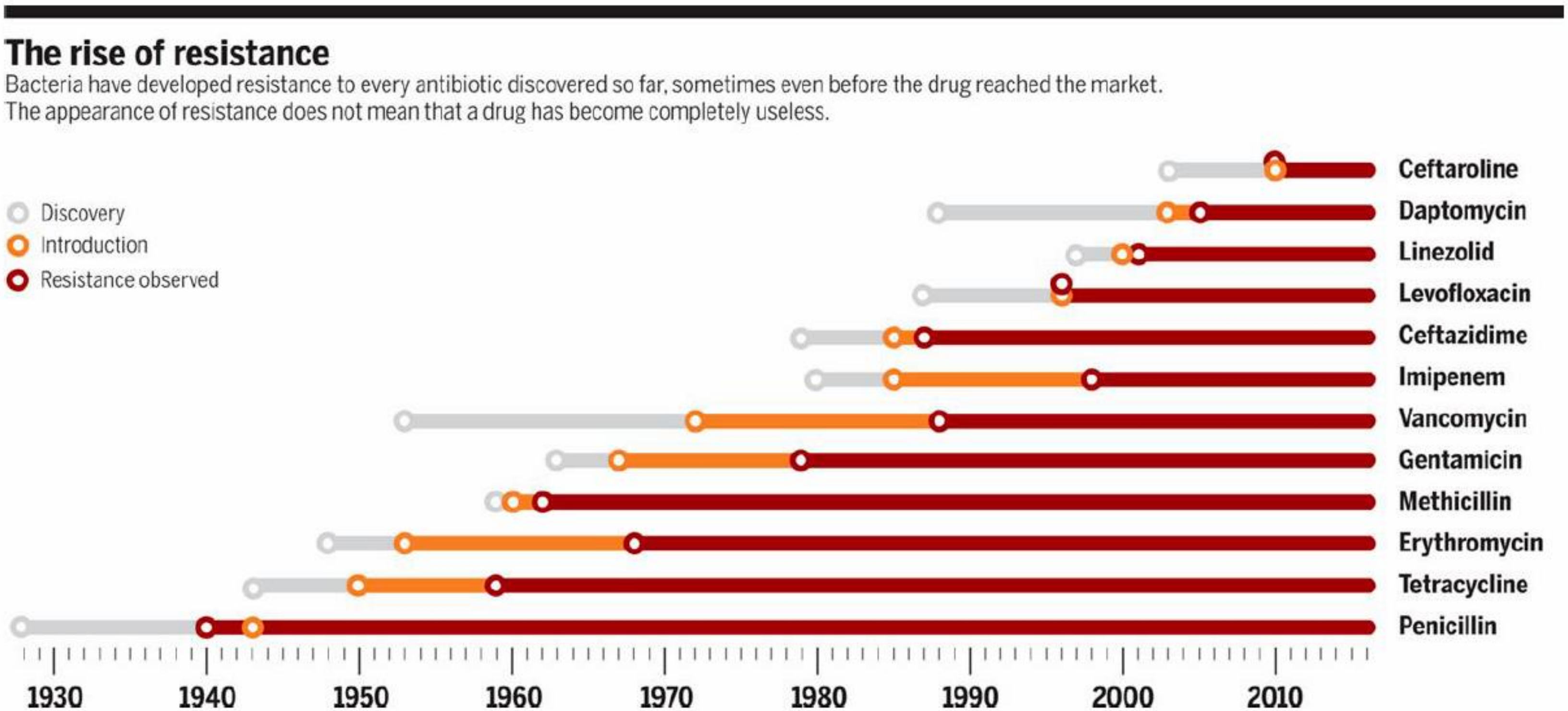
# Wastewater treatment plant effluents and their implications for antimicrobial resistance in surface water and water reuse

Thomas Berendonk

# The rise of resistance




Kai Kupferschmidt Science 2016;352:758-761


World Health Organization

dia centre | Publications | Countries | Programmes | Governance | About WHO

## Media centre

### High levels of antibiotic resistance found worldwide, new data shows

News release

29 JANUARY 2018 | BANGKOK - WHO's first release of surveillance data on

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## Pharmaceuticals (Antibiotics) and antibiotic resistance genes are emerging contaminants

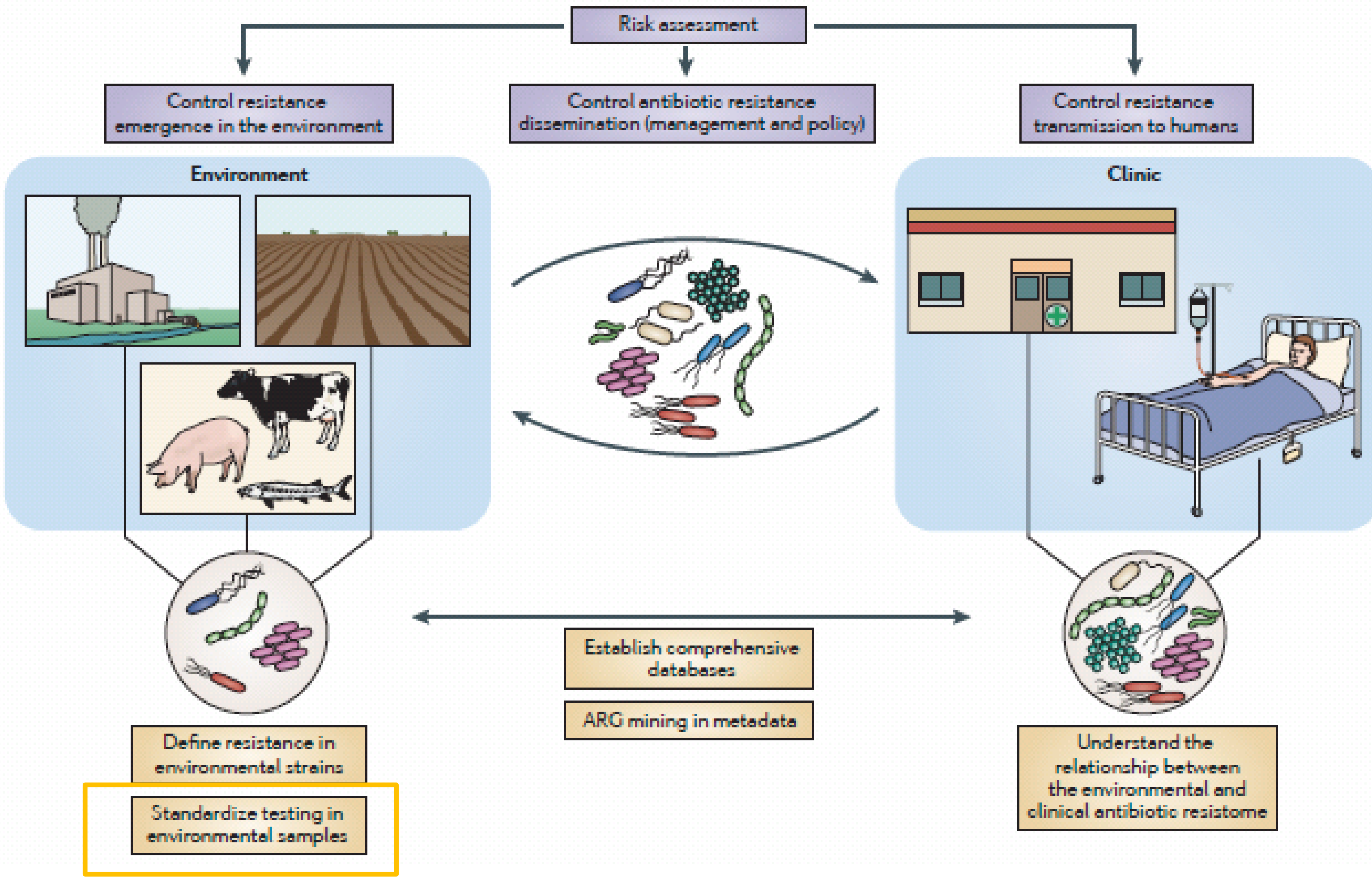
**Chemical contaminants**

Parent compounds and physicochemical and biological transformation products originated during treatment and/or in the environment

**Biological contaminants**

Antibiotic resistance genes associated with mobile genetic elements and/or antibiotic resistant bacteria (ARG/ARB)

# Antibiotic resistance and the environment




Berendonk et al.
Nature Reviews Microbiology 13 2015

# Overview

WG 2 Uptake and translocation of organic microcontaminants and antibiotic resistant bacteria and their genes in crops

Understanding the fate of antibiotic resistant bacteria within urban WW, soil, ground/surface water, and crops.

# **Primary Objective** of NEREUS



A **multi-disciplinary network** to determine which of the current challenges related to wastewater reuse are the most concerning ones in relation to public health and environmental protection, and how these can be overcome.

# 380 members 43 countries

## COST Countries

- Austria
- Belgium
- Bosnia & Herzegovina
- Bulgaria
- Croatia
- Cyprus
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Greece
- Ireland
- Israel
- Italy
- Lithuania
- Luxembourg
- Malta
- Montenegro
- Netherlands
- Norway
- Poland
- Portugal
- Romania
- Serbia
- Slovakia
- Slovenia
- Spain
- Sweden
- Switzerland
- Turkey
- United Kingdom

## International Partner Countries

- Australia
- Pakistan
- Singapore
- South Korea
- United States of America
- Mexico

## Near Neighbor Countries

- Georgia
- Jordan
- Tunisia
- Ukraine

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# Action's Working Groups (WG)

| Working Group | | Title | Leader / Vice Leader |
|---|---|---|---|
|  | WG1 | Microbiome and mobile antibiotic resistome in treated WW and in downstream environments | Eddie Cytryn<br>Thomas Berendonk<br>Christophe Merlin |
|  | WG2 | Uptake and translocation of organic microcontaminants and ARB&ARGs in crops | Josep Maria Bayona<br>Benny Chefetz |
|  | WG3 | Effect-based bioassays required for WW reuse schemes | Jaroslav Slobodnik<br>Norbert Kreuzinger |
|  | WG4 | Technologies efficient/economically viable to meet the current WW reuse challenges | Luigi Rizzo<br>Sixto Malato |
|  | WG5 | Risk assessment and policy development | Lian Lundy<br>Mario Carere |

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# NEREUS Working Group 2

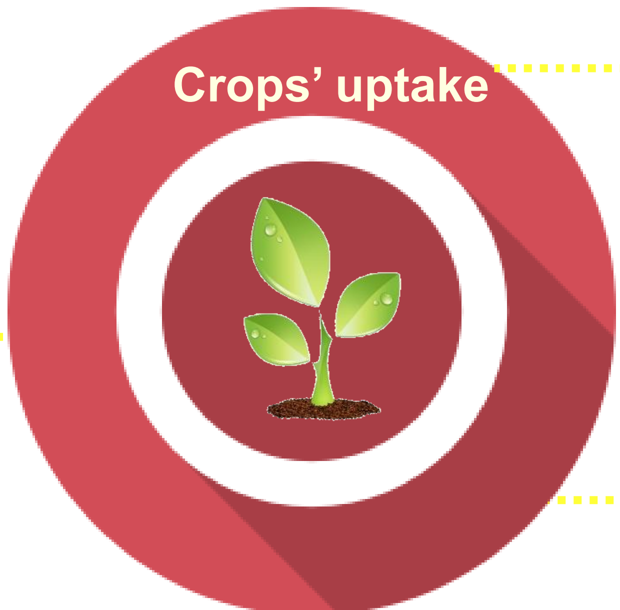
## Uptake and translocation of organic microcontaminants and ARB&ARGs in crops

### Objectives

- Consolidate knowledge on the uptake and translocation of microcontaminants and ARB&ARGs in crops
- Identify the main physicochemical characteristics affecting the uptake and translocation of microcontaminants and ARB&ARGs
- Develop a set of recommendations regarding the minimisation of biomagnification processes and environmental and human health impacts associated with wastewater reuse

# Crops’ uptake - pharmaceuticals

The uptake of pharmaceuticals by crops may be influenced by a variety of factors, both **biotic and abiotic.**




**Plant characteristics & Physiology**

e.g. root system, shape and size of leaves, lipid content

**Physicochemical properties of organic compounds**

e.g. water solubility, vapour pressure, molecular weight, octanol-water partition coefficient, etc.

**Environmental characteristics & Growth conditions**

e.g. temperature, soil type, water content in soil, agricultural practices

# Crops’ uptake - pharmaceuticals

- Experimental results revealed that the potential for CEC uptake by crop plants decreased in the order of **leafy vegetables > root vegetables > cereals and fodder crops > fruit vegetables**

**BUT**, it is difficult to draw concrete conclusions on the effects of soil and wastewater properties on the uptake of pharmaceuticals:

- Data in the literature are available for **different** plant-growth methods (controlled laboratory or greenhouse or field or simulated conditions)
- **Different** plants and pharmaceuticals in each study
- **Different** experimental conditions and equipment in each study

**??Priority list** of crops that have the highest and lowest potential for contaminants’ uptake.

# Crops’ uptake - pharmaceuticals

**The uptake of CEC by fruit trees is not yet evaluated**.

Fruit trees, such as *citrus*, *bananas*, *apple*, have high net irrigation requirements and evapotranspiration rates, which may render them as plants with moderate to high potential for CEC uptake (similar to that of fruit vegetables).

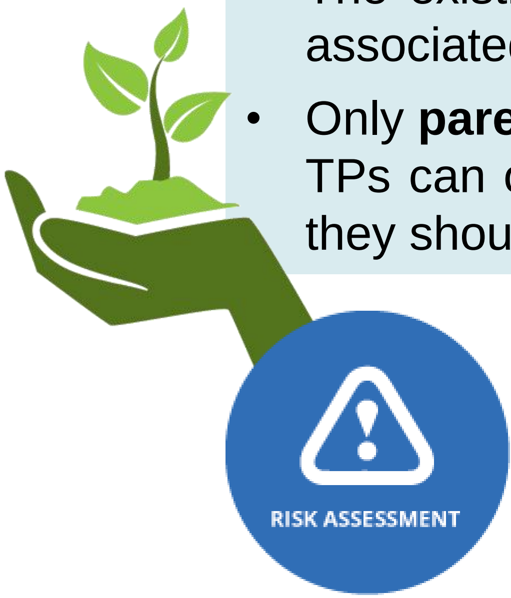
**Field studies** (fruit trees included), must be performed in order to shed light on the uptake of CEC by crops under **realistic agricultural conditions**.

The quantification of the examined CEC in both the **edible parts** of the examined plants and in the **growing medium** (WW-irrigated or biosolids- or manure-amended soil, substrates, etc.) is of high importance, as it will allow for the better understanding of crop plants’ potential for CEC uptake.

# Crops' uptake - pharmaceuticals

**Lack of risk assessment regarding irrigation of vegetables with WW**

- The existing risk assessment methodologies do not estimate the risk associated with **mixtures of compounds**
- Only **parent compounds** are considered. Phase I and II metabolites or TPs can occur at higher concentration than the parent compound and they should be taken into account.




**Predictive models** to accurately estimate the CEC/TPs concentration in crops grown in different environmental and agronomical practices would be extremely useful for risk assessment.

Further research need to be addressed to develop and accurate model to predict **ARB&ARGs** in the root-soil interphase and in the endophytic bacteria.

**Deliverable 2.2**
**Prioritization of microcontaminants (chemical and biological) and key factors affecting their uptake process**

## Chemical contaminants

1. High frequency of detection in treated effluents. It is related to high patterns of use and recalcitrance during the wastewater treatment process.
2. Environmental, agricultural or health concern. At least one of the following criteria should be met by the target CECs:
    a. DT50 in soil > 14 d (O'Connor, 1996)
    b. Phytotoxicity at environmental relevant concentrations
    c. Promote a selective pressure to soil microbiota
    d. Potential human health effects according with threshold contaminant concentration (TCC) criteria.
3. Significant uptake rate by crops. Usually, bioconcentration factors (RCF=[root]/[growing medium]; LCF=[leaf]/[ growing medium]; FCF=[fruit]/[ growing medium]) are higher than 1.

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nereus COST ACTION ES1403

**Deliverable 2.2**
**Prioritization of microcontaminants (chemical and biological) and key factors affecting their uptake process**

## biological contaminants

- Prevalence in RWW
- Prevalence in soils irrigated with RWW
- Prone to be uptaken by crops

| ARBs |
|---|
| *Escherichia coli* |
| *Klebsiella pneumoniae* |
| *Aeromonas* spp. |
| *Pseudomonas aeruginosa* |
| *Enterococcus faecalis* |
| *Enterococcus faecium* |
| *Staphylococcus* |
| *Salmonella* |

| Genetic determinants and the proteins they encode |
|---|
| *qnrS* (quinolone pentapeptide repeat family) |
| *vanA* (vancomycin resistance operon gene) |
| *mecA* (penicillin binding protein) |
| *tetM* (ribosomal protection protein, associated with tetracycline resistance) |
| *aph* (aminoglycoside phosphotransferase) |
| *$bla_{TEM}$ and $bla_{CTX\text{-}m}$* (β-lactamases frequently identified in Enterobacteriaceae) |
| *$bla_{OXA}$* |
| *$bla_{KPC}$* (Klebsiella pneumonia carbapenemase) |
| *sul1, sul2* (sulfonamide-resistant dihydropteroate synthase) |
| *ermB* and *ermF* (rRNA adenine N-6-methyltransferase, associated with macrolide resistance) |
| *intI1* (integrase gene of class 1 integrons) |
| *tetW* (tetracycline resistant protein) |

# ANSWER Project (H2020-MSCA-ITN-2015/675530)

The **Marie Skłodowska-Curie ITN project "Antibiotics and mobile resistance elements in wastewater reuse applications: risks and innovative solutions (ANSWER)"**, aims at training **15 ESRs** to address the risks associated with **Antibiotics, Antibiotic-Resistant Bacteria and Antibiotic Resistance Genes (A&ARB&ARG)** and **wastewater reuse**.

http://www.answer-itn.eu/

ANSWER

**STRONG NETWORKING of the consortium with the scientific and regulatory community:**

Agriculture and Agri-Food Canada

United States Environmental Protection Agency

efsa European Food Safety Authority

IWA International Water Association

norman

State of Israel Ministry of Health משרד הבריאות, ישראל

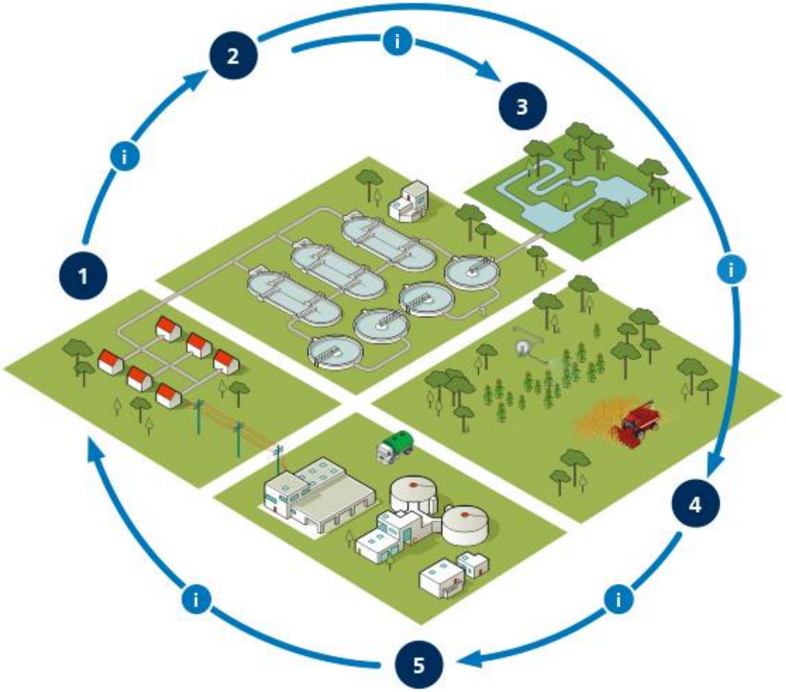
# ANSWER Project (H2020-MSCA-ITN-2015/675530)

## Main scientific objectives

- understanding the fate of A&ARB&ARG within urban WW, soil, ground/surface water, and crops.
- validation of a battery of bioassays for A&ARB&ARG effects evaluation and hazard identification.
- development of a modelling framework capable of predicting the fate of A&ARB&ARG in activated sludge, soil, surface water and crops.
- assessment of the efficiency of innovative technologies (light-driven oxidation and photocatalytic technologies) to minimize A&ARB&ARG.
- development of a database which will allow for an automated prioritization of chemical/biological risk factors to be used by stakeholders for future policy development.

ANSWER

# Water reuse in Braunschweig (Germany)

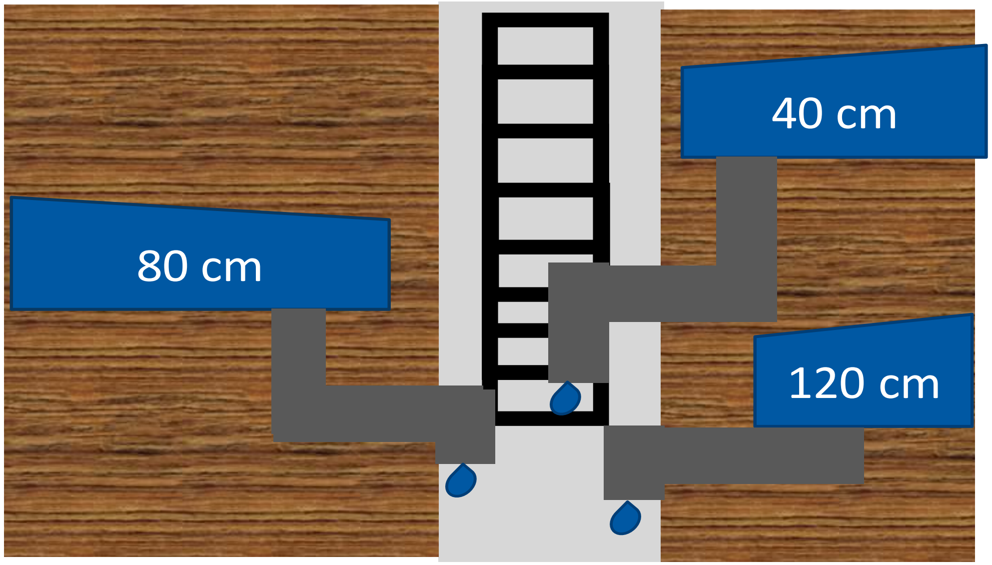



ANSWER

# Braunschweig - Sampling

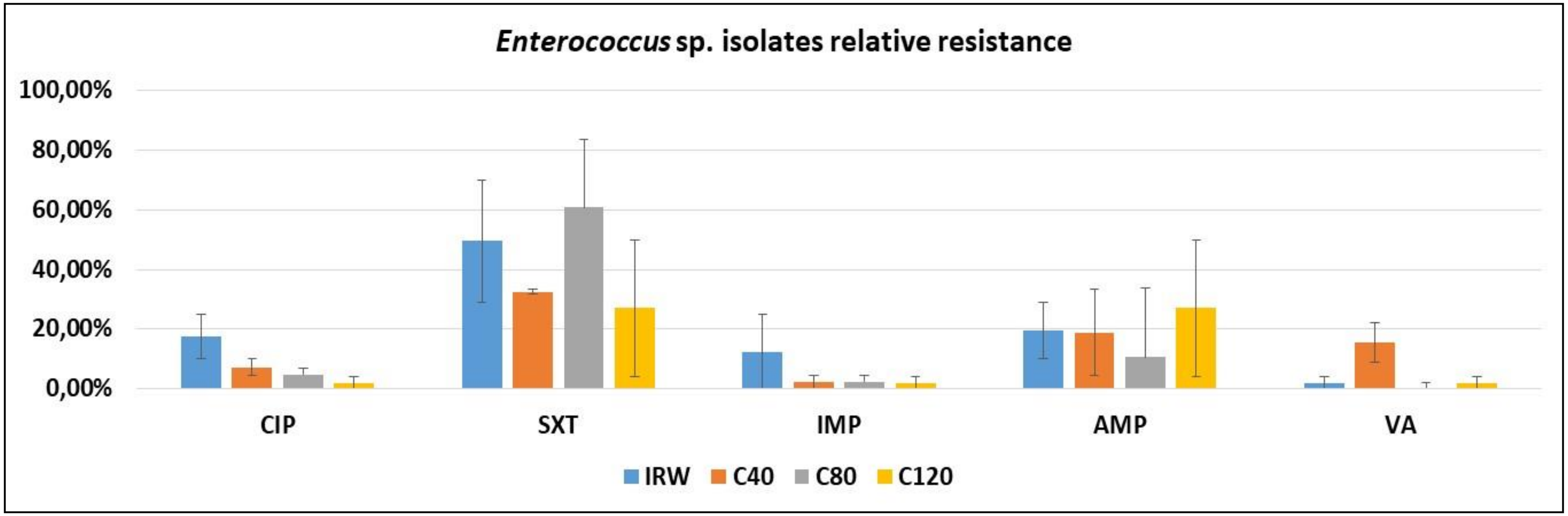
4 Lysimeter (A, B, C, D)

3 Horizons von of each Lysimeter
(40, 80 and 120 cm)



ANSWER

# Resistente Isolate

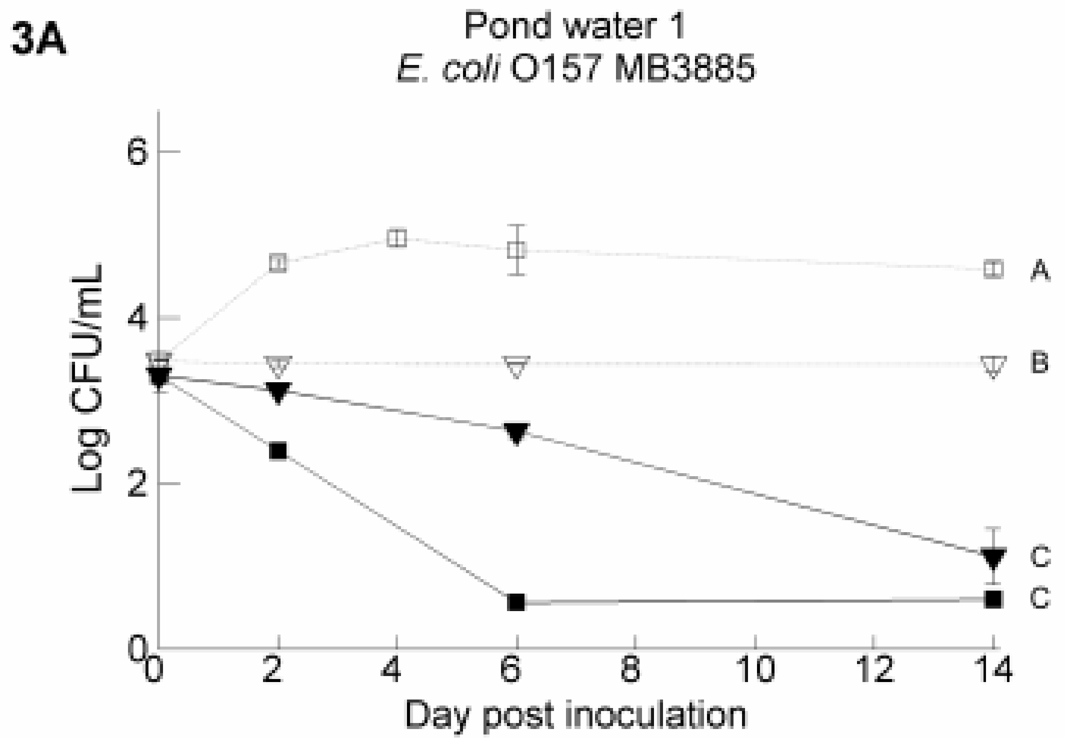



CIP = Ciprofloxacin
SXT = Sulfomethoxazole/Trimetophorim
IPM = Gentamycin, Imipenem
AMP = Ampicillin
VA = Vancomycin

ANSWER

# Survival of E.coli in water for irrigation ?

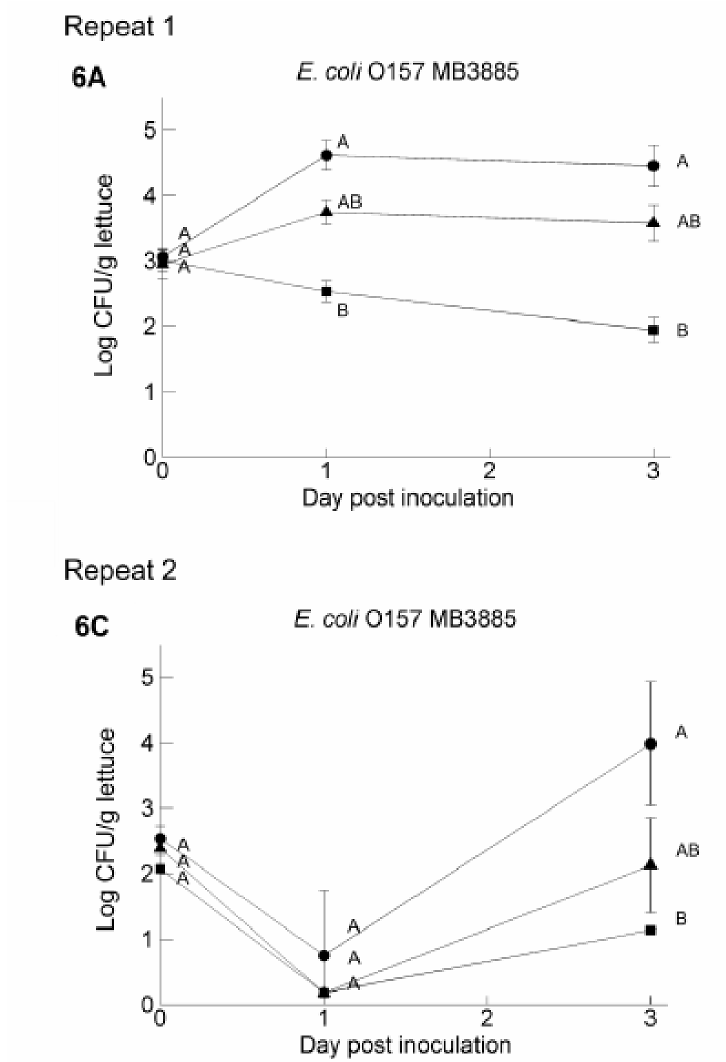
**Enteric Pathogen Survival Varies Substantially in Irrigation Water from Belgian Lettuce Producers**




Van der Linden *et al.* Int. J. Environ. Res. Public Health 2014, 11

ANSWER

# Survival of E.coli on lettuce ?




Van der Linden *et al.* Int. J. Environ. Res. Public Health 2014, 11

ANSWER

# Survival of E.coli after drip irrigation ?

Effects of water managements on transport of *E. coli* in soil-plant system for drip irrigation applying secondary sewage effluent

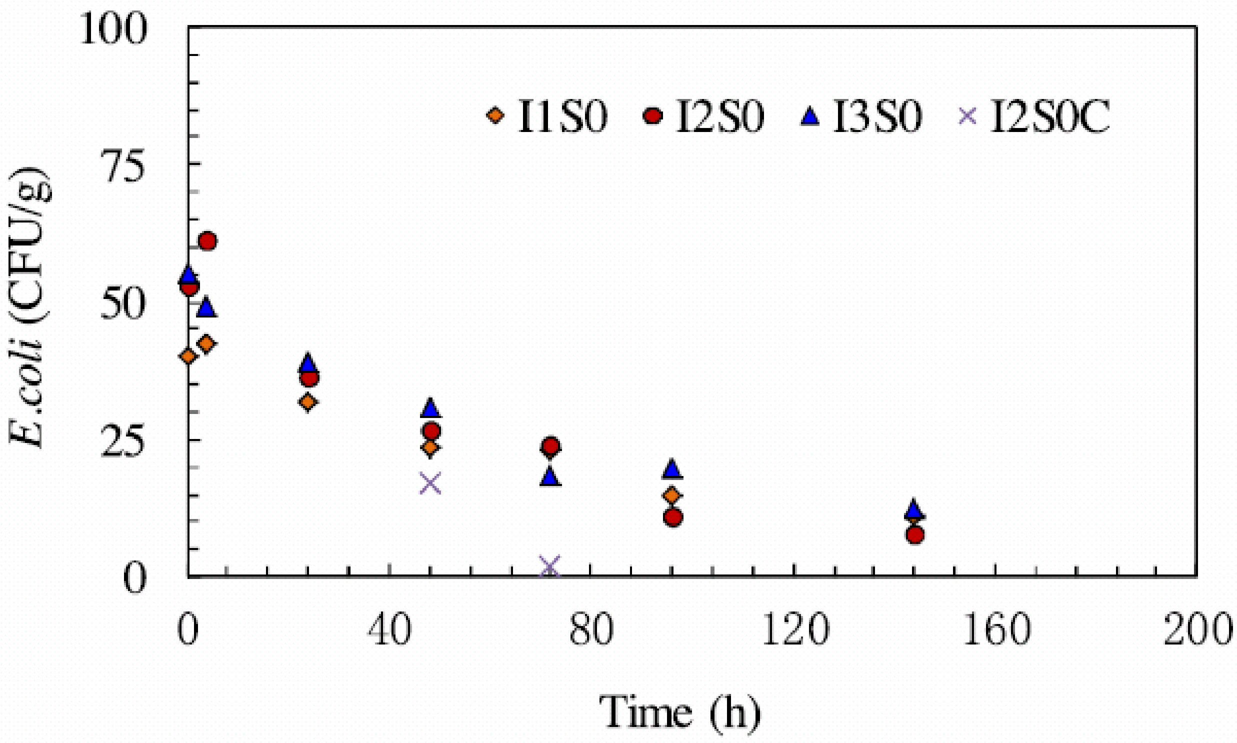



**Fig. 3.** The concentration of *E. coli* in surface soil as function of time after irrigation ceased for the treatments of surface drip irrigation on 27 September in the 2014.

Li and Wen Agricultural Water Management 178 (2016) 12-20

ANSWER

# The transferable resistome of produce

- Blau et al. 2018 preprint

  ⇨ „From mixed salad and cilantro … plasmids were captured exogenously. Importantly, whereas direct detection of IncI and IncF plasmids in TC-DNA failed, these plasmids became detectable in DNA extracted from enrichment cultures.“

- Uptake of ARG or ARB ?

Ex- COST Action DARE members

NORMAN-network

JPI-water StARE Stopping antibiotic resistance evolution

ANSWER

GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

NaWaM RiSKWa FONA

Risikomanagement von neuen Schadstoffen und Krankheitserregern im Wasserkreislauf

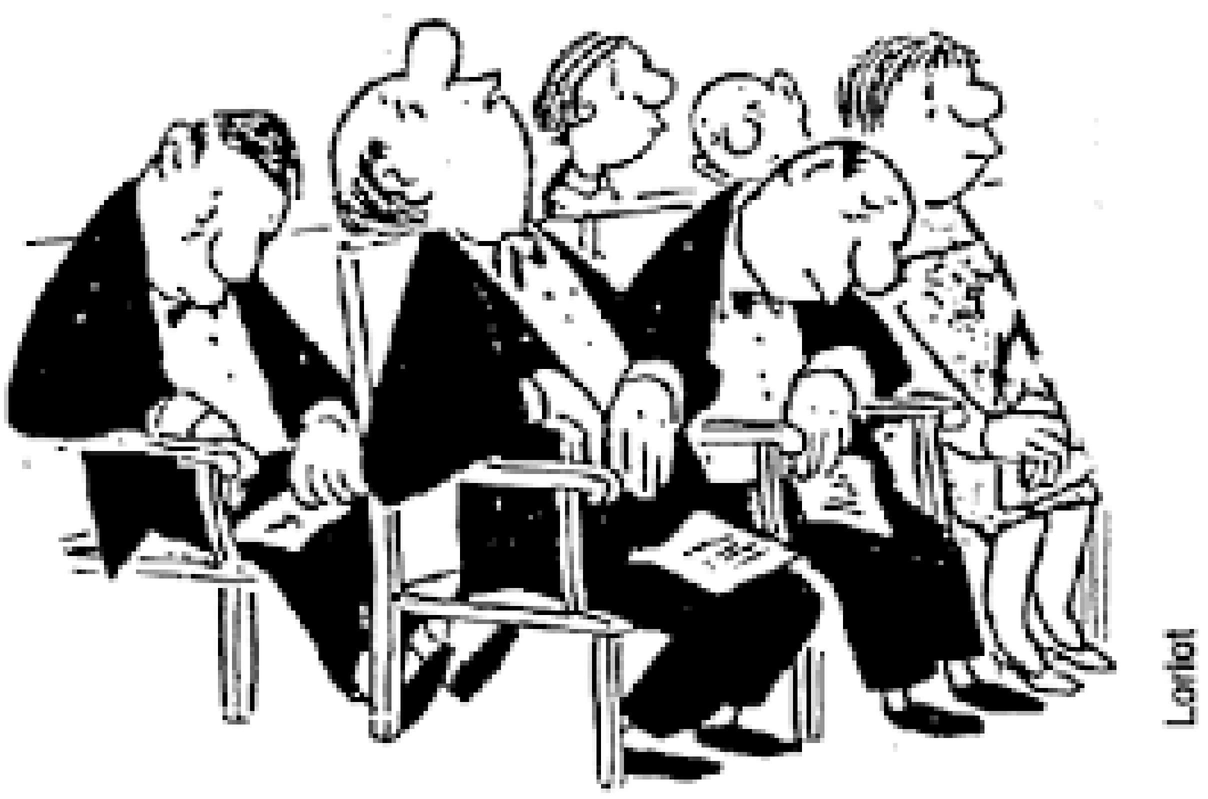
nereus COST ACTION ES1403

"HYREKA" http://hyreka.ihph.de/

GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

*The End*

## Thank you for listening



# Survival of E.coli in different waters?



## Survival of *Escherichia coli* O157:H7 in waters from lakes, rivers, puddles and animal-drinking troughs

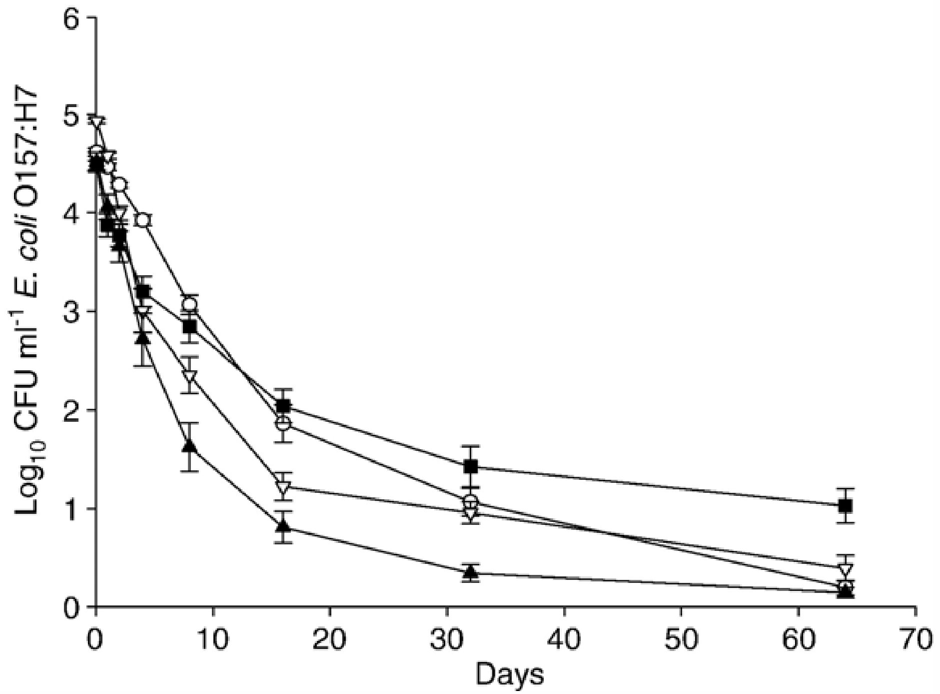
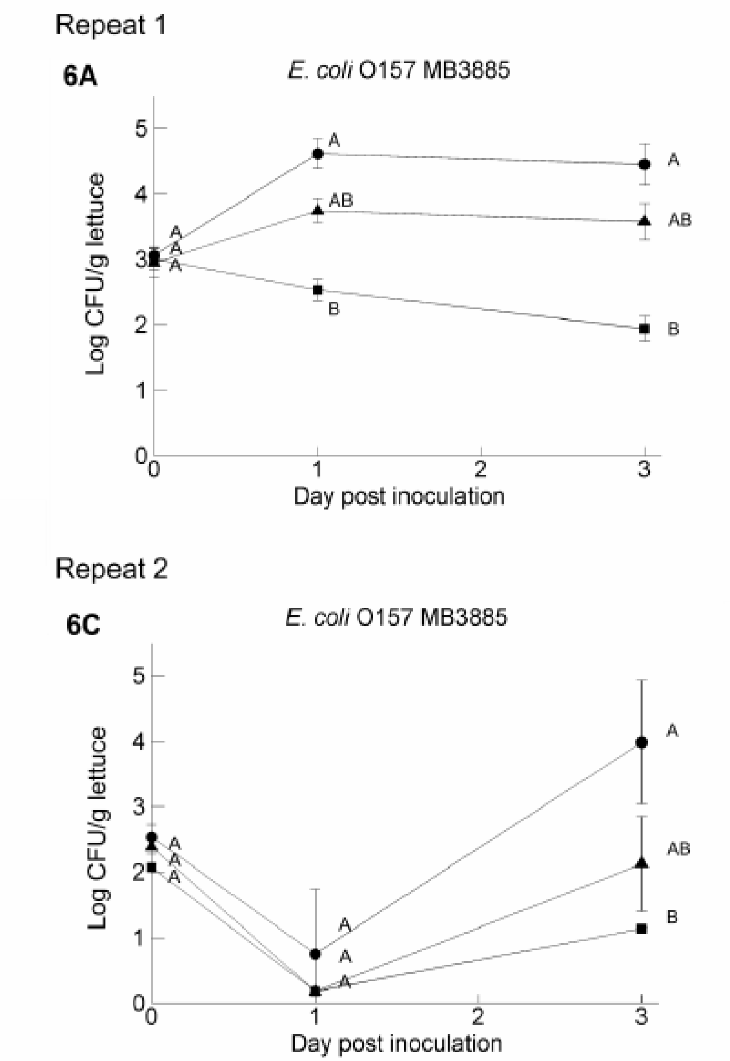



**Fig. 1 – Survival of E. coli O157:H7 ($log_{10}$ … faecally contaminated puddle (■), river … animal-drinking trough (▲) waters. Data are $log_{10}$ (y+1)**

Avery *et al. Science of the Total Environment 389 (2008) 378-385*

# Survival of E.coli on lettuce ?

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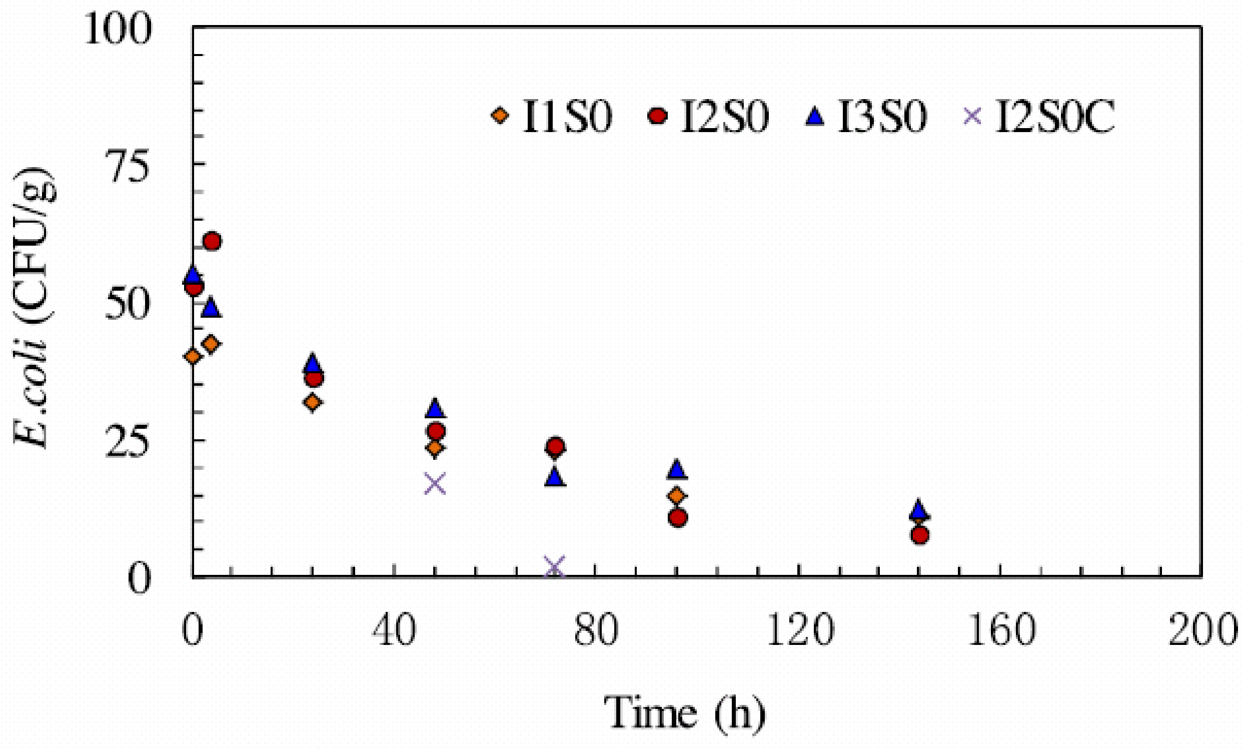
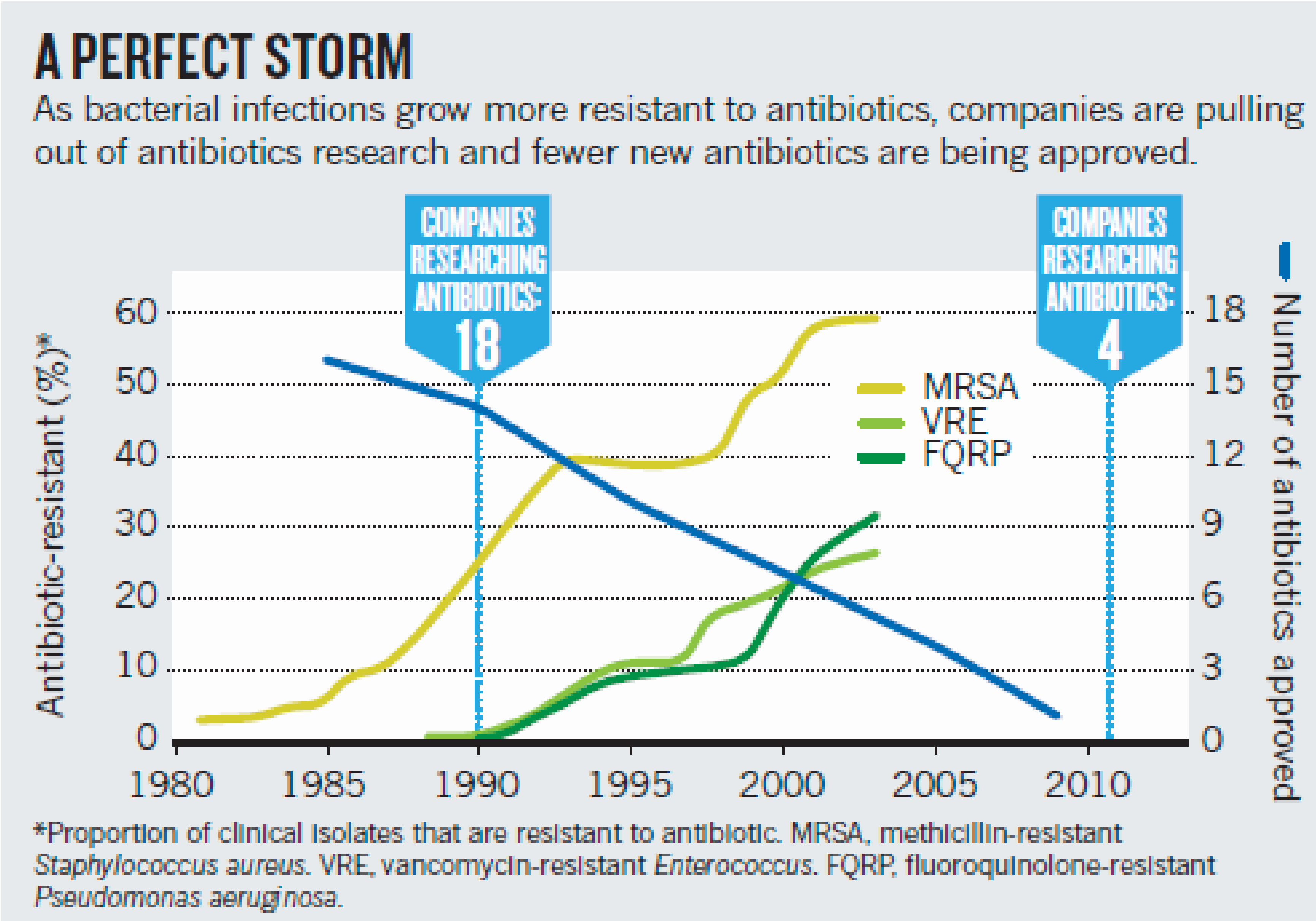



**Fig. 3.** The concentration of *E. coli* in surface soil as function of time after irrigation ceased for the treatments of surface drip irrigation on 27 September in the 2014.

Li and Wen Agricultural Water Management 178 (2016) 12-20

# The rise of resistance




Matthew A. Cooper | NATURE | VOL 472 | 7 APRIL 2011

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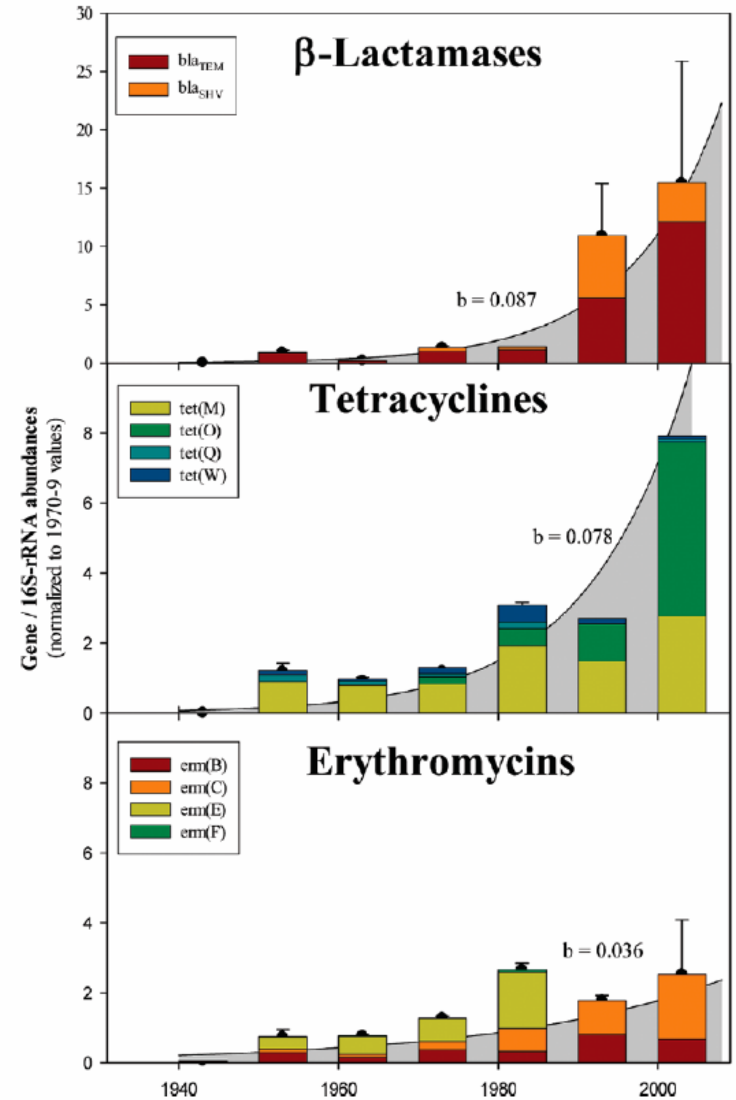
# Evidence of Increasing Antibiotic Resistance Gene Abundances in Nature

Relative increase of antibiotic resistance genes among soils collected at five sites in The Netherlands from 1940 to 2008.




(Knapp et al., 2010)