163.*

First independent assessment of pharmaceutical company action on AMR

Antimicrobial Resistance Benchmark 2018



access to medicine FOUNDATION

Review on Antimicrobial Resistance
Tackling drug-resistant infections globally

We need minimum standards to improve waste management in antimicrobial production

We need to improve standards of waste management to avoid scenarios where very high concentrations of antibiotics or APIs are released into the environment. There are different ways that this might be achieved. Our preferred route would be to have a minimum regulatory standard. However, while this is established, we believe there is a case for other participants in the supply chain to act now, improving transparency and standards for how antibiotic waste is treated.

A good starting point for such standards might be a recent study[65], which proposed maximum limits for concentrations of common antibiotics in water.

65. Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environment International*,2015,86:140-149, doi:10.1016/j.envint.2015.10.015.

National Action Plan on Antimicrobial Resistance (NAP-AMR) 2017 – 2021

© *Government of India, April 2017*

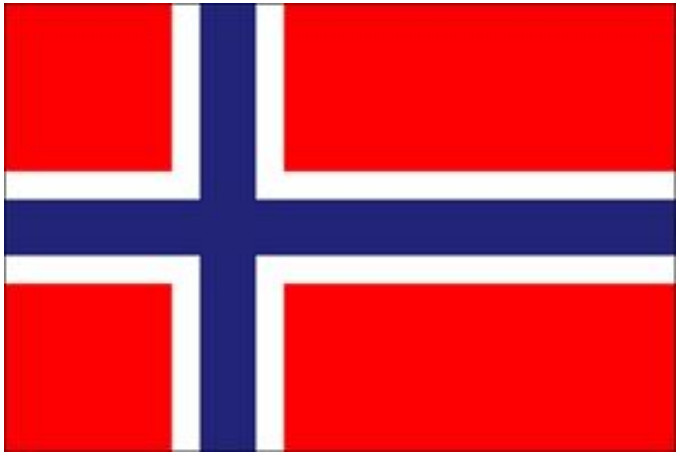
2.3.4.4. Develop standards for antibiotic residues in industrial effluents and waste from farms, human health care and veterinary care settings (**CPCB**, MoEFCC) **M**

- Define standards and monitor antibiotic residues and bacterial load in effluents (**S**-**M**-**L**); disinfection at treatment plant to remove bacteria (**S**)

b. Surveillance of antimicrobial resistance – in human, animal/food and environment sectors – for evidence-informed policy-making

…BUT India is the only country so far that has declared their intent of setting national discharge limits

Creating incentives for pollution control during procurement of drugs



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Removing counter-incentives

In many countries, reimbursment of costs for medicines (from insurance companies or tax money) is only given if the very cheapest interchangable product is selected

This provides counter-incentives for manufacturers to invest in efficient waste water treatment

Ersättning vid läkemedelsskador
och miljöhänsyn i läkemedelsförmånerna

STATENS OFFENTLIGA UTREDNINGAR
SOU 2013:23

What could be appropriate actions?

Support better fecal management/sewage infrastructure, particularly in low-resource settings

Encourage research on risks associated with exposure to “low” levels of antibiotics

Promote antimicrobial pollution standards nationally and globally

Encourage transparency throughout the production chains of antibiotics

Recognize the risks of only premiering lowest price in generic substitution systems

Intensify work on including environmental criteria in procurement of drugs

Thank you for listening!

UNIVERSITY OF GOTHENBURG

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