Universiteit Utrecht

Antibiotic resistance in the environment – inclusion in the authorization of pharmaceuticals?

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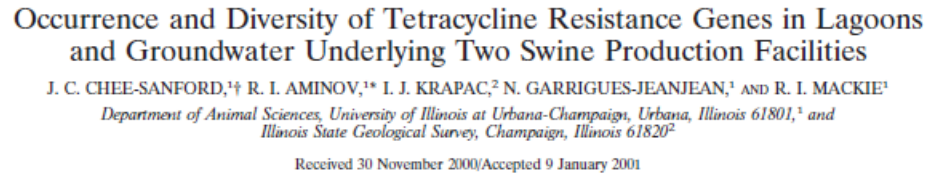
E·C·T RESEARCH AND SERVICES IN ECOTOXICOLOGY

This presentation:

- Occurrence of resistance in the environment
- Role of antibiotic residues for selection of resistance
- Transmission to humans – human risks
- Regulatory needs?

Resistance in the environment – "Emerging contaminants"



Occurrence and Diversity of Tetracycline Resistance Genes in Lagoons and Groundwater Underlying Two Swine Production Facilities

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Environ. Sci. Technol. **2006,**

Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado†

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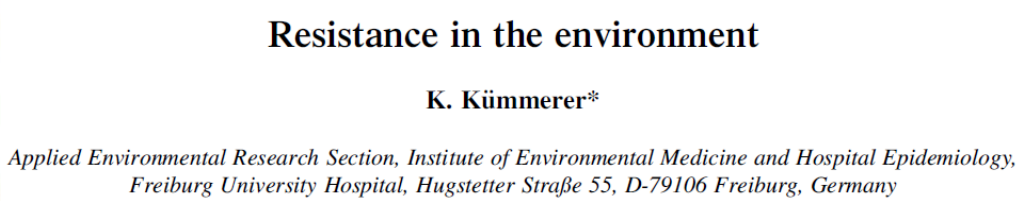

Resistance in the environment

K. Kümmerer*

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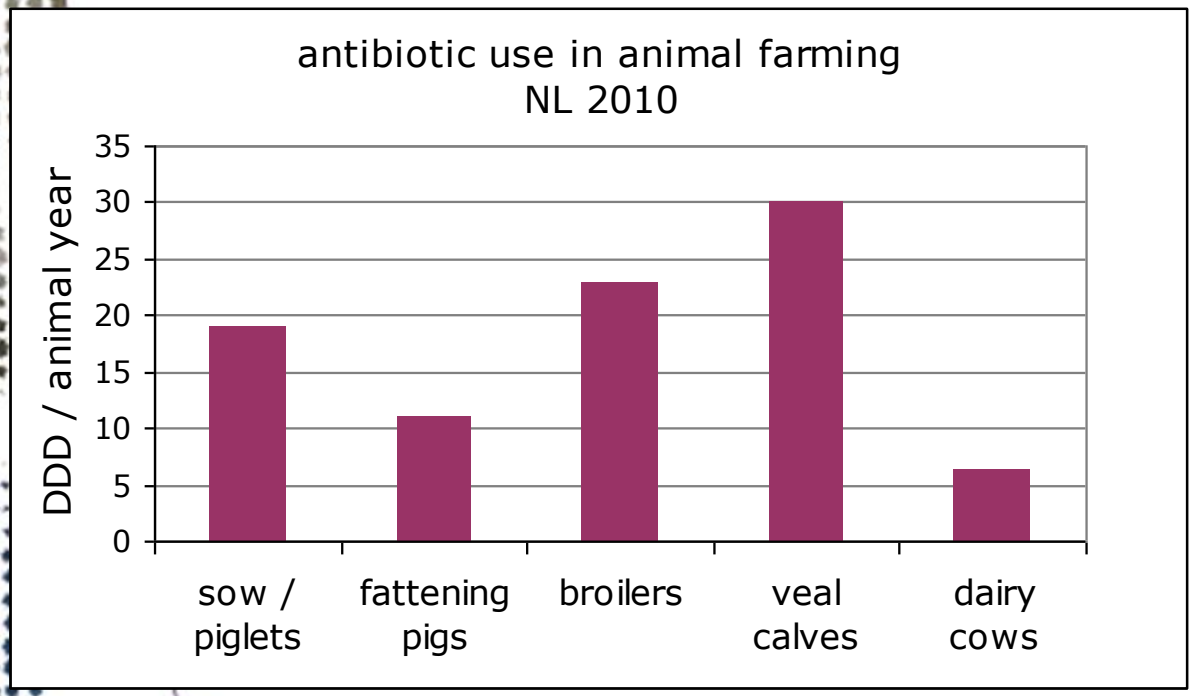
- Research subject since 2000 (but: fish farms)

Chee-Sanford AEM 2001 / Kümmerer JAC 2004 / Pruden, EST 2006

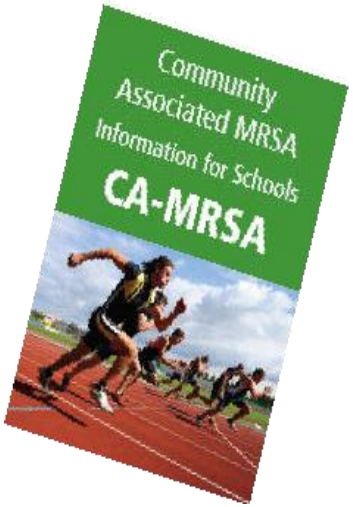
Antibiotic use – the Dutch example



- Intensive usage of antibiotics in pig rearing / broiler production / veal calves
- Human: 4 DDD / year
- Hospital: 70 DDD / 100 patient days

MARAN-LEI 2011, MARAN 2012

Healthcare-related resistance



- Occurrence: hospitals /community
- Mostly focused on resistant pathogens
  - ESBL *(E. coli / Klebsiella)*
  - *Staphylococcus aureus (*MRSA*)*
  - *Pseudomonas aeruginosa*
  - *Enterococcus faecium*
  - …

New Study Shows Rise In Drug Resistance of Dangerous Infection in U.S. Hospitals

Created by **ANONYMOUS**

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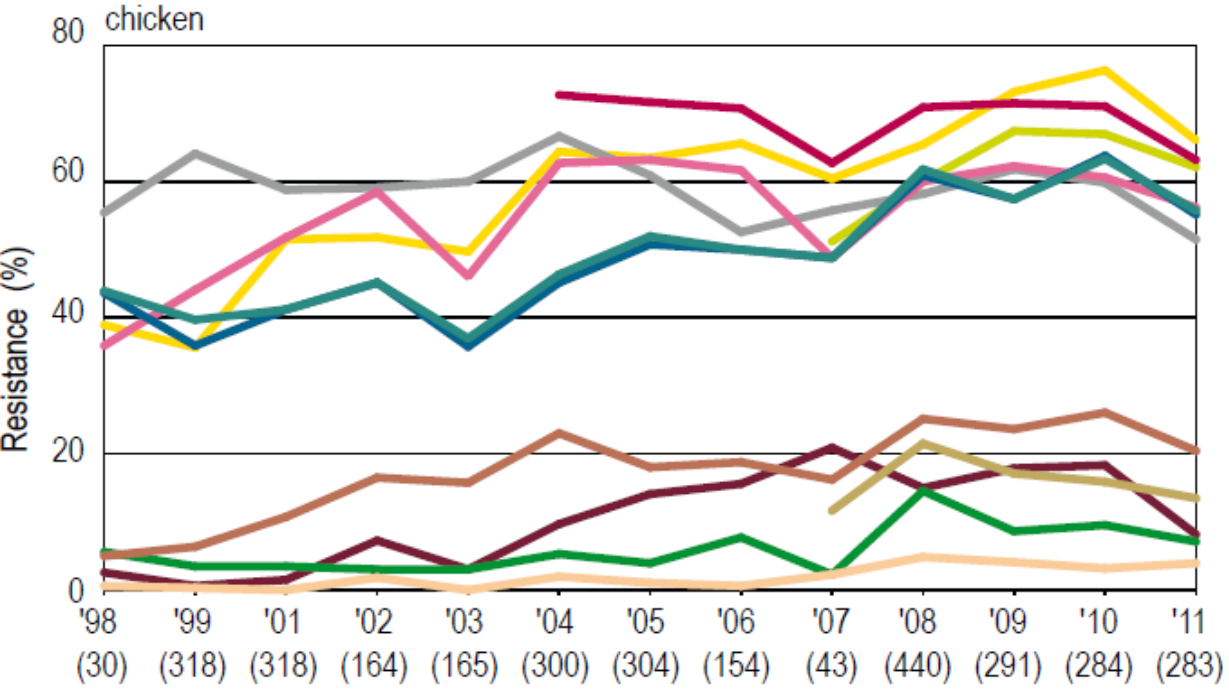
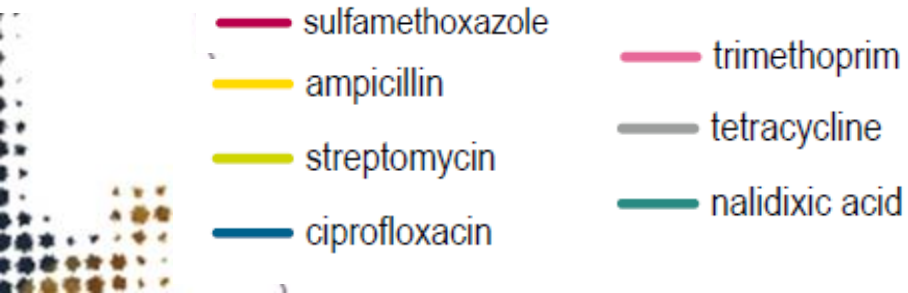
Washington, D.C. -- A new study in the journal Infection Control and Hospital Epidemiology reports a surge in drug-resistant strains of Acinetobacter,

Resistance in animal farming





- Reflects usage intensity
- Multiresistance is increasing
- Clinically important resistances observed:
  - MRSA
  - ESBL

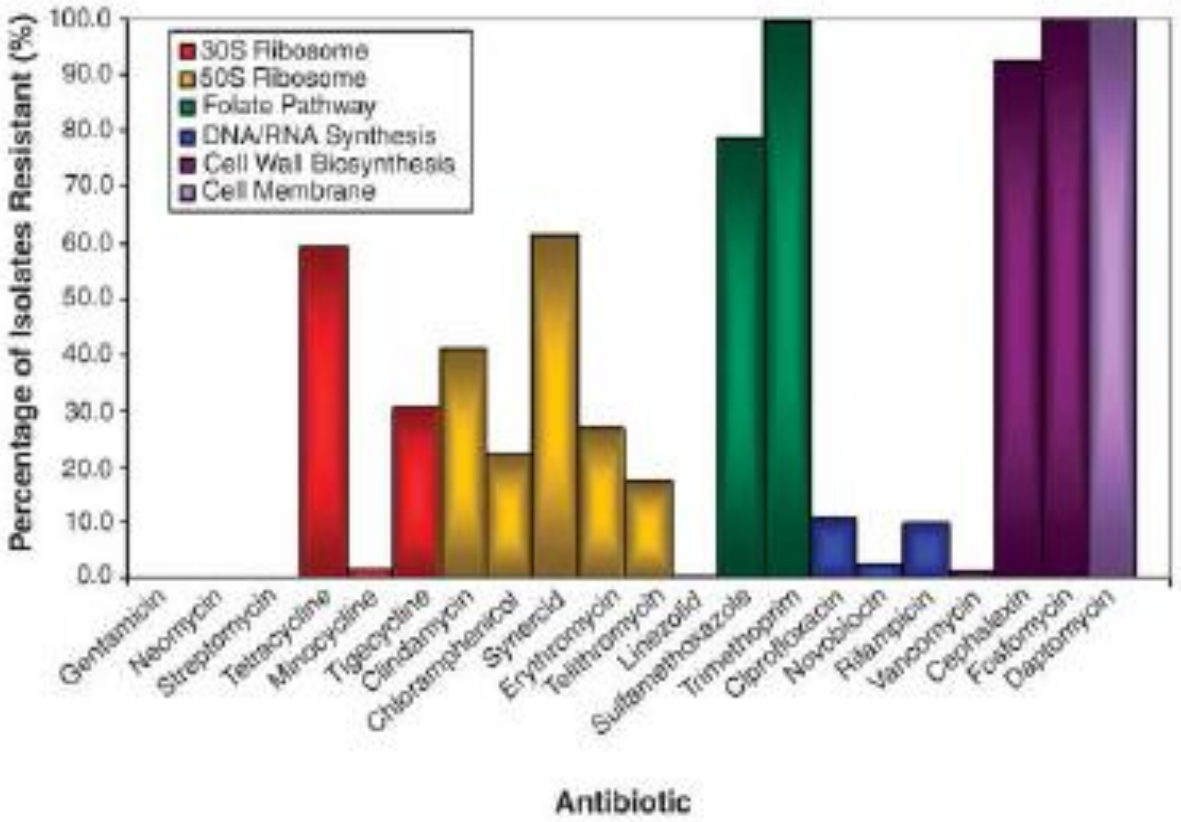
Resistance: a natural phenomenon

- Producers of antibiotics:
  - Fungi
  - Actinomycetes
- Competition
- Communication

Gillings, FMic 13 / Martinez, 08 Science

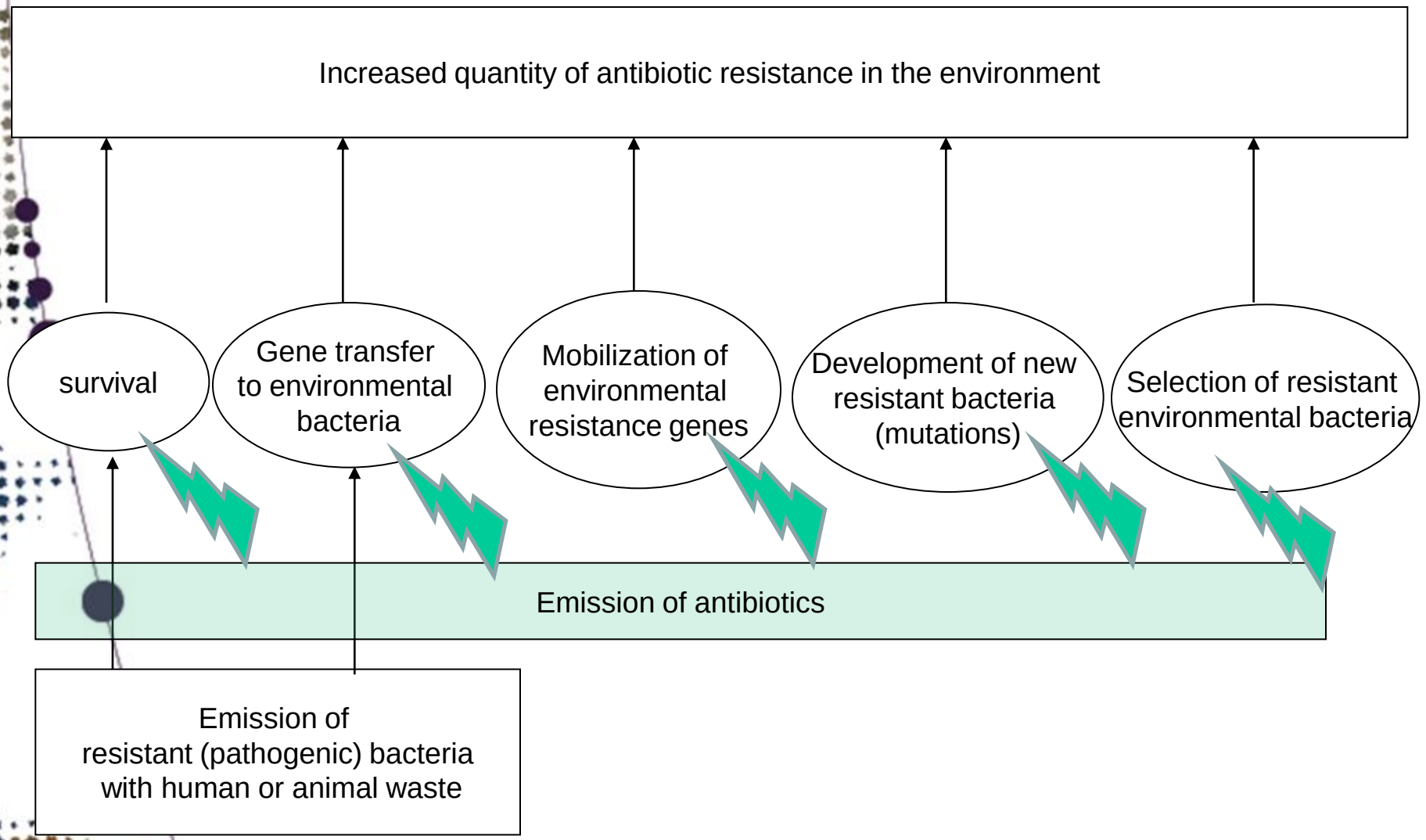
Extent of ‘natural’ resistance

- Streptomyces soil isolates
- Resistance pattern

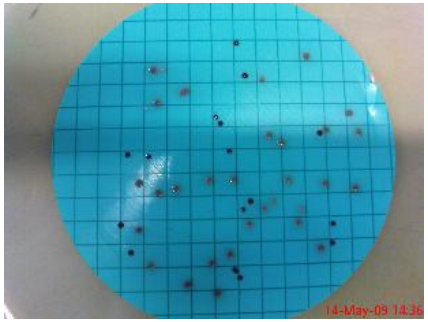


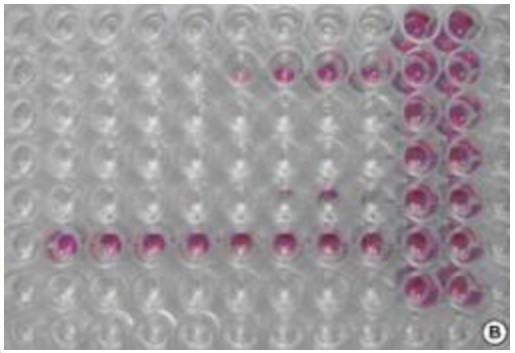
D’Costa, Science 2006

Pathways of resistance in the environment



Excursion: detection of resistance
1) Culture-based analyses: bacteria





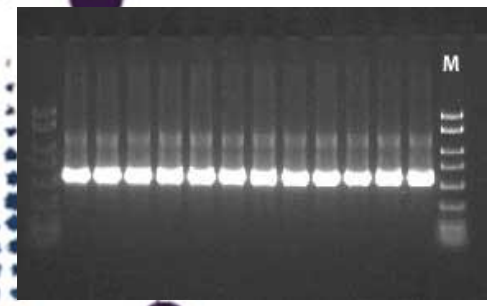
increasing antibiotic concentration

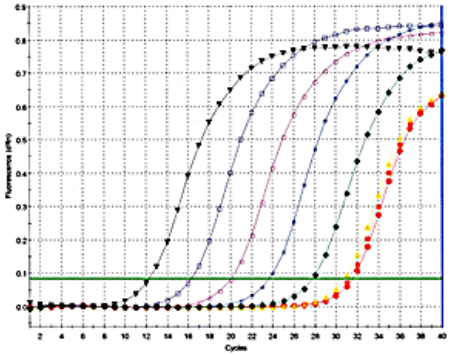
- From clinical science
- Isolation on agar plates with antibiotics
- Determination of resistance: minimum inhibitory concentration

➔ Focus on selected bacteria

➔ Enables in-depth study of resistance mechanisms

2) Gene-based analyses



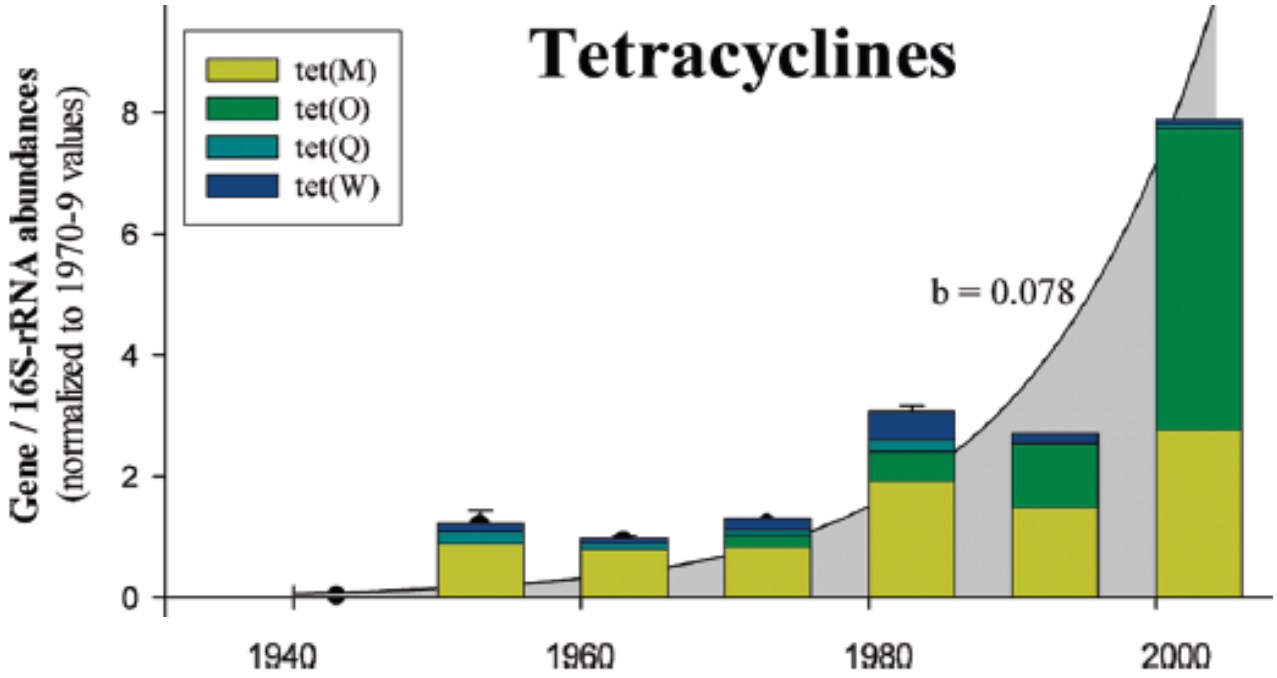


- In environmental studies
- DNA (RNA) isolation from environmental samples
- Detection of genes: Polymerase chain reaction
- Prior information on resistance genes needed

➔ Include unculturable bacteria (90-99%)

➔ No information on bacterial carriers

Occurrence of resistance - soil

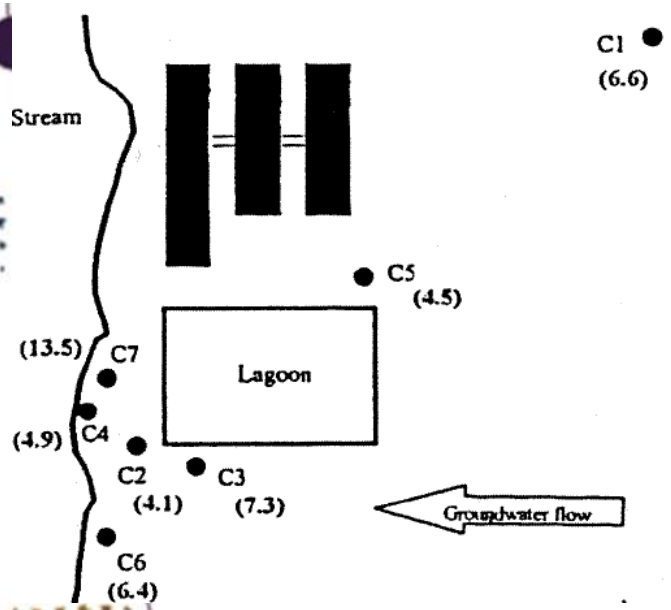


➔ Evidence for quantitative changes in resistance

Knapp, EST 2010

Occurrence of resistance – ground water

Sample	Tetracycline resistance genes							
	tet(W)	*tet*(O)	*tet*(Q)	*tet*(M)	*tet*(S)	*tet*(T)	*tetB*(P)	*otrA*
Site C lagoon	+	+	+	+	+	+	+	+
C1 bkg[a]	–	–	–	–	–	–	–	–
C3	–	–	–	–	–	–	–	–
C2	+	–	+	+	–	+	–	–
C4	–	–	–	–	–	–	–	–
C6	+	+	+	+	–	+	–	+
C7	+	+	+	+	–	–	–	–



- Tetracycline resistance: >40 genes
- Genes downstream a pig lagoon

➔ More genes downstream the pig lagoon

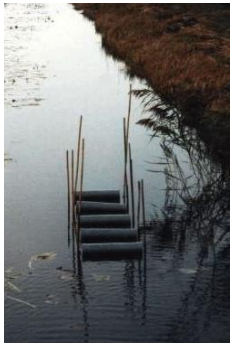
Chee-Sanford, AEM 2001

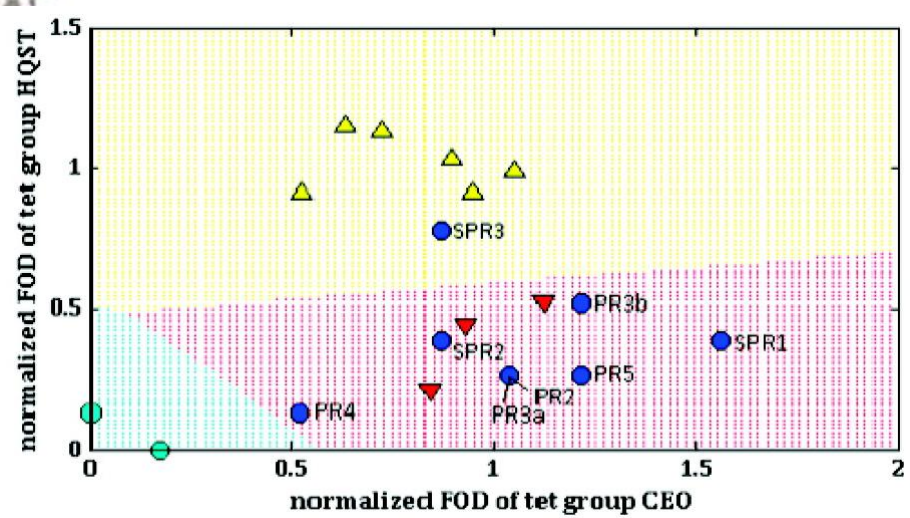
A watershed example

- Detection frequency of tetracycline and sulfonamide genes
- "Profiles" compared with profiles of WWTP, animal lagoons, and pristine sites

Storteboom, EST 2010

Watershed example





Tet(C), (E), (O): typically WWTP

- Only 1 sample site animal farming-influenced
- Genes mirror sources ➔ no selection in the river

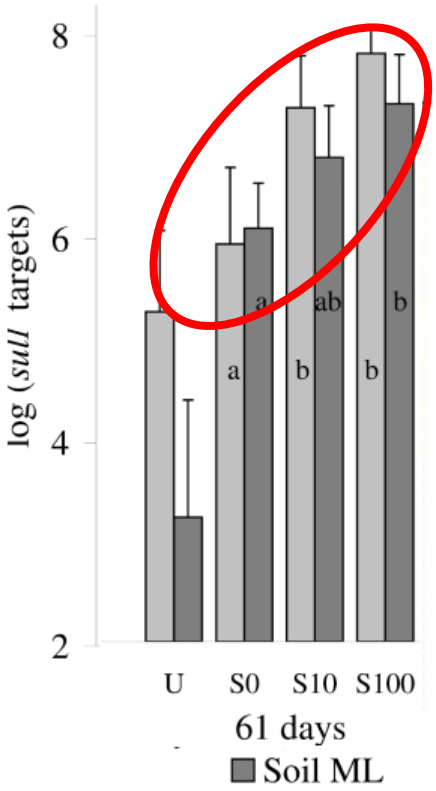
➔ Evidence for increase in resistance

Role of antibiotic residues for resistance in the environment?

- Literature study
- 1500 hits (resistance & environment **& antibiotic**)
- Of which ~25 are relevant

Role of antibiotic residues for resistance in the environment?

Antibiotic effect

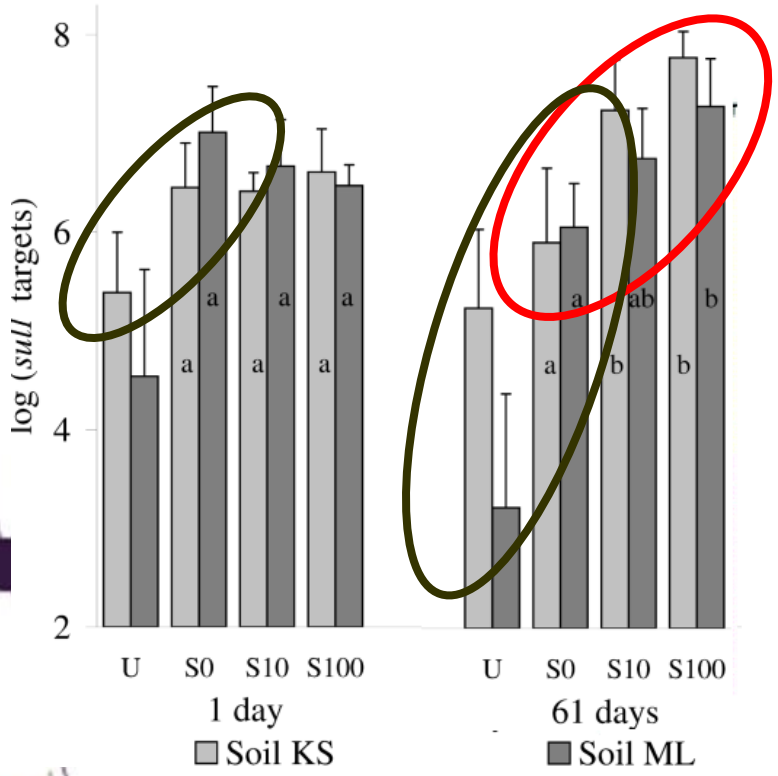


- Literature study
- 1500 hits (resistance & environment & antibiotic)
- Of which ~25 are relevant
- Yes, antibiotics can further select resistance

Heuer, EM 2007

Role of antibiotic residues for resistance in the environment?

Manure effect Antibiotic effect



- Yes, antibiotics can further select resistance
- Often, manure / WWTP sludge also increase resistance

Heuer, EM 2007

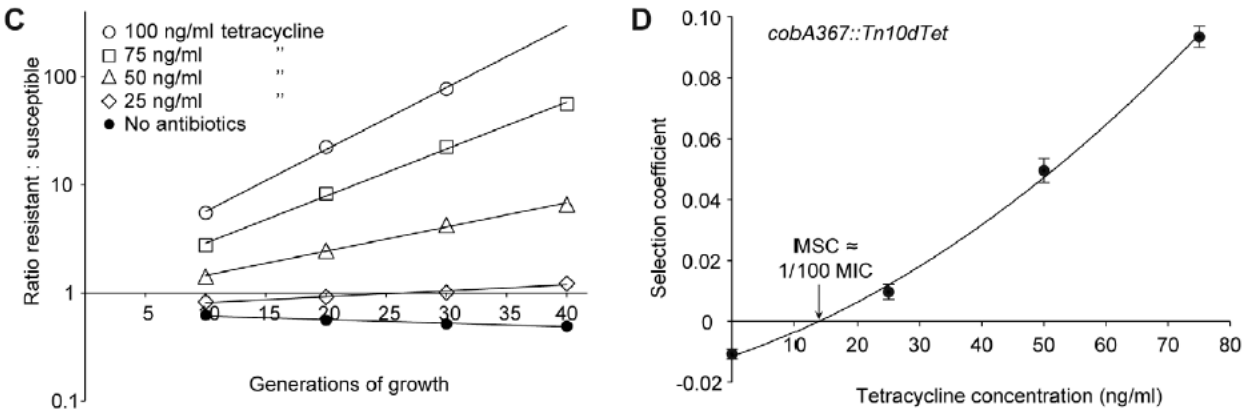
Role of antibiotic residues for resistance in the environment ?

- If relatively high antibiotic concentrations are used, effects on resistance in the environment are found
- Few concentration-response studies with wide range of concentrations
- In these studies, effects found at slightly higher concentrations than MEC

Threshold concentrations?

source	environmental matrix	compound	statistically significant at [ug/L]	endpoint	MEC [ug/L]	MEC / lowest effect concentration
Stepanauskas 2006	surface water microcosms	tetracycline	3000	% ampicillin resistance of isolated strains increased from 0% to 42%	1.34	0.0004
Munoz-Aguayo 2007	chemostats with river water samples, fed 1/10 LB broth	chlor-tetracycline	800	total counts of resistant bacteria increase by factor 100	2.42	0.0030
Knapp 2008	mesocosms fed with lake water	oxy-tetracycline	20	selection rate (first order rate constant for the increase of the sum of resistance genes in time) increases from 0.015 to 0.025	2.2	0.1100

Threshold concentrations? Minimum selective concentrations

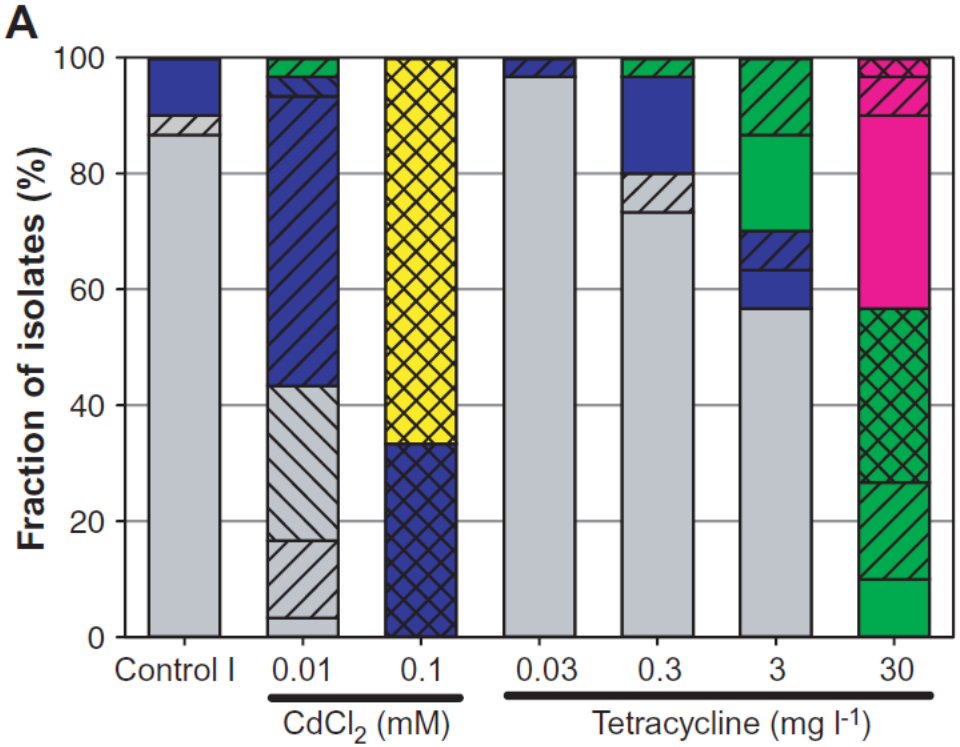


	MSC ug/L	MIC50 ug/L	NOEC ug/L	measured aqueous environmental concentration, hotspot ug/L	measured surface water concentration ug/L	measured environmental concentration, non-aqueous hotspot ug/kg
ciprofloxacin	0.1 - 2.3	100	8	0.007-2.37 effluent	0.7	340 swine lagoon
tetracyclin	15	1585	145	Nd-1.00 effluent	1.34	66 manure

Gullberg, Plos Pathogens 2011

Role of co-selecting agents: metals

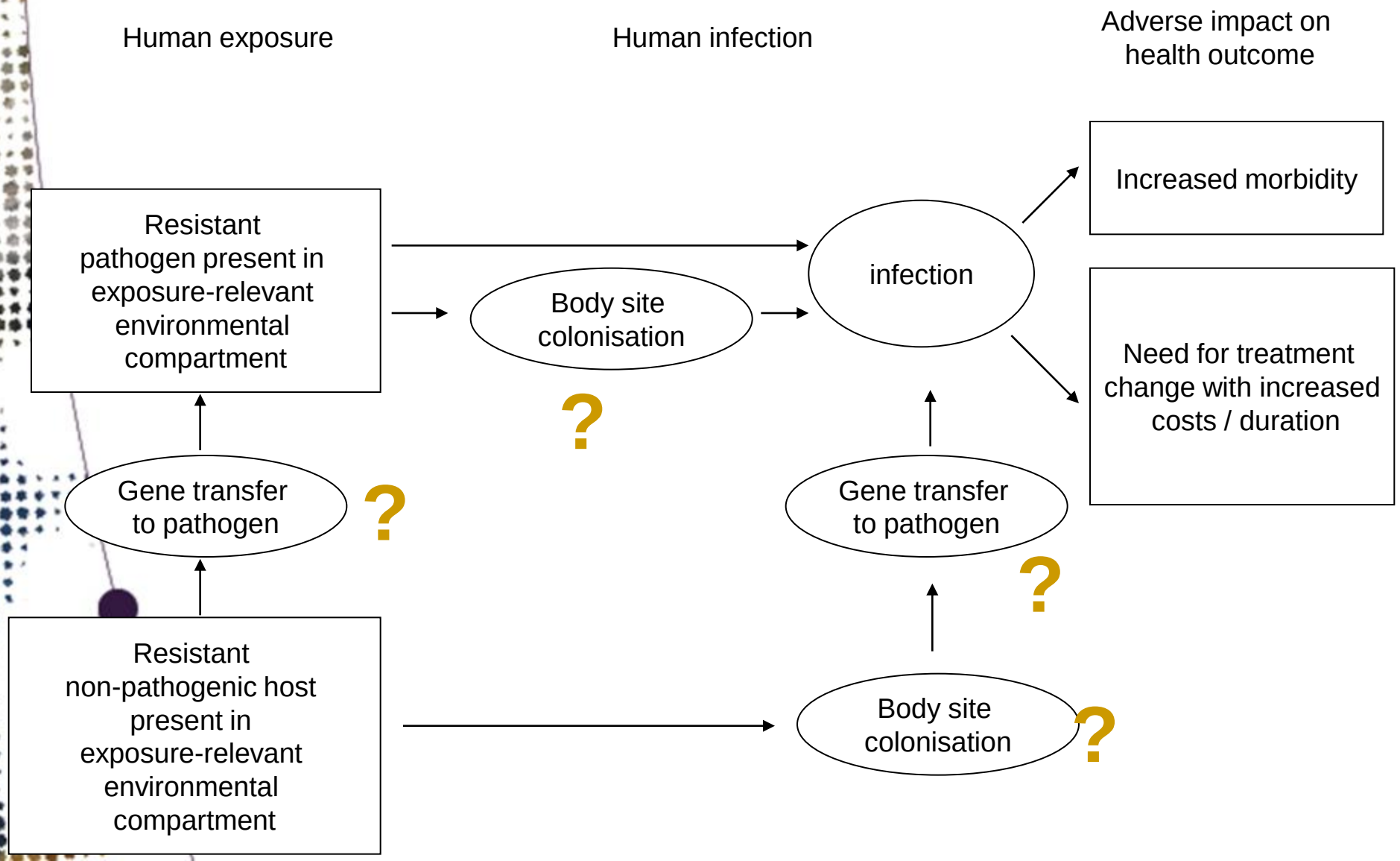
Coloured: resistant



- Heavy metals shown to coselect for antibiotic resistance
- At relevant concentrations

Stepanauskas, EM 2006

Transmission to humans - chain of events



Human risks: considerations

- Implications of resistance in the environment for public health (adverse effects): largely unstudied

➔ Needed: data on processes // relative risk of human uptake of resistance through food, environment

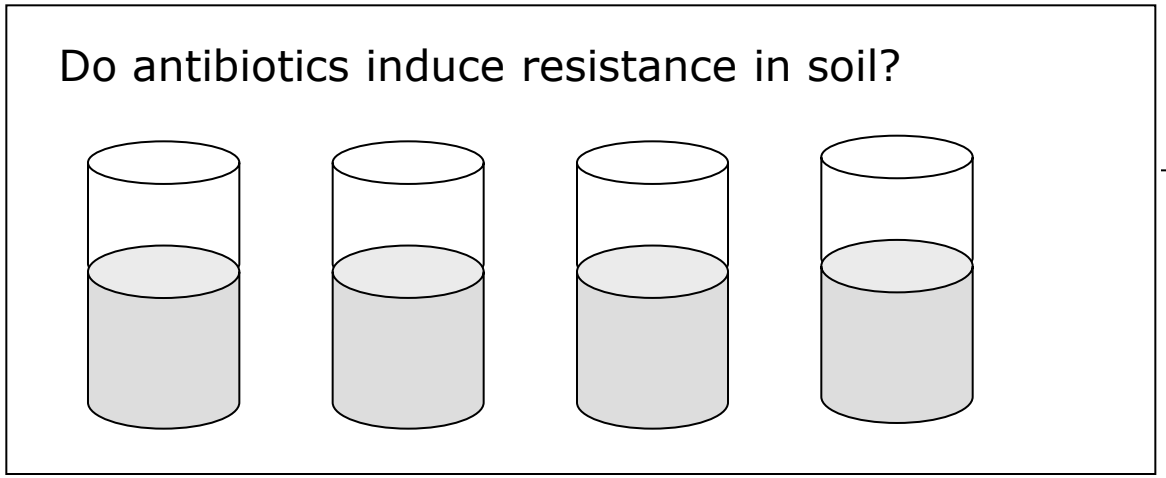
- But also: rare events can possibly have serious implications (gene transfer from soil bacterium to commensal to pathogen) – and are difficult to prove

➔ Current research topic

Regulatory context – need for new test systems?

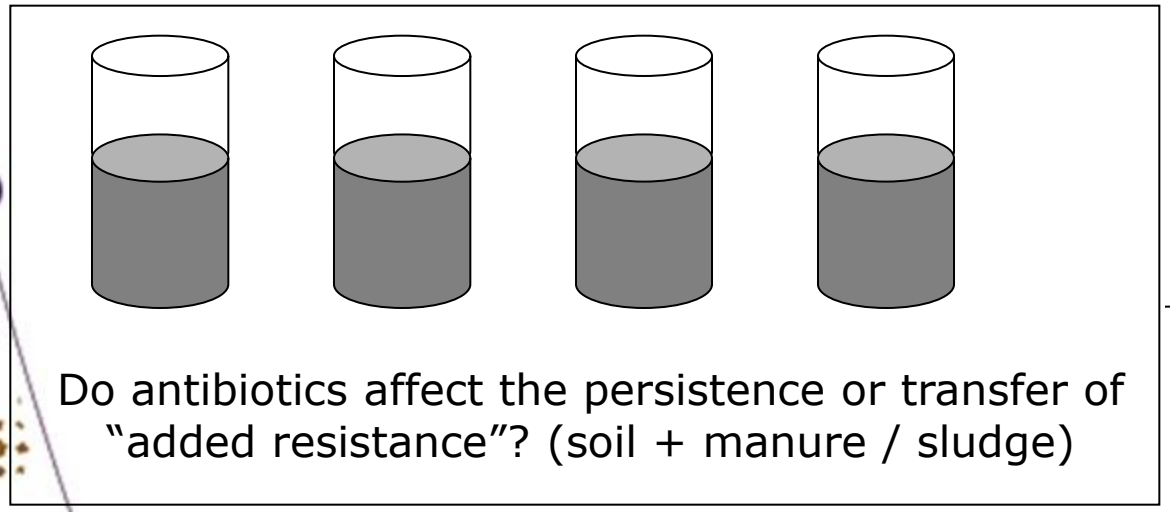
- Resistance: currently not assessed in environmental risk assessment of human and veterinary antibiotics
- VICH GL27, guidance on resistance – endpoint: resistance development in animals
- VICH GL36, microbiological ADI - endpoint: human intestines (through antibiotic residues)
- Some analogies with risk assessment of genetically modified organisms

Suggestions for test systems - design



Control without antibiotic

antibiotic concentration range



1. qPCR
2. Culture-based assays
3. Horizontal transfer of genes

Suggestions for test systems - methods

test system	resistance profiling of bacterial isolates	Derivation of minimum inhibitory concentrations (MICs) of isolates	Selective plating of bacteria	Detection of resistance genes in bacterial isolates	Qualitative detection of resistance genes in environmental DNA	Quantitative detection of resistance genes in environmental DNA	Analysis of clone libraries
specific for resistance in environmental bacteria	+-	+-	+-	+-	--	--	--
generally applicable (no need for pre-information)	++	++	++	--	--	--	++
relevance	--	--	--	--	+	+	+
Limit of detection	-	-	++	-	+	+	
specificity	+-	+-	+-	+-	+	+	+
reproducibility	+-	+-	+-	+-	+-	++	+-
sensitivity: effect size of distinguishable effects	+	+	+-	+	-	+-	
standardisation	+	+	+	--	--	--	--
validation / quality controls	+-	+-	--	-	-	-	
cost effectiveness (material)	+-	+-	++	-	++	++	--
test throughput	+-	+-	+	+-	+	+	--
complexity of test method	++	+-	++	-	-	-	--
need for specialized equipment	++	+-	++	++	++	+	--

Suggestions for test systems - methods

test system	Selective plating of bacteria	Quantitative detection of resistance genes in environmental DNA
generally applicable (no need for pre-information)	yes	no - resistance gene sequence needed for primer design
relevance	small: only around 1% of total environmental bacteria is culturable	high: contribution of non-culturable bacteria, but limited to known genes
reproducibility	unknown for environmental samples, in food matrices with well-defined species approx. <1 log unit	high (< 0.5 log unit)
standardisation	standardised tests existing for cultivation of intestinal bacteria, but not for "mixed" environmental bacteria	no standardized methods for PCR from environmental samples existing. Standards for DNA extraction published
validation / quality controls	only existing for defined bacteria	positive / negative controls are common during PCR
complexity of test method	low	medium - high

Additionally:

- In addition: resistance monitoring
- National monitoring systems in place for human and veterinary indicator bacteria and pathogens
- Extension to (hotspot) environmental compartments

Food for thought – Concept paper for a guideline on antimicrobial risk assessment EMA/CVP

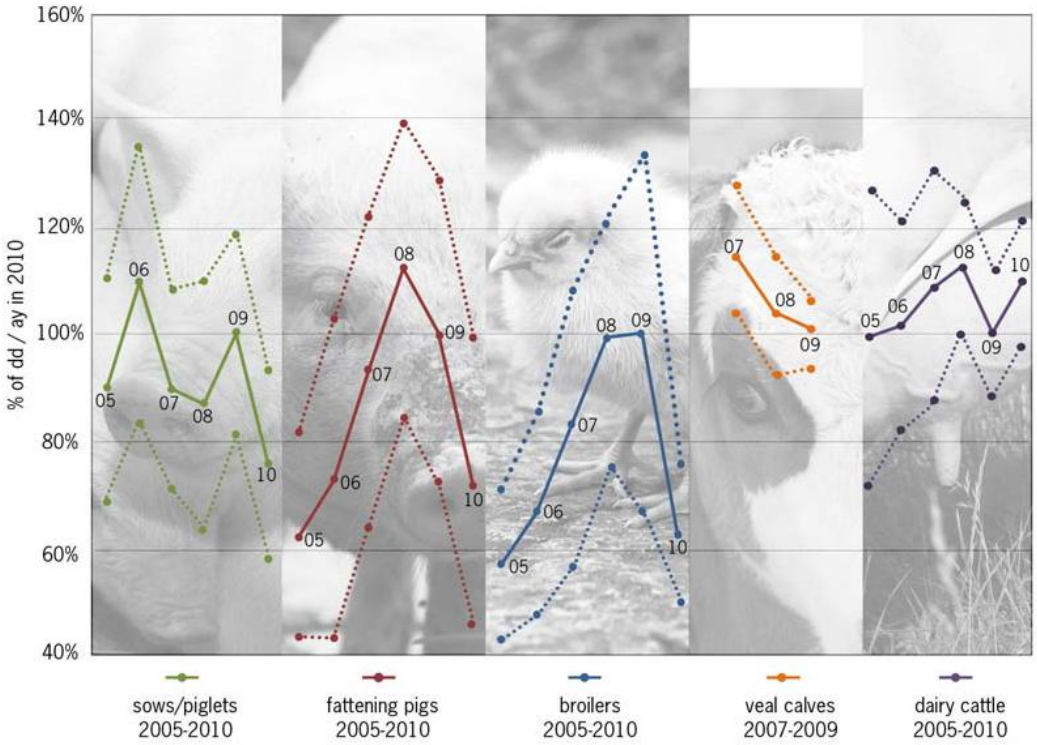
- The AMR related public health risks linked to the use of a certain VMP will be semi-quantifiable at best.
- To go beyond the pre-harvest stage and estimate the exposure to humans (directly or via food) might be too complicated knowing the number of possible different scenarios.

Summary / conclusions

- ! Evidence for occurrence of resistance in the environment
  - ! Detectable by culturing / genetic methods
- ! Role of antibiotic residues?
  - ! In principle, yes
  - ? At environmentally realistic concentrations?
- ! Why are we concerned?
  - ! Public health as protection goal
  - ? Evidence for public health relevance?
- ? Regulatory needs
  - ? Placing in risk assessment framework: role of antibiotic residues?
  - ? Test systems?

Questions?

- Expert workshop on inclusion of resistance in the environmental RA of pharmaceuticals – 4 July, Berlin
- Jens.Schoenfeld@uba.de, h.schmitt@uu.nl



VICH GL36 – microbiological ADI

- Assesses effects of antibiotic residues on intestinal resistance
- Continuous and semi-continuous cultures and fed-batch cultures of fecal inocula provide a means to evaluate long-term exposure of bacteria to the drug.
- Enumeration techniques on media with and without the antimicrobial drug, applying phenotypic and molecular methodologies.