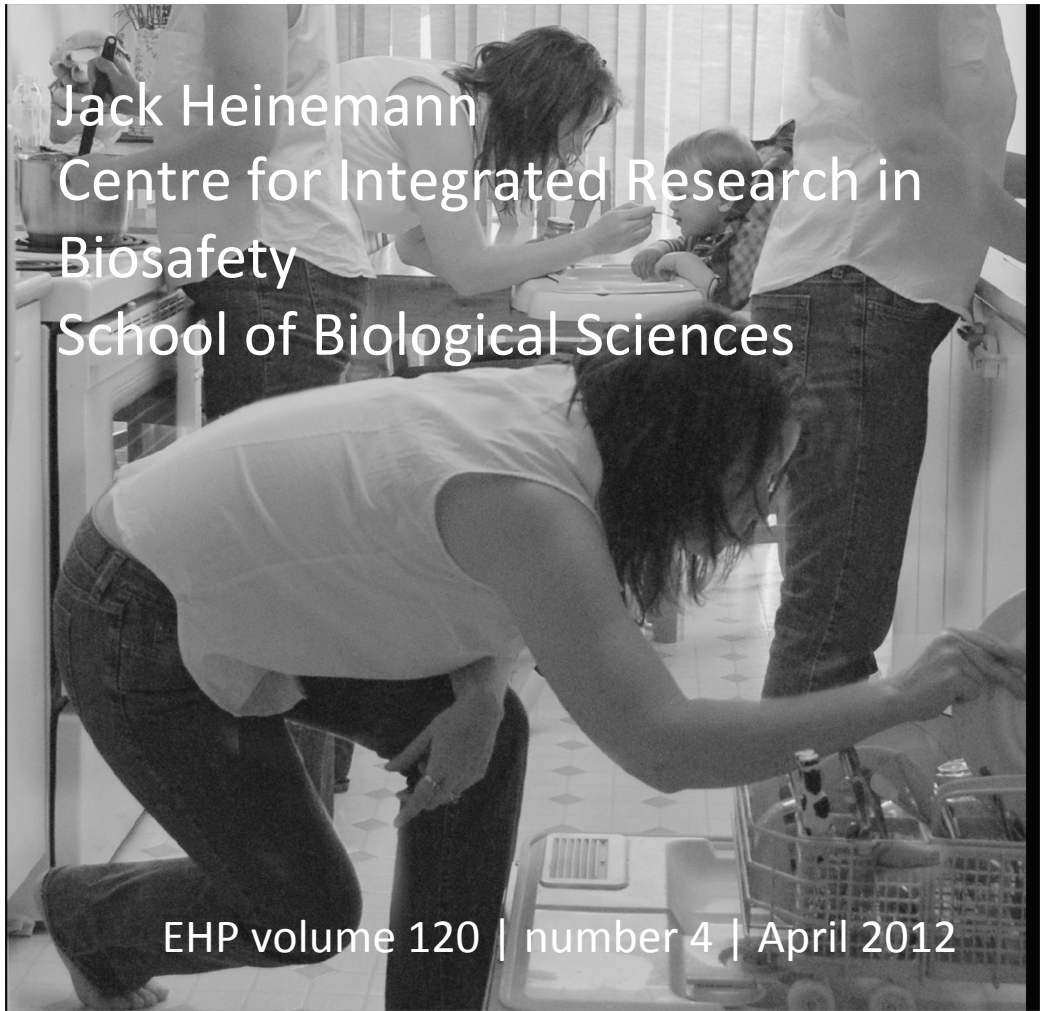
How industrial agriculture threatens the existence of the symbiotic human, home and farm microbiome



UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

A chemically intensive world

80-100,000 different chemicals in commerce

- Consumer products are a primary source of chemical exposures
- Little information is available on the chemical ingredients of products
- Or concentrations at which they are present

A chemically intensive world

80-100,000 different chemicals in commerce

Exposure analysis is limited because of eg,:

1. trade secrets hiding ingredients
2. unknown contaminants in products
3. accumulation of degradation/synthesis (eg, from cooking, microbial conversion) products
4. residues from packaging

A chemically intensive world

Pesticides

- US EPA permits over 200 different pesticides to be used for lawn care, and these are often mixed together and sold as chemical combinations.
- The US Fish and Wildlife Service reported that “homeowners use up to 10 times more chemical pesticides per acre on their lawns than farmers use on crops.”
- The packaging of many lawn-care chemicals is porous, releasing vapors at retail outlets

Biggest use herbicides

Dicamba

Glyphosate

2,4-D

Every bushel matters

The most advanced herbicide-tolerant technology available, Enlist™ corn provides exceptional weed control and application flexibility.

The Enlist corn trait takes weed control program to the next level — building on the Roundup Ready system by adding tolerance to a new 2,4-D. Enlist corn allows you to use Enlist Duo herbicide to maximize your corn yield.

Weed Control System
Enlist™
Corn

An antibiotic intensive world

Agricultural use

- > ½ of all antibiotic use is in agriculture
- <20% of antibiotic administered is metabolised
- >MIC concentrations found in manure

10 ppb or less of antibacterial drugs, pesticides and veterinary drugs could increase antibacterial resistance in bacteria.
Kleiner et al 2007

Table 1

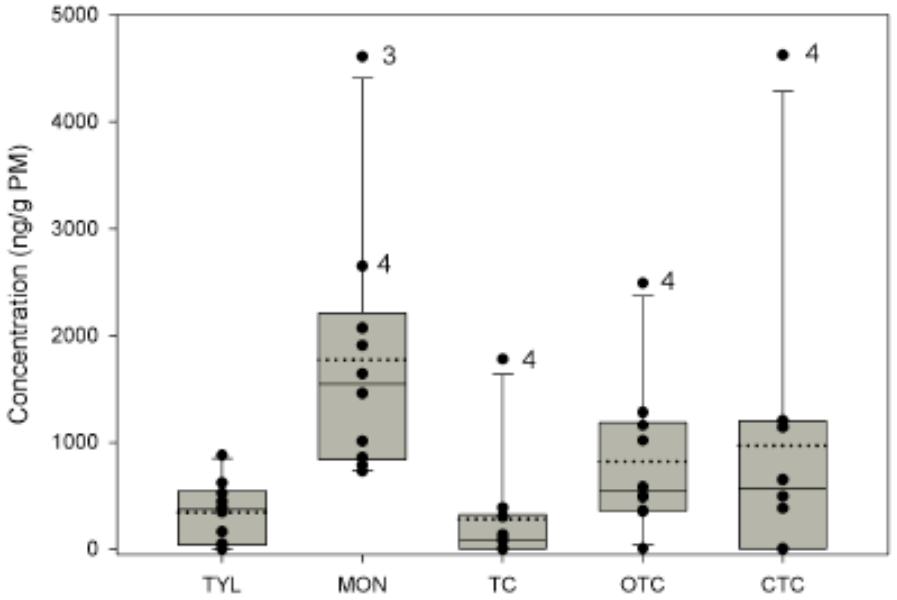
Detection of antibiotic compounds in recent studies of pig manure samples

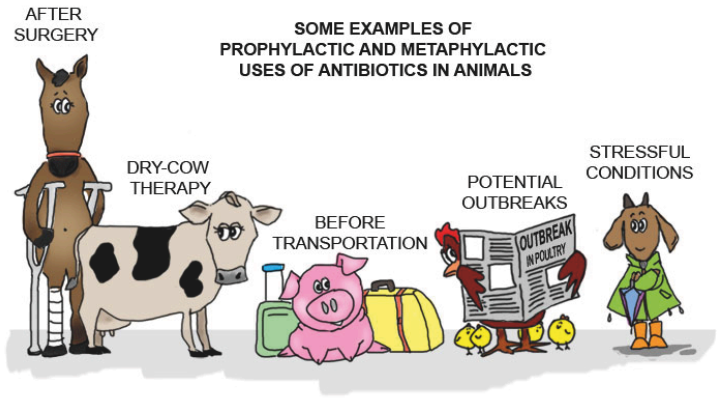
Country	Antibiotic compound	Sample size	Detection frequency [%]	Max. concentration [mg kg^{-1}]	Study
China	Enrofloxacin	61	49	33	[80]
	Sulfamonomethoxine	61	48	4	
	Oxytetracycline	61	41	59	
Germany	Tetracyclines	305	54	53	[17•]
	Sulfonamides	305	51	38	
Austria	Chlortetracycline	30	57	46	[13]
	Sulfadimidine	30	60	20	

An antibiotic intensive world

Agricultural use

- multiple antibiotics at therapeutic concentrations found in wind-borne particulate matter down wind of large farms in US (resistance genes too)





Intersections between antibiotics and herbicides

Agriculture/environment

Urban

Biocides can induce antibiotic resistance

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8771–8774, December 1985
Microbiology

Nonheritable resistance to chloramphenicol and other antibiotics induced by salicylates and other chemotactic repellents in *Escherichia coli* K-12

(aspirin/acetate/benzoate/multiple drug resistance/chemotaxis)

JUDAH L. ROSNER

Exposure of Escherichia coli ATCC 12806 to Sublethal Concentrations of Food-Grade Biocides Influences Its Ability To Form Biofilm, Resistance to Antimicrobials, and Ultrastructure

Question: do common commercial formulations of herbicides induce a response?

Journals.ASM.org

Transient and Sustained Bacterial Adaptation following Repeated Sublethal Exposure to Microbicides and a Novel Human Antimicrobial Peptide

Sarah Forbes,[a] Curtis B. Dobson,[b] Gavin J. Humphreys,[a] Andrew J. McBain[a]
Manchester Pharmacy School[a] and Faculty of Life Sciences,[b] The University of Manchester, Manchester, United Kingdom

The organisms

Escherichia coli

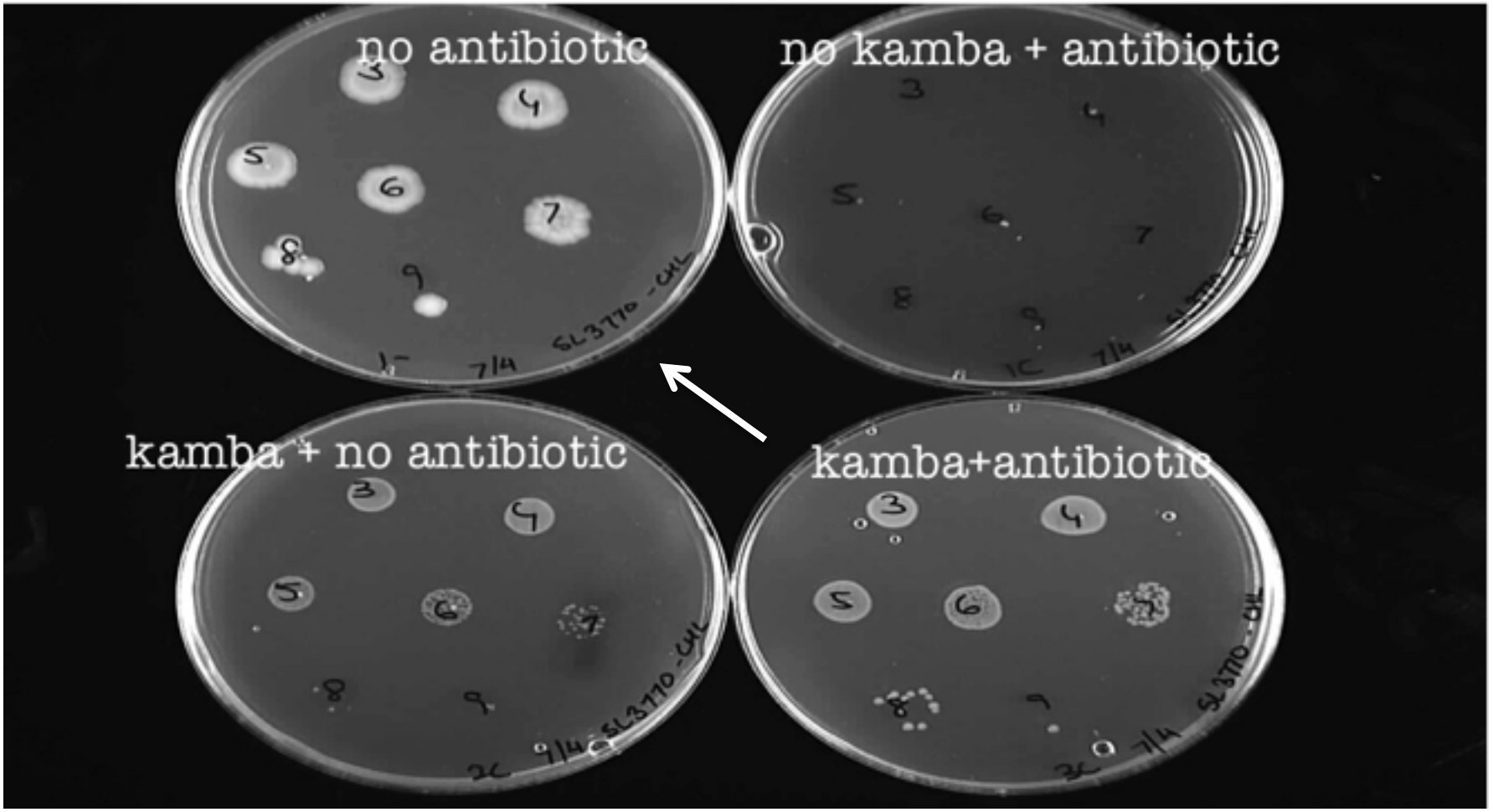
- Gram negative
- Enterobacteriaceae
- Part of the normal intestinal flora of warm blooded animals
- Some strains are pathogens:
  - Food poisoning
  - Gastroenteritis
  - Urinary tract infections

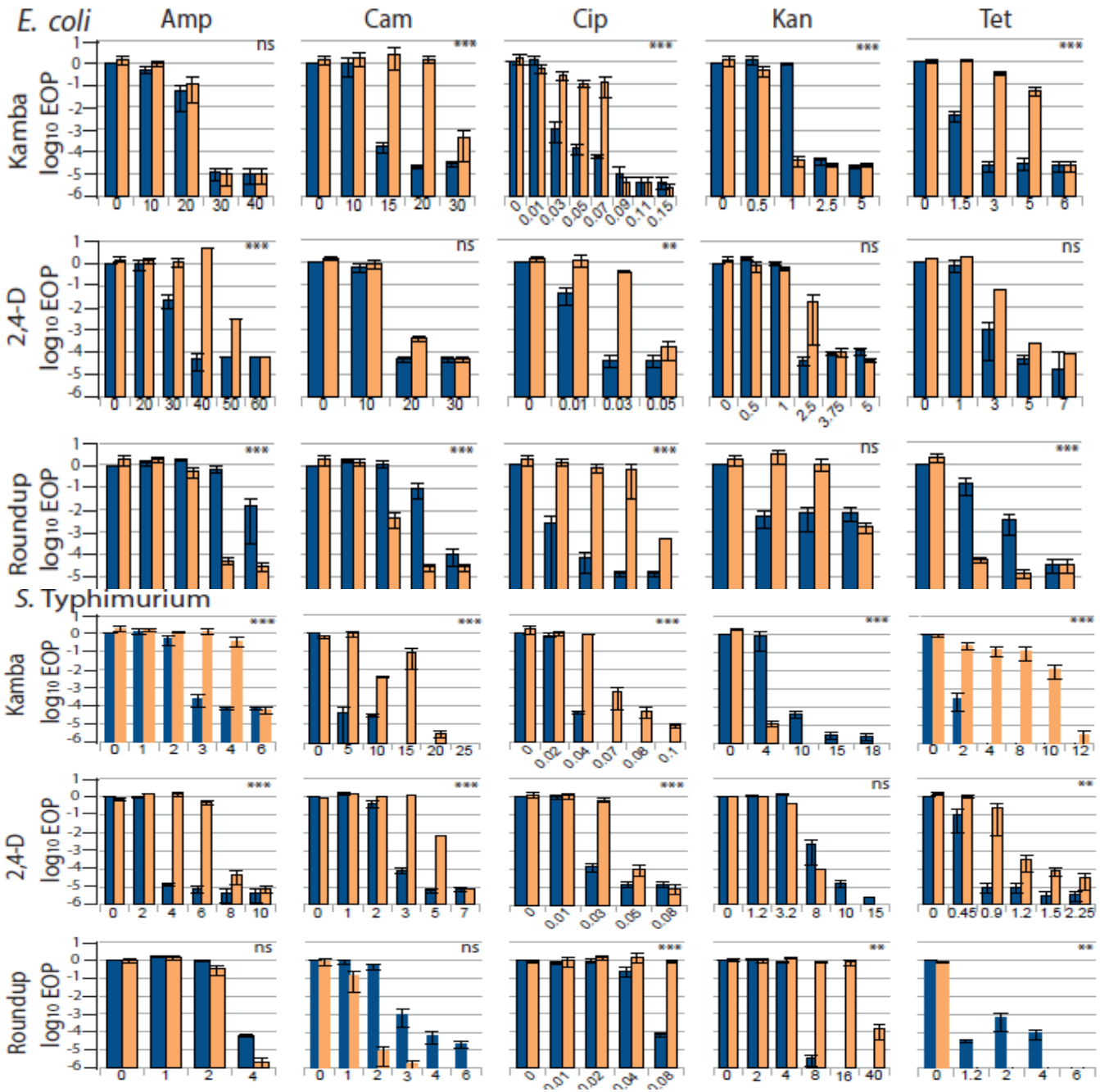
Salmonella enterica serovar Typhimurium

- Gram negative
- Enterobacteriaceae
- Common in intestines of warm blooded animals
- Pathogens:
  - Food poisoning
  - Typhoid fever

Kurenbach, B., Marjoshi, D., Amabile-Cuevas, C. A., Ferguson, G. C., Godsoe, W., Gibson, P. & Heinemann, J. A (2015) *mBio* **6**, e00009-00015.

Efficiency of plating (EoP)




E. coli
Amp
Cam
Cip
Kan
Tet
Kamba
2,4-D
Roundup
log10 EOP
S. Typhimurium
ns
*
**
***

Three effects

✗ no observed effect

↑ significantly increased *resistance*

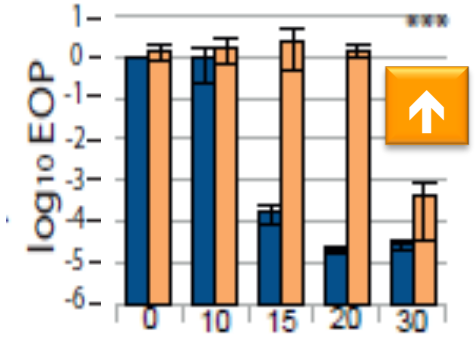
↓ significantly increased *susceptibility*

E. coli	**Amp**	**Cam**	**Cip**	**Kan**	**Tet**
Kamba	✗	↑	↑	↓	↑
2,4-D	↑	✗	↑	✗	✗
Roundup	↓	↓	↑	✗	↓
S. enterica					
Kamba	↑	↑	↑	↓	↑
2,4-D	↑	↑	↑	✗	↑
Roundup	✗	✗	↑	↑	↓

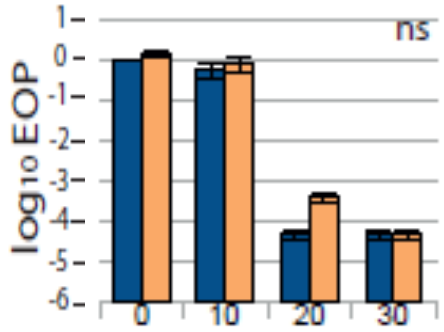
Killing curves - chloramphenicol

E. coli

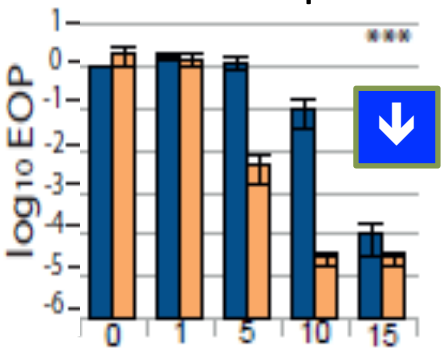
Kamba



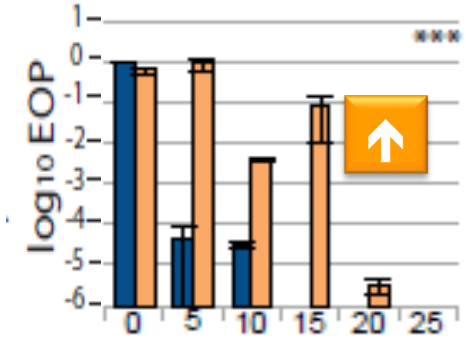
2,4-D

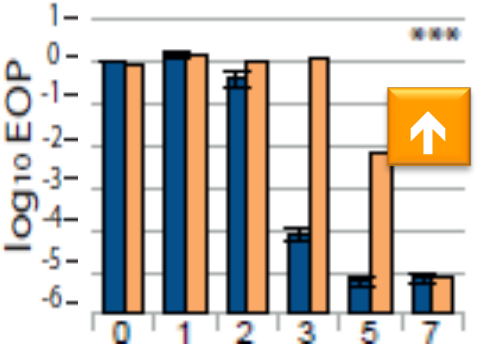


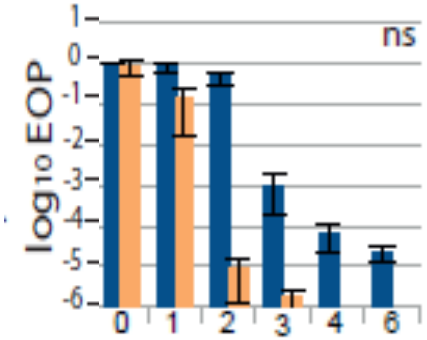
Roundup



S. enterica







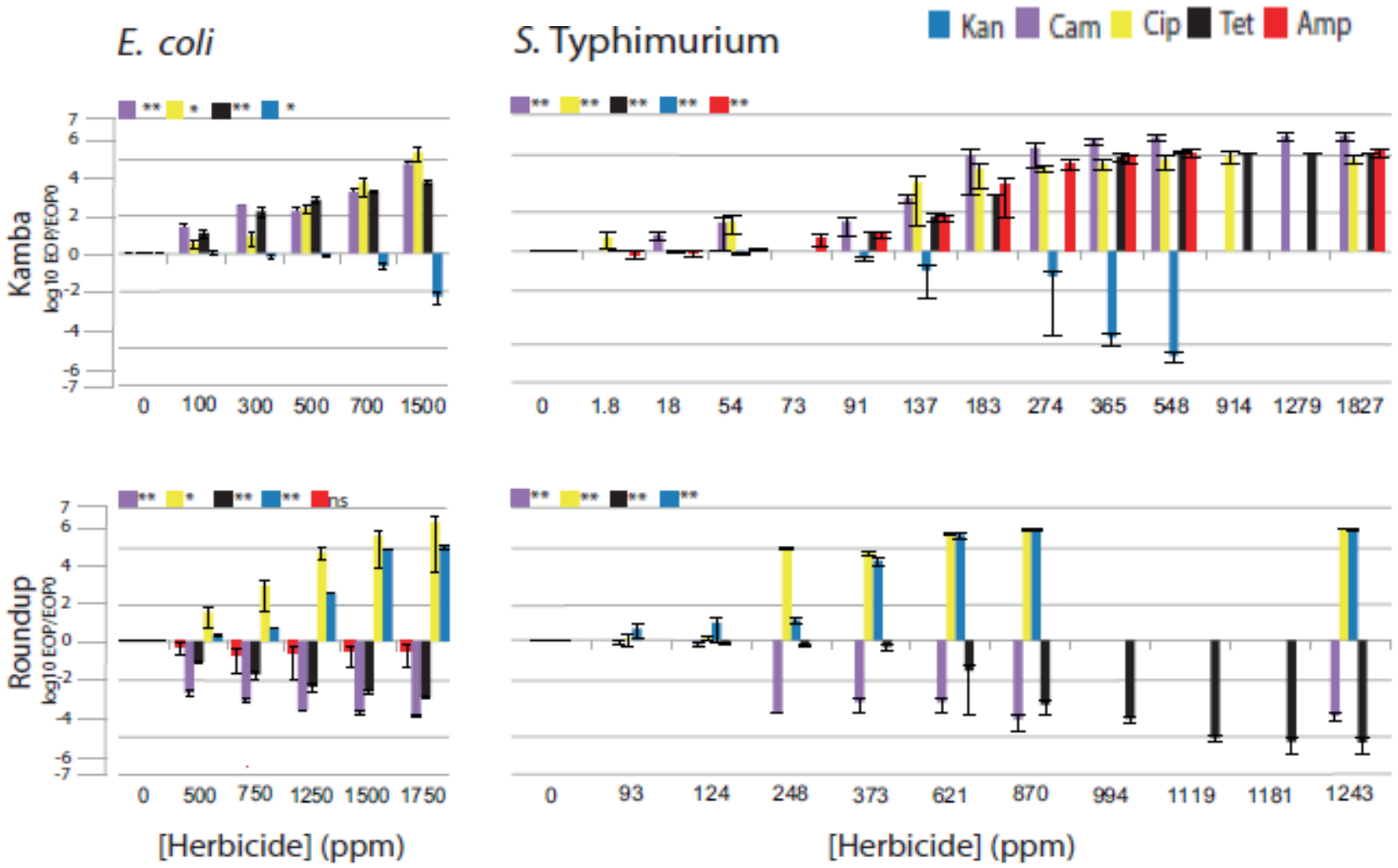
Blue: no herbicide

Orange: with herbicide

Fold change in “MIC”

Antibiotic	Herbicide	*E. coli*	*S. enterica*
Ampicillin	Kamba	0	**2.3**
	2,4-D	**1.5**	**2**
	Roundup	NA	0
Chloramphenicol	Kamba	**2**	**2.2**
	2,4-D	0	**2.3**
	Roundup	1.5	2.5
Ciprofloxacin	Kamba	**1.7**	**2.7**
	2,4-D	**1.7**	**1.7**
	Roundup	**1.8**	**5.8**
Kanamycin	Kamba	2.5	2.5
	2,4-D	1.5	1.2
	Roundup	NA	**5.0**
Tetracycline	Kamba	**2.0**	**3.3**
	2,4-D	**1.7**	**2.5**
	Roundup	3	1.66

[Herbicide] causing significant response



Response is in relevant range for use

Effects were detected at concentrations that are **above** currently allowed MRL on food

But they were seen within application levels used in agriculture and urban areas

Cocktail effects

<250 ppm Kamba = no effect

+

<250 ppm aspirin = no effect

---

250 ppm combined = effect

Conclusions

Both herbicides and antibiotics are used in unprecedented quantities with complex interaction pathways

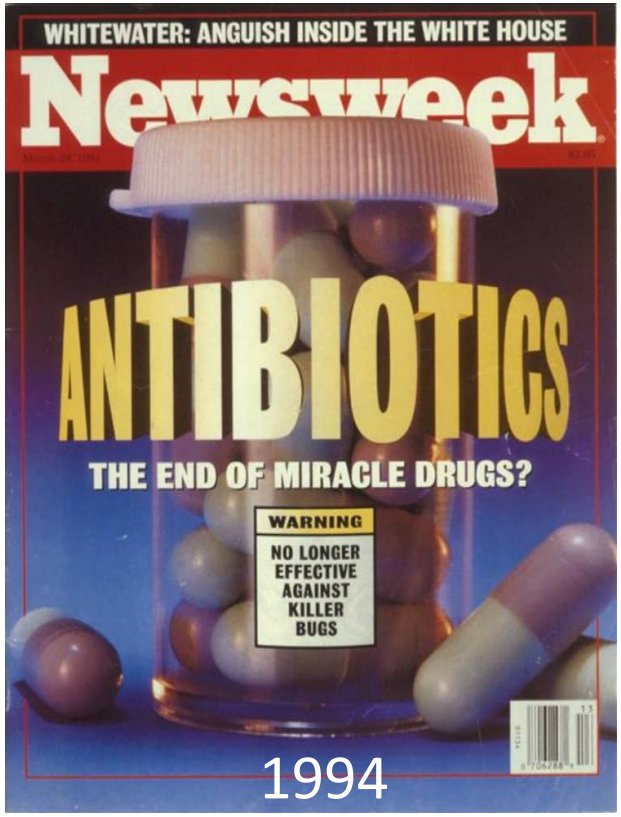
Commercial herbicide formulations induce an antibiotic response in medically relevant bacteria

The effect is large enough (2-6x MIC) to theoretically significantly undermine therapy

Different chemicals can combine to cause the effect

The initial mechanism is induction of efflux (and possibly decrease permeability)

Later, the population can transition to mutational resistance



Relevance

A focus on antibiotics is not enough to preserve antibiotic use

- To bacteria, a toxin is a toxin is a toxin (only we put into categories of medicine, pesticide, pollution)
- Regulatory risk assessment of products should be based on more than active ingredient alone
- Chemical safety regulation should consider combinatorial effects

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