

Antibiotic Resistance Genes as Environmental Pollutants in Agricultural Soils

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ARGs as Environmental Pollutants



Agenda

1. Relevance of antibiotic resistance genes (ARG) in agricultural soils

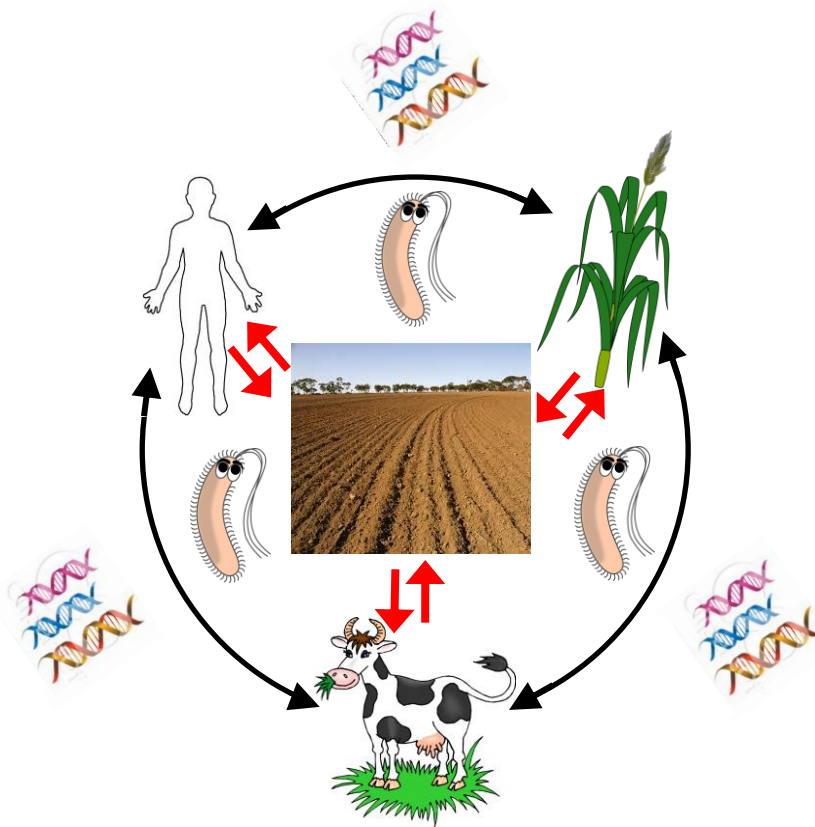
2. Definition: ARG = Environmental Pollutant

3. Case Study:

Naturally occurring background levels of 2 ARGs - **nptII/nptIII** -
in maize and potato fields in Austria

1. Relevance of ARGs in Soil

Agricultural Soils - Gene Exchange Platform



Horizontal Gene Transfer

- human-, animal-, soil and plant-associated bacteria

Anthropogenic Impact

- Manure, tiling, selection pressure

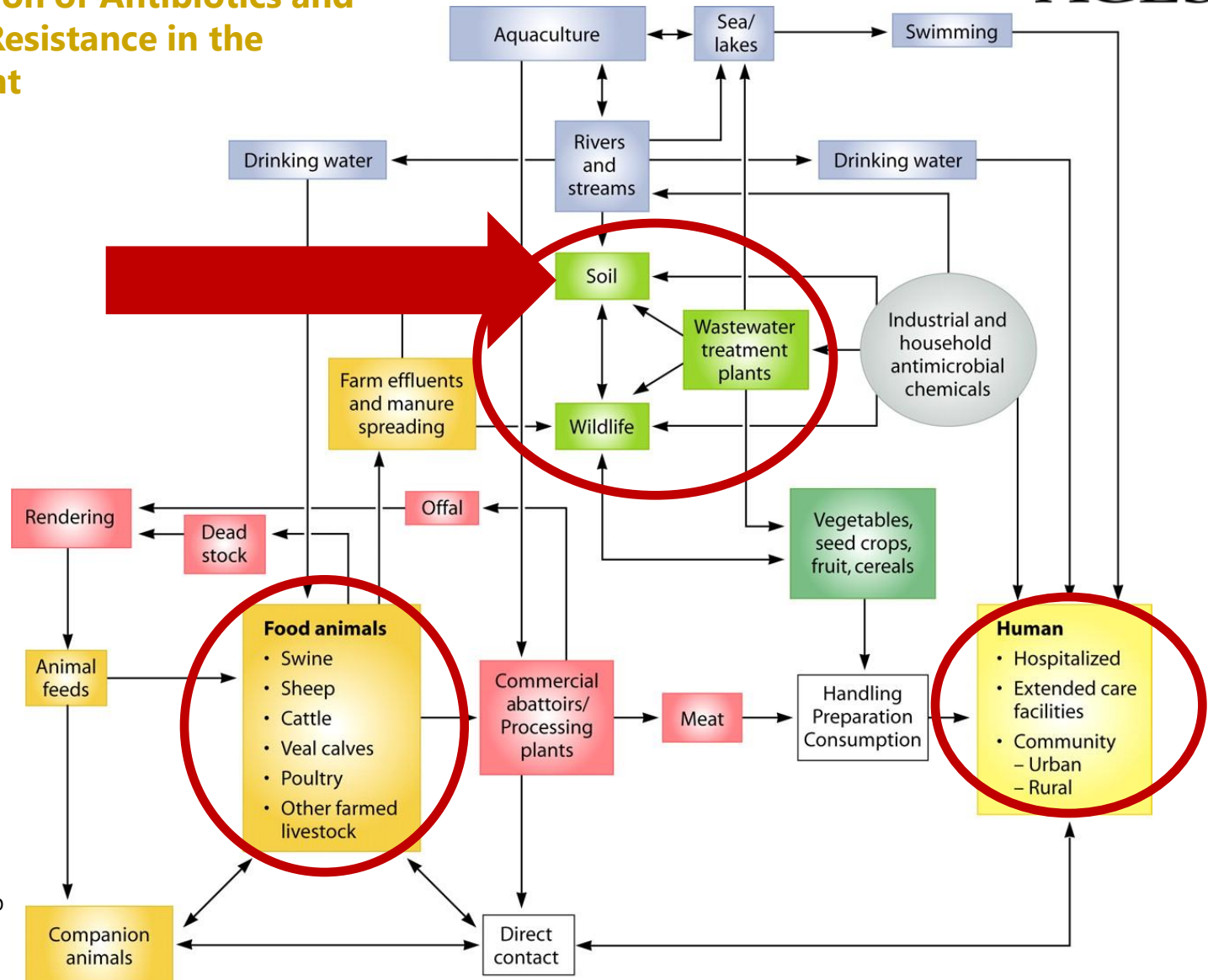
Reservoir - Environmental Resistome

- Antibiotic resistant bacteria
- Antibiotic resistance genes
- Source for clinically relevant ARGs

1. Relevance of ARGs in Soil

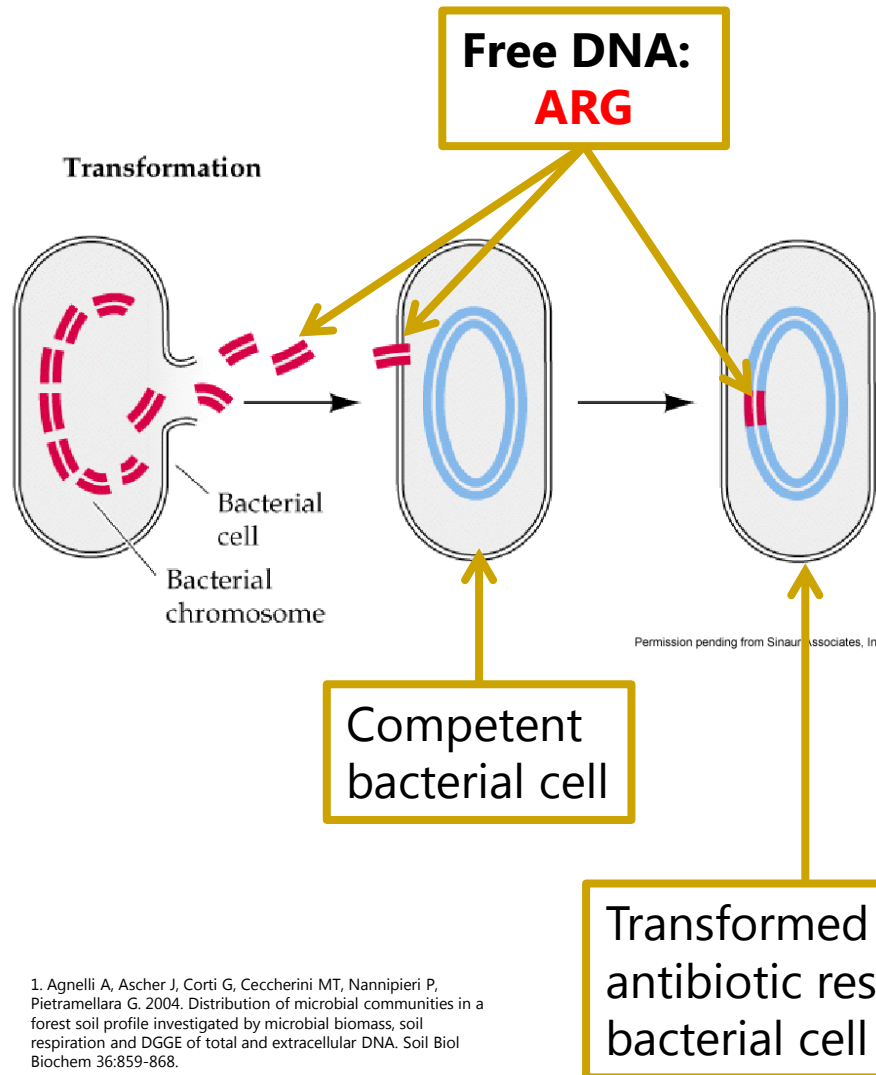
Dissemination of Antibiotics and Antibiotic Resistance in the Environment

Gene
exchange
platform



1. Relevance of ARGs in Soil

Horizontal Gene Transfer - Bacterial Transformation



Uptake of free extracellular DNA

- Active excretion
- Plant root exudate
- Pollen
- Plant decay, dying cells
- Up to 60% of total soil DNA = **free extracellular DNA¹⁾**

Competent Recipient Bacteria

1. Agnelli A, Ascher J, Corti G, Ceccherini MT, Nannipieri P, Pietramellara G. 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biol Biochem 36:859-868.

2. Definitions

Antibiotic Resistance Gene = Environmental Pollutant



Pruden, Martinez, Keen:

- ↪ Mammalian origin/
anthropogenic activities
- ↪ Recently emerged
(evolutionary time scale)
- ↪ Association with **mobile genetic elements**
- ↪ **Prevalence** above the normal value usually encountered in the habitat (**quantitative aspect**)

3. Case Study: nptII/nptIII - Pollutant?

Natural baseline in GMO-pristine Agricultural Soils



☞ nptII/nptIII (ARGs):

- Most abundant antibiotic resistance marker genes in plant biotechnology
- Inactivate critically important aminoglycoside antibiotics¹⁾
- Present in commercially cultivated GM crops:
maize MON863, potato EH92-127-1, cotton MON531, MON1445
- GM crop cultivation: **artificial exposure of soil resistome with additional copies of ARGs of anthropogenic origin**

☞ Research Question:

Establishing naturally occurring baseline of nptII/nptIII in maize and potato fields before cultivation of corresponding GM crops to facilitate impact evaluation

3. NptII/nptIII-Environmental Pollutants



Soil Sampling, DNA extraction, qPCR

☞ 100 composite soil samples

- 50x maize fields
- 50x potato fields
- **Representative** selection for Austrian maize and potato growing regions
- 10 single soil extractions/field (predefined, universally deployable sampling scheme; extraction layer: 0 – 25 cm, rhizosphere sensu lato around 10 individual plants; **representative**)
- Approx. 700 g soil/field: 50 g sieved (2 mm)

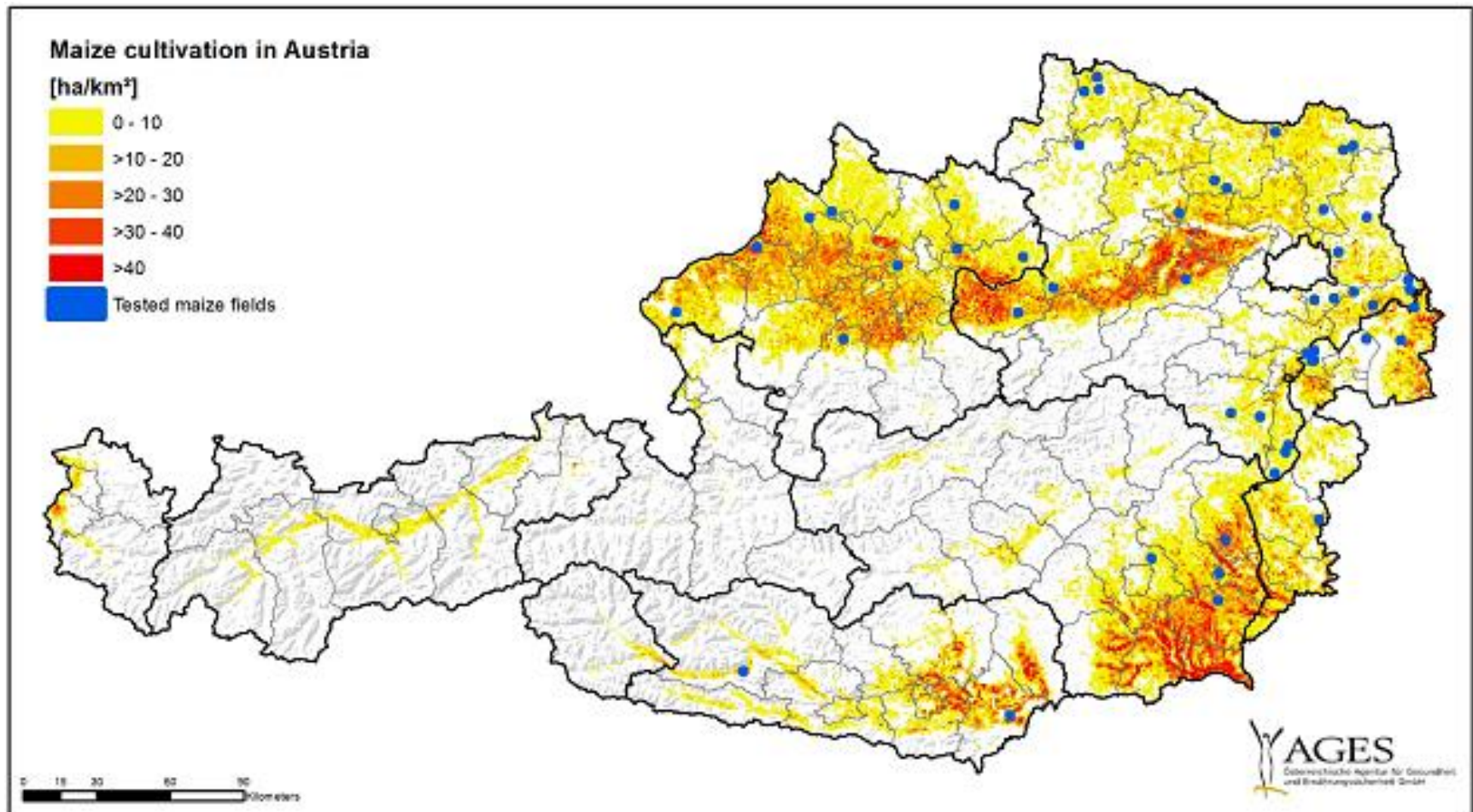
☞ Total soil DNA: 1 g soil

☞ Quantitative TaqMan real time PCR: nptII/nptIII

☞ Strict safety protocol to avoid cross-contaminations

3. NptII/nptIII-Environmental Pollutants

Sampling Area for Austrian Maize fields



3. Case Study: Results

Prevalence and Copy Number of nptII/nptIII

Austrian Agricultural Soils	Copy number / g soil	
	nptII	nptIII
Minimum	31	13
Maximum	856	61 600
Mean	340	4 750
Median	150	1 190
Positive maize + potato fields [95%]	6% [2.2%;12.6%]	85% [76.5%;91.4%]

3. Case Study: Results

NptII/nptIII – Environmental Pollutants?



Classification of **nptII** as environmental pollutant
based on locally obtained data for **Austria**:

Criteria/ environmental pollutant	Observation (nptII relevant)	Explanation
Mammalian/ anthropogenic origin	yes	<i>Klebsiella pneumoniae</i> , human pathogen Transgenic plant: artificially modified sequence
Recently emerged resistance determinant	yes	Anthropogenic propagation
Association with mobile genetic element	yes	Transposon Tn5
Prevalence above the normal value in exposed habitats	yes	Low prevalence in analysed receiving ecosystems in Austria

Classification of **nptIII** as environmental pollutant
based on locally obtained data for **Austria**:

Criteria/ environmental pollutant	Observation (nptIII relevant)	Explanation
Mammalian/ anthropogenic origin	yes	<i>Staphylococcus aureus</i> , <i>Enterococcus faecalis</i> , human commensals/pathogens Transgenic plant
Recently emerged resistance determinant	yes	Anthropogenic propagation
Association with mobile genetic element	yes	Tn5405, Tn3854; conjugative plasmids
Prevalence above the normal value in exposed habitats	variable	Depends on the analysed receiving ecosystem in Austria

4. ARGs as Environmental Pollutants

Conclusions

- ☞ **nptII** → environmental pollutant:
 - if released as ARM gene in the tested ecosystems
- ☞ **nptIII** → environmental pollutant:
 - low background level environments
- ☞ Case-by-case evaluation
 - different „ARG – environment“ combinations have to be re-evaluated
- ☞ Quantitative data: naturally occurring ARG baseline
- ☞ Representative Sampling
- ☞ Agricultural soils: Hotspot for **ARGs**, ARB and AB transfers
- ☞ Free extracellular DNA:
 - relevant for ARG risk assessment

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