Antibiotic stewardship requires an integrated approach to minimising the chemical exposome.

He taura whiri kotahi mai anō te kopunga tai no i te pu au

From the source to the mouth of the sea all things are joined together as one

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UC
UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

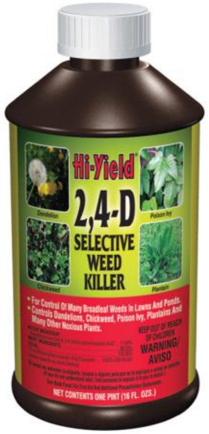
Chemical environment

- 80-100,000 different chemicals in commerce
- universal human exposure to the 5,000 produced in greatest volume

Dicamba

Glyphosate

2,4-D



source: The Lancet Commission http://dx.doi.org/10.1016/S0140-6736(17)32345-0

Exposures to common herbicide formulations induce antibiotic resistance

↑ significantly increased *resistance*

↓ significantly increased *susceptibility*

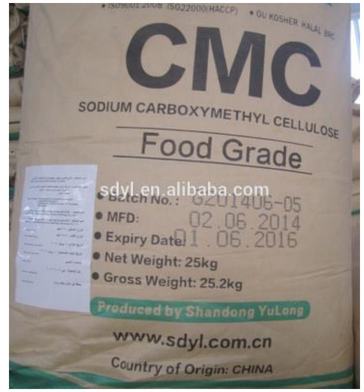
X no observed effect

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

Δ MIC: 0-300%

mBio **6**, e00009-00015, doi:10.1128/mBio.00009-15 (2015)

Exposures to common ingredients in herbicide formulations induce antibiotic resistance



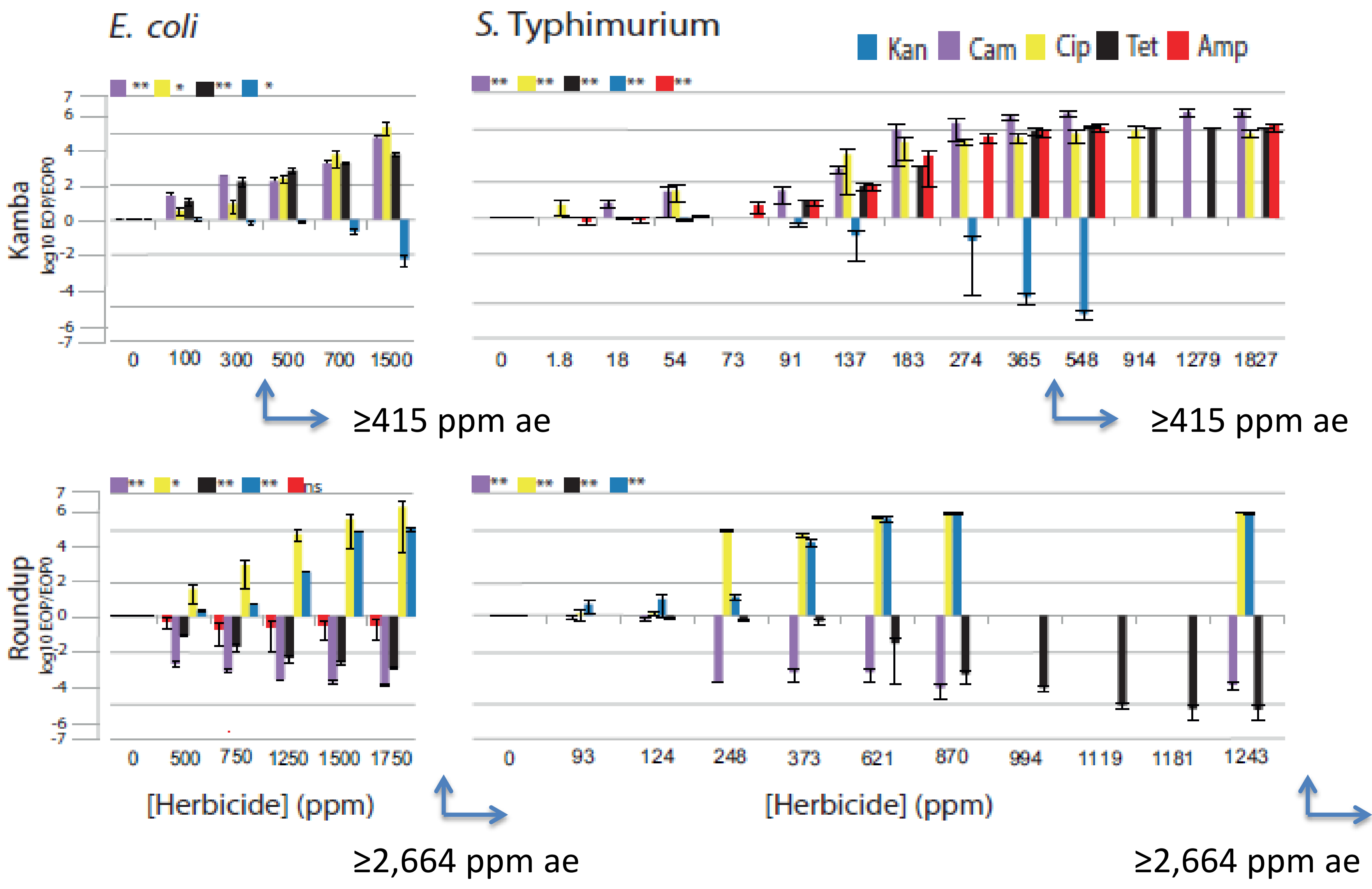
Δ MIC: 0-300%

Table 2. Fold-change shift in antibiotic effectiveness following exposure to herbicide ingredients

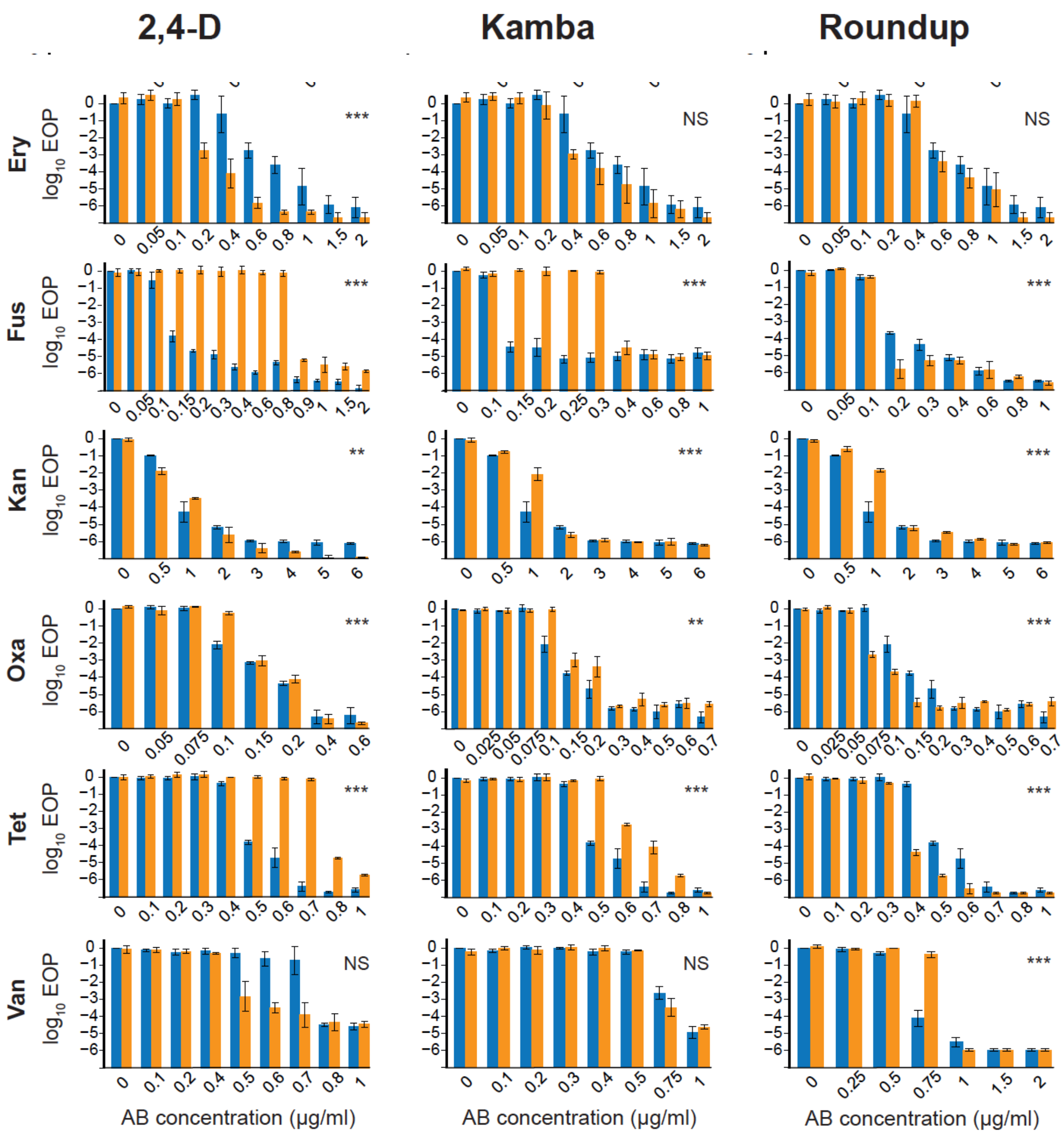
	Amp	Cam	Cip	Kan	Tet
S. enterica					
Active ingredients					
Dicamba	1.3 (1500)	7 (1500)	3.5 (1500)	0 (1500)*	2.7 (1500)
2,4-D	NS	2.5 (600)	1.8 (5000)	4 (6000)*	2.2 (500)
Glyphosate	1.8 (3000)	1.5 (3000)*	2 (200)	5 (200)	1.4 (3000)*
Surfactants					
Tween80	NS	2.3	1.2	1.8	1.8
CMC	1.7	NS	NS	1.5	1.4
E. coli					
Surfactants					
Tween80	NS	1.6	0	1.5*	NS
CMC	1.25*	NS	NS	4	NS

Microbiology. 2017 Nov 17. doi: 10.1099/mic.0.000573.

[Herbicide] causing significant response



Herbicide effects on *Staph aureus*



erythromycin

fusidic acid

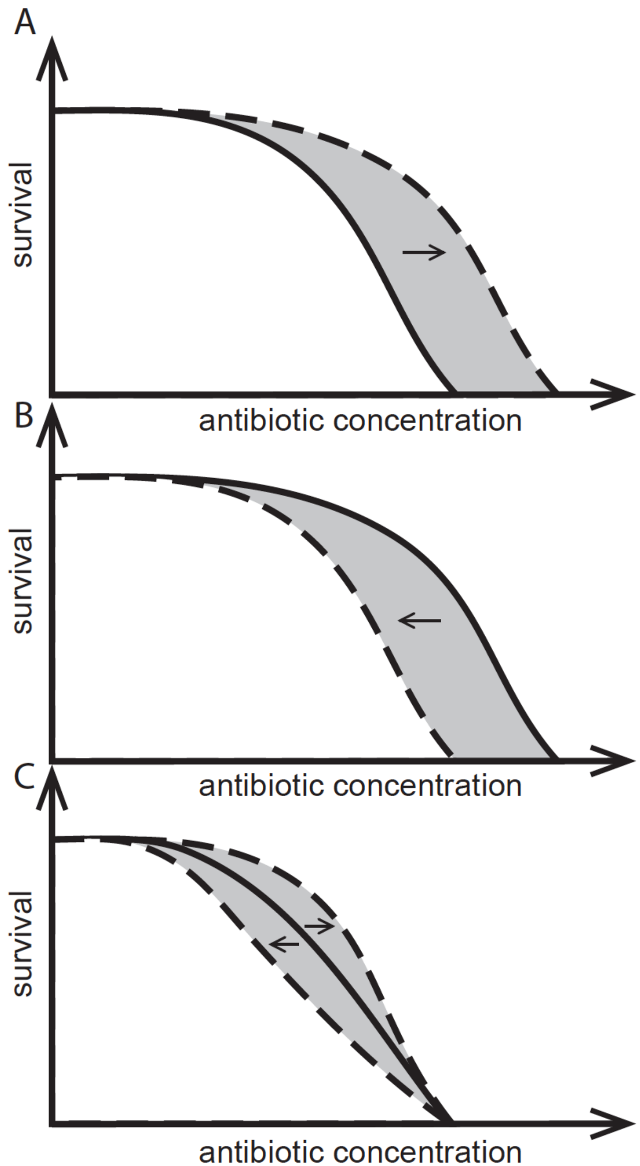
kanamycin

oxacillin

tetracycline

vancomycin

Selection space



↑ significantly increased *resistance*

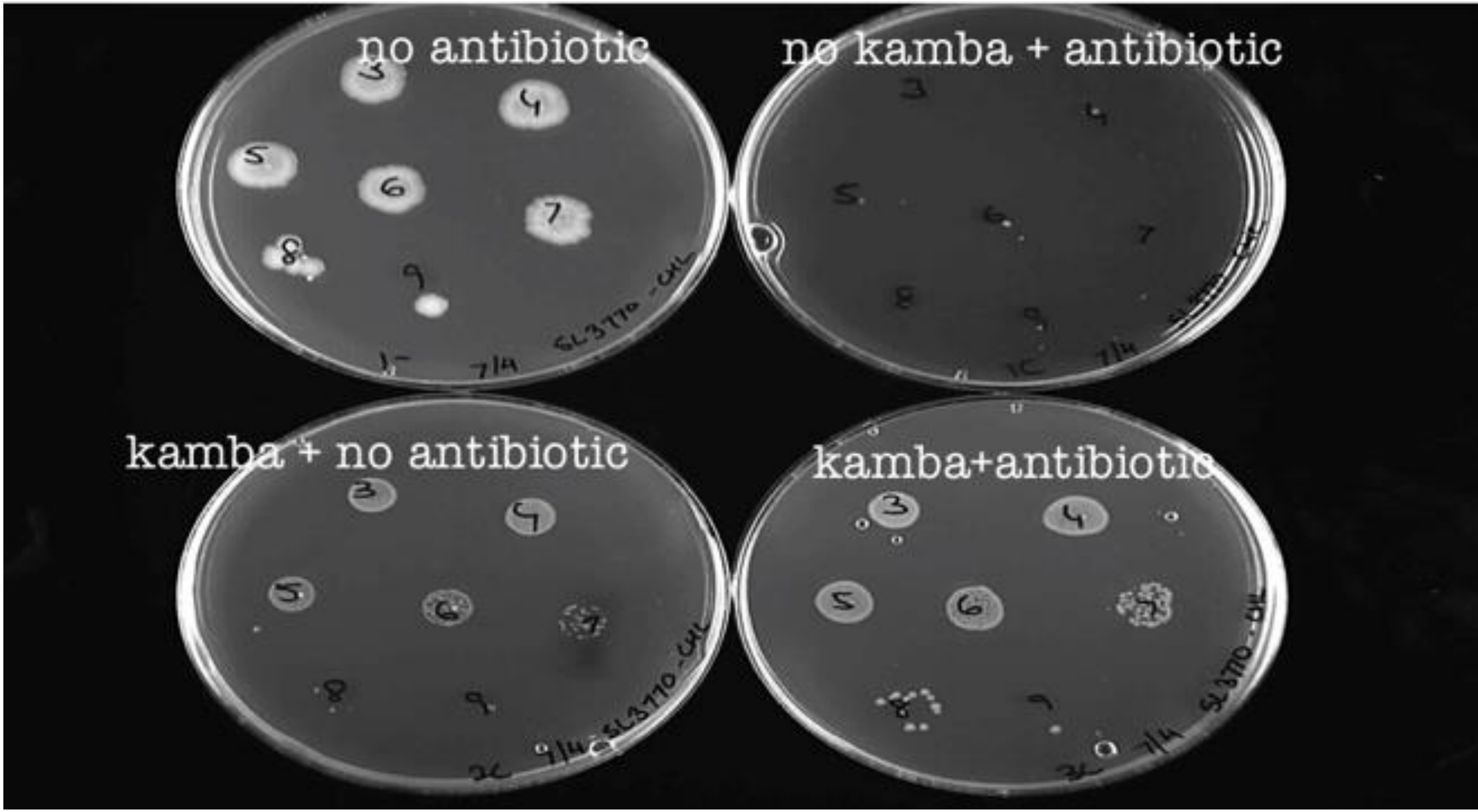
↓ significantly increased *susceptibility*

X no observed effect

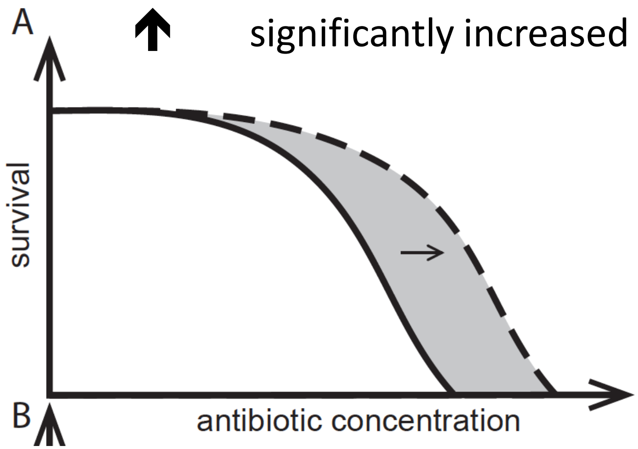
E. coli	**Amp**	**Cam**	**Cip**	**Kan**	**Tet**
Kamba	X	↑	↑	↓	↑
2,4-D	↑	X	↑	X	X
Roundup	↓	↓	↑	X	↓
S. enterica					
Kamba	↑	↑	↑	↓	↑
2,4-D	↑	↑	↑	X	↑
Roundup	X	X	↑	↑	↓

PeerJ. 2018 Oct 12;6:e5801. doi: 10.7717/peerj.5801. eCollection 2018.

Efficiency of plating (EoP)



Selection space

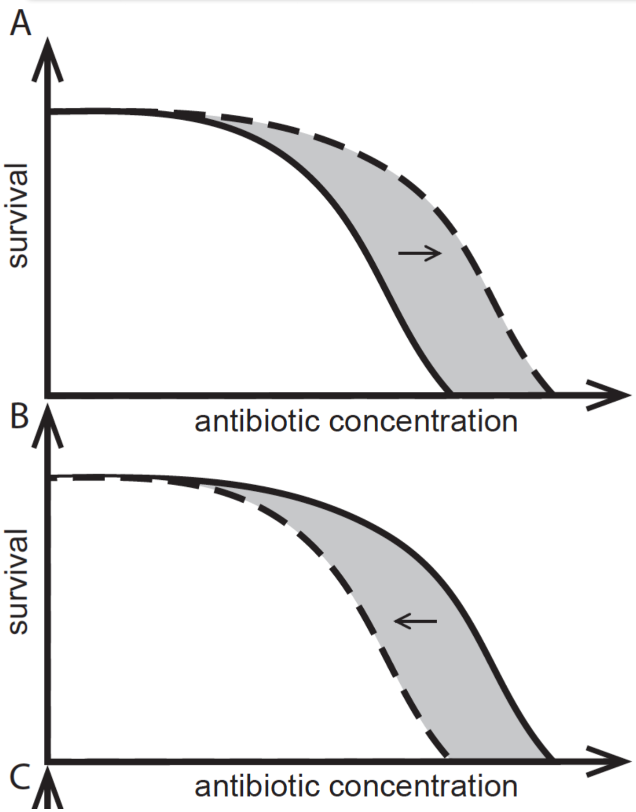


Rates of acquired ciprofloxacin resistance

	LB	LB+Herbicide	LB+Herbicide+Cip
S. enterica			
Kamba	$3.57 \times 10^{-6}(1.27 \times 10^{-6})$[b]	$2.01 \times 10^{-4}(1.95 \times 10^{-4})$	1.30×10^{-2} (1.29×10^{-2})[c]
Roundup	$3.57 \times 10^{-6}(1.27 \times 10^{-6})$[b]	2.91×10^{-5} (2.47×10^{-5})	2.79×10^{-2} (1.71×10^{-2})[a,c]
E. coli			
Roundup	1.80×10^{-9} (1.62×10^{-9})	1.97×10^{-10} (5.46×10^{-11})	2.72×10^{-5} (2.67×10^{-5})[d]

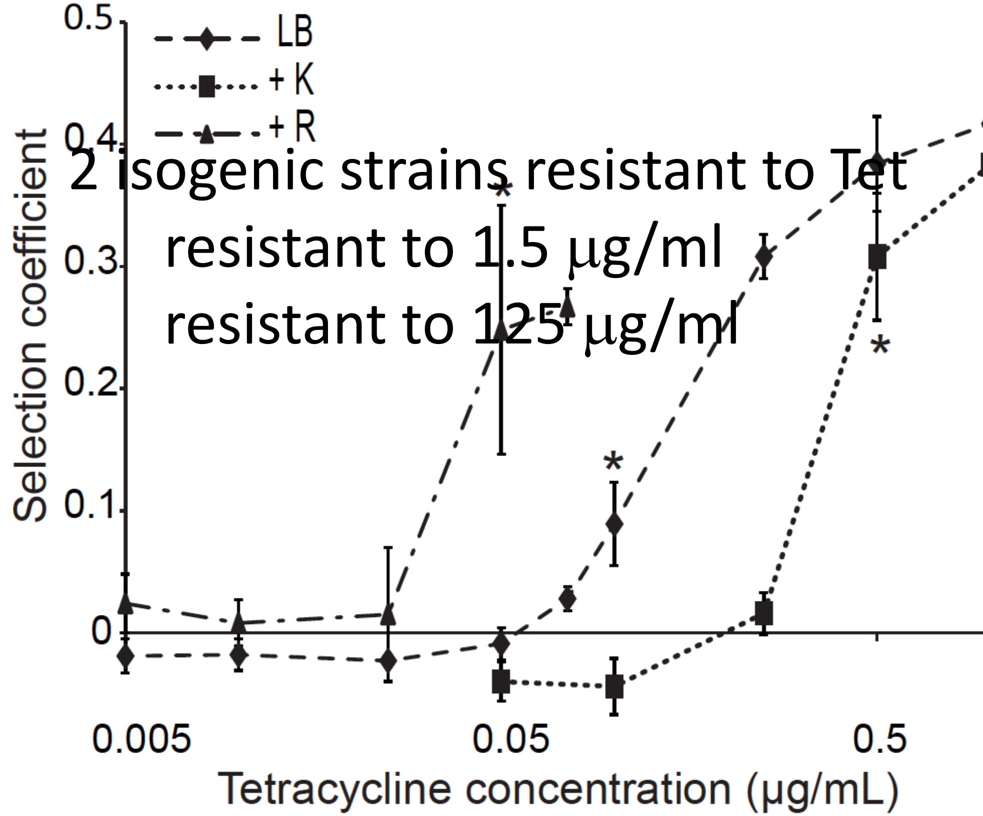
PeerJ. 2018 Oct 12;6:e5801. doi: 10.7717/peerj.5801. eCollection 2018.

Competition between genotypes at varying tetracycline concentrations



K+Tet ↑ significantly increased *resistance*

R+Tet ↓ significantly increased *susceptibility*



2 isogenic strains resistant to Tet

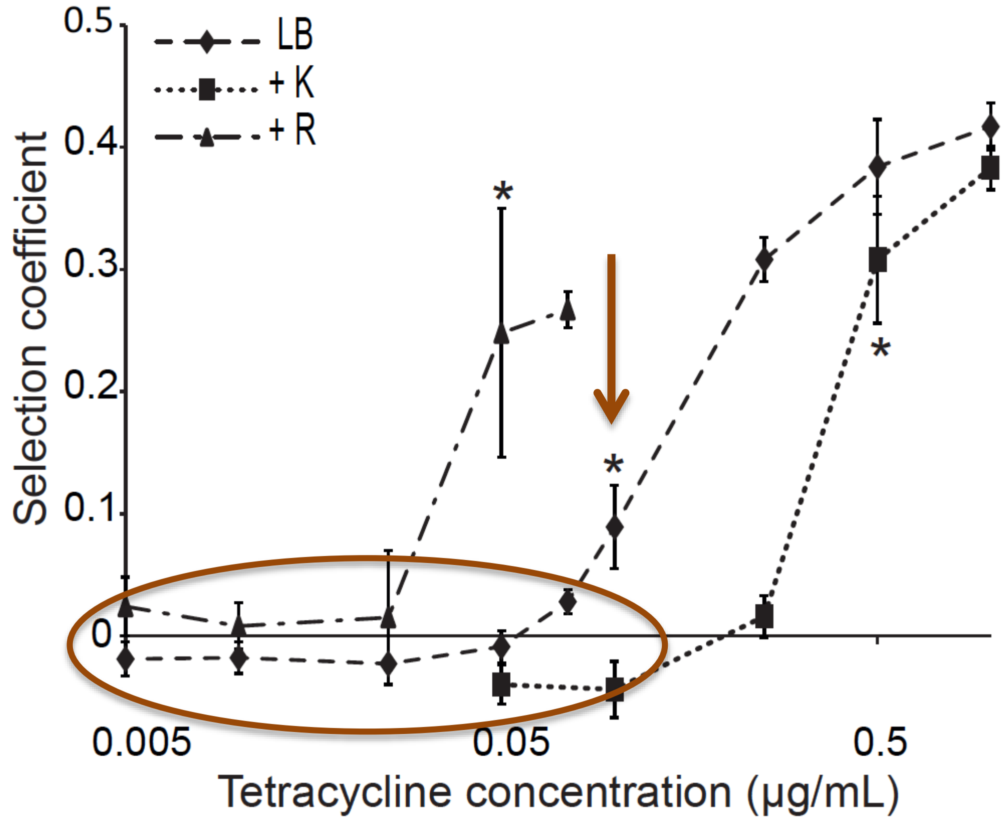
resistant to 1.5 μg/ml

resistant to 125 μg/ml

PeerJ. 2018 Oct 12;6:e5801. doi: 10.7717/peerj.5801. eCollection 2018.

Competition without herbicide

- Strains equally fit up to ~0.1 μg/ml tetracycline
- Competition between genotypes at ≥ ~0.2 μg/ml



PeerJ. 2018 Oct 12;6:e5801. doi: 10.7717/peerj.5801. eCollection 2018.

Herbicides alter selection parameters

↑ significantly increased *resistance*

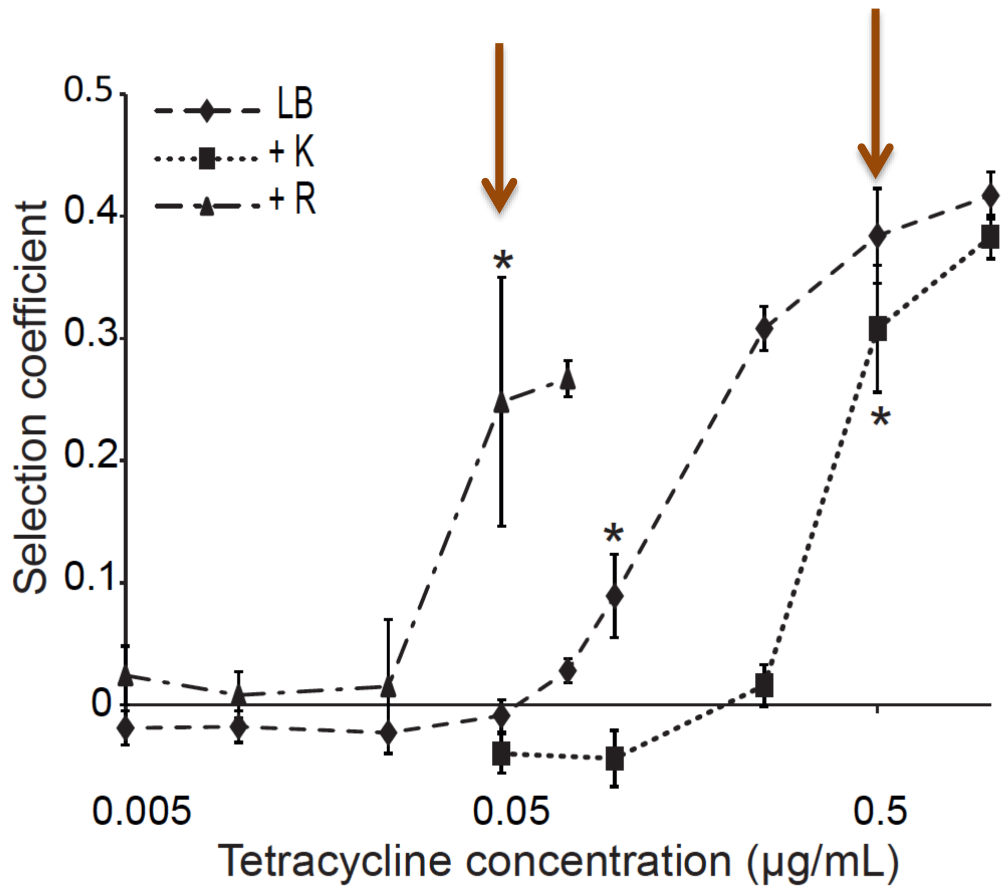
1. Competition between genotypes occurs at higher concentrations of antibiotic and favours genotypically most resistant

↓ significantly increased *susceptibility*

2. Competition between genotypes occurs at lower concentrations of antibiotic and favours genotypically most resistant

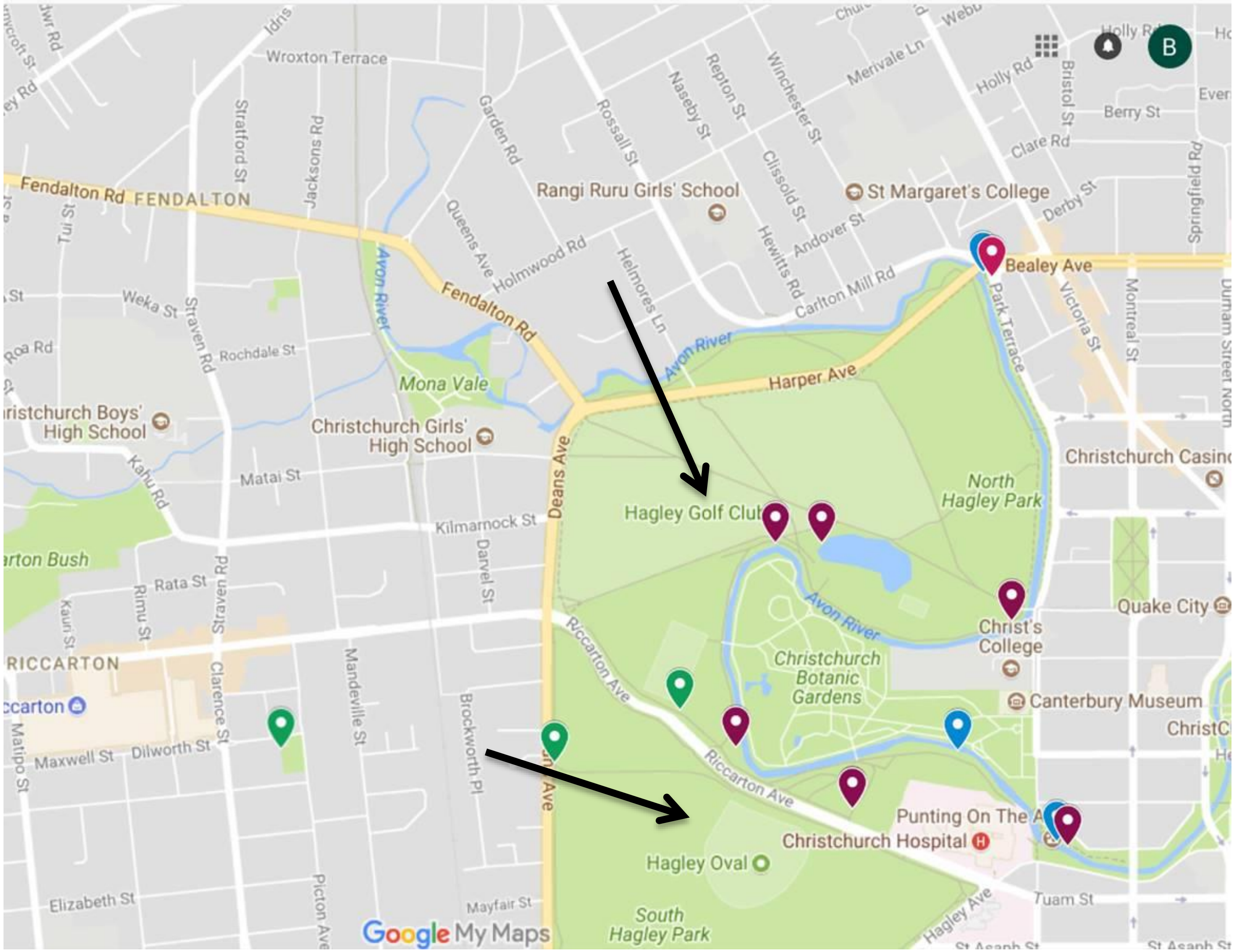
K+Tet ↑ significantly increased *resistance*

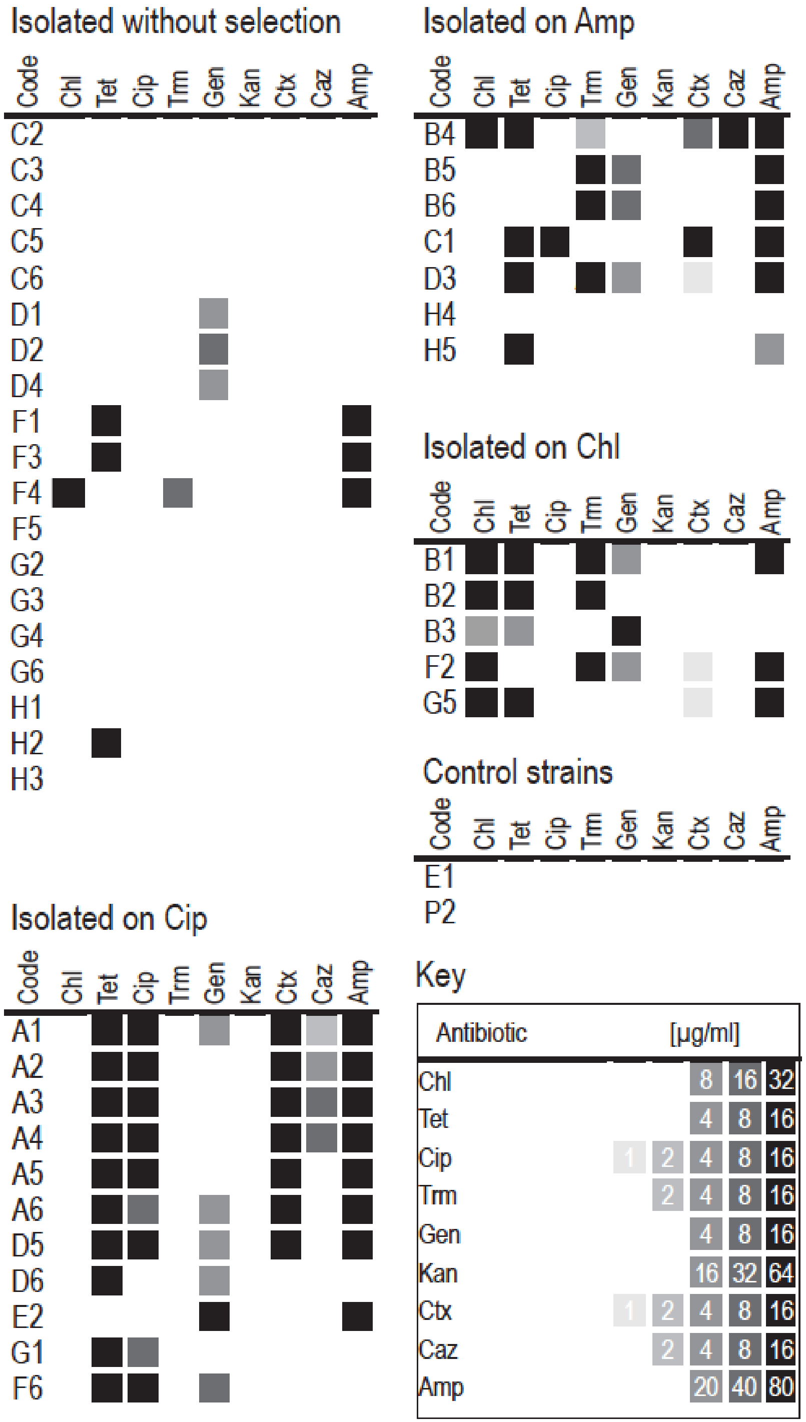
R+Tet ↓ significantly increased *susceptibility*



PeerJ. 2018 Oct 12;6:e5801. doi: 10.7717/peerj.5801. eCollection 2018.

Think local: Avon/Ōtākaro





Frequency of multi-resistance

high frequencies of MDR

high MICs in MDR strains

MICs

Conclusions

Commercial herbicide formulations, active ingredients/surfactants induce an antibiotic response in medically relevant bacteria

The effect is caused above MRL but well below application rate

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

The effect always favours evolution of the genotypically resistant strains with the highest MIC in any competition.

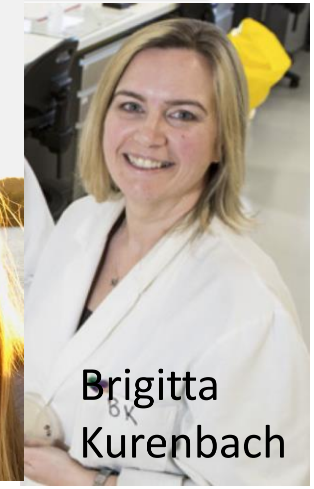
Real world consequences?

Antibiotic resistant *E. coli* were routinely isolated from the Avon River.

Frequencies of MDR from the Avon River were as high as 98%.

Acknowledgements

Amy Hill



Delphine Marjoshi

Gayle Ferguson, Massey U
Mark Silby, U Mass-Dartmouth
Adam Bitzer, U Mass-Dartmouth

Acknowledged for cooperation:
Christchurch City Council
Ecan
Canterbury Medical Officer

William Godsoe, Lincoln U
Mitja Remus-Emsermann, UC
Jon Harding, UC
Carlos Amabile-Cuevas, Mexico City
Brent Gilpin, ESR Ltd.
Mark Silby, U Mass-Dartmouth

Funding for this research
University of Canterbury
Philanthropic donors

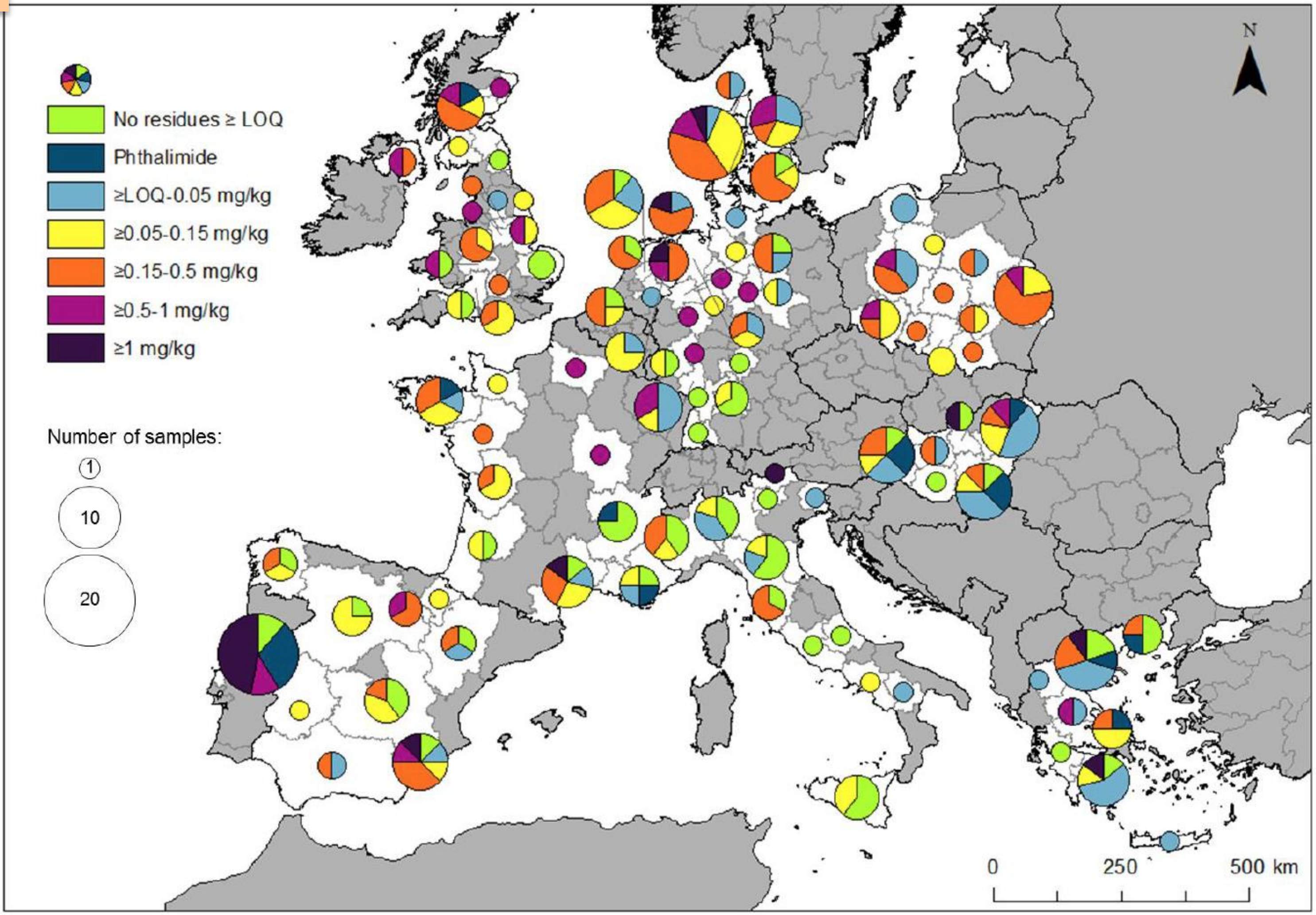
Transmissible resistance-ceftazidime

Strain	Recipient	Antibiotics	Transconjugants CFU/ml	Transmission Frequency
TC-L3Cip-3	RR1-S	STR/CTX	5.8×10^4	0.05%
TC-CMB28	RR1-S	STR/CTX	5.6×10^3	0.02%

Table 1: conjugation frequency of two strains with presumptive F-plasmids mated with a streptomycin resistant recipient.

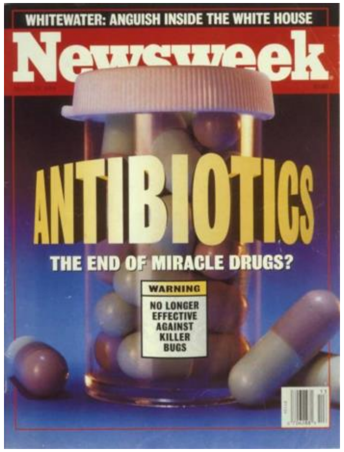
Impact Evidence

Pesticides



Silva et al STOTEN, in press

Dominant paradigms for a future with antibiotics



Invention faster than resistance

New drugs developed faster than old drugs fail

Challenges:

high efficacy

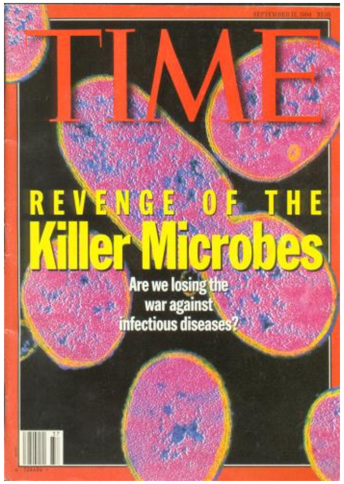
low human toxicity

Resistance slower than invention

Old and new drugs work longer

Opportunity:

more time to find new drugs



Example of scale of use

635,029,318 kg of glyphosate was used worldwide

Table 1. Herbicide use by sector in millions of kilograms* of active ingredient.

Active ingredient	United States	
	Agriculture	Other**
glyphosate	127	6
2,4-D	16	6
atrazine	31	not reported
pendimethalin	5	3
dicamba	2	1

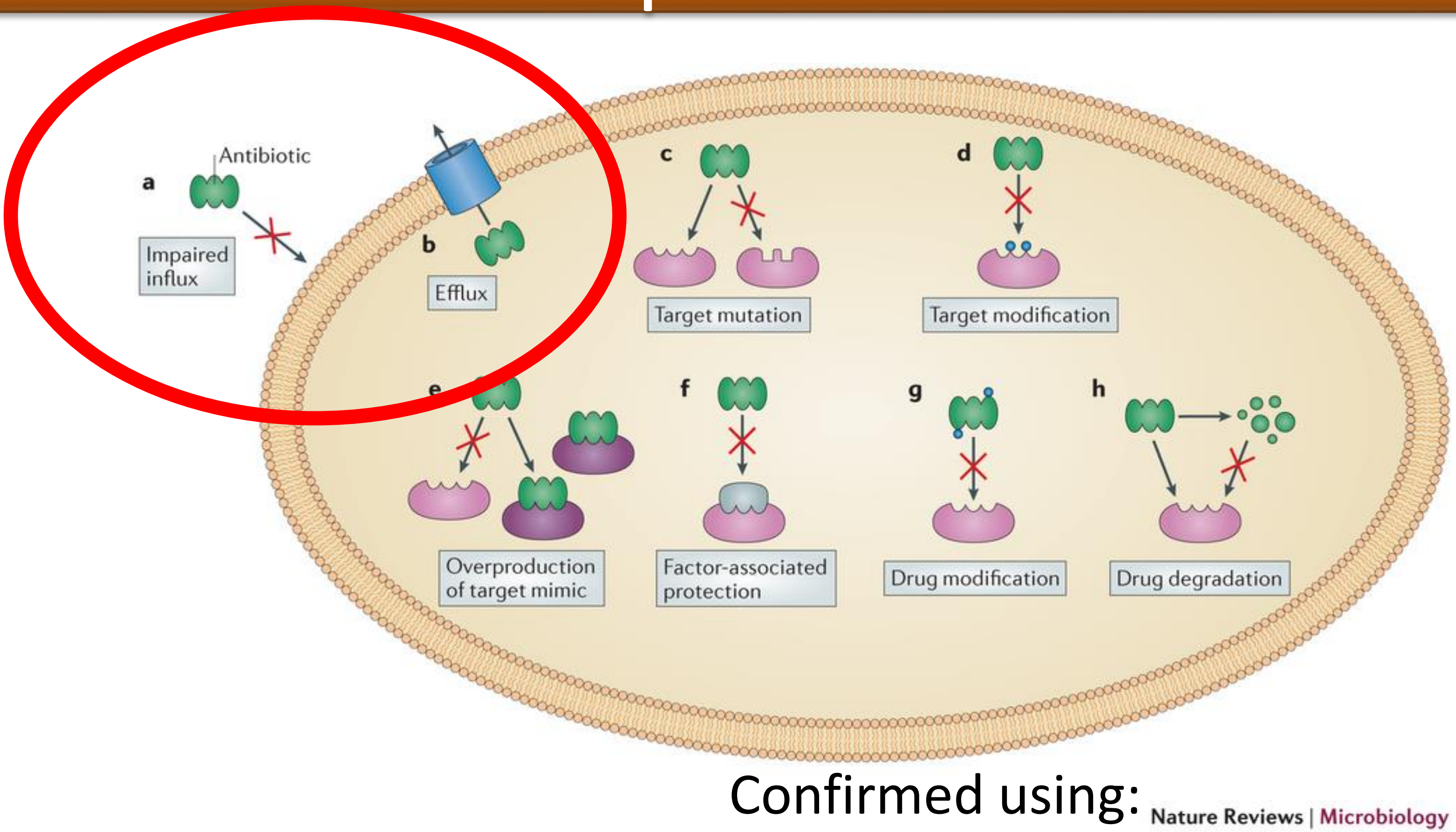
* Based on mid range of estimates for 2012.

**Home and garden combined with government and industry.

Source: (24)

Swanson et al., 2014; US Geological Survey, 2012

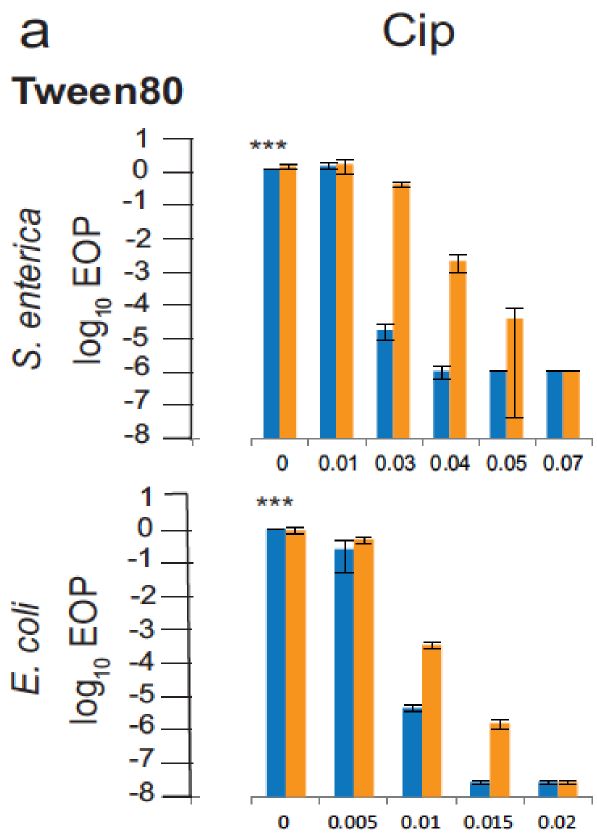
Biochemistry of resistance: adaptive response



Confirmed using:

1. a specific efflux pump inhibitor
2. efflux pump gene knockouts
3. transcriptomics.

Tween80 used in ciprofloxacin formulations



MIC: increases 250%

MIC: increases 75%

Microbiology. 2017 Nov 17. doi: 10.1099/mic.0.000573.

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

Herbicides induce adaptive response

1. PAβN reverses resistance

Condition	EoP (- PAβN)	EoP (+ PAβN)
LB	1	1.093 (0.093)
Kamba	1.42 (0.49)	0.292 ($7x10^{-3}$)
Cam	$2.3x10^{-3}$ ($1.7x10^{-3}$)	$<10^{-7}$**
Kamba + Cam	1.01 (0.17)	$<10^{-7}$**
Roundup	0.8 (0.44)	$<10^{-7}$**
Kan	$8.7x10^{-5}$ ($4.07x10^{-5}$)	0.052 (0.034)
Roundup + Kan	1.44 (0.67)	$<10^{-7}$**

Microbiology. 2017 Nov 17. doi: 10.1099/mic.0.000573.

Herbicides induce adaptive response

2. Efflux pump gene deletions can neutralise resistance
3. Transcriptomics (not shown)

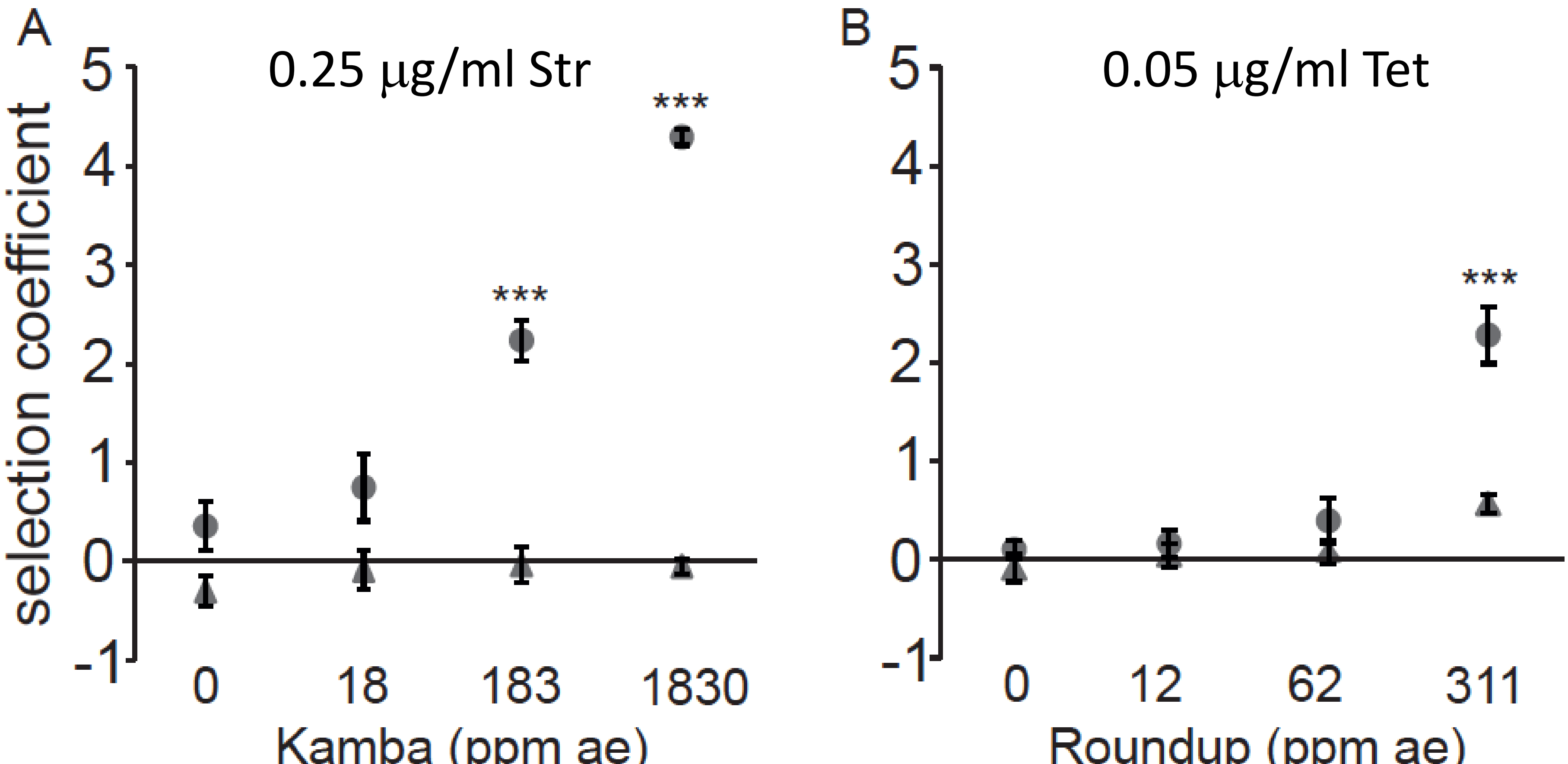
Table 4. Responses of gene deletion strains

Strain	Kamba						Roundup					
	Cip			Tet			Cip			Tet		
	P-value*	R^2 (%)	Fold-change MIC	*P*-value*	R^2 (%)	Fold-change MIC	*P*-value*	R^2 (%)	Fold-change MIC	*P*-value*	R^2 (%)	Fold-change MIC
BW25113 (WT)	***	6	3	***	4.9	2†	***	10.8	5	***	5.7	2
CR7000 (Δ*acrA*)	***	2.1	1.25	***	1.6	1.25	*	0.7	0	NS	0.3	0
CR5000 (Δ*acrB*)	NS	0.6	0	*	3	1.25†	***	1.8	0	***	1.2	0
JW5503 (Δ*tolC*)	***	6	2	***	0.8	0	NS	0.1	0	***	1.3	2
JW2454 (Δ*acrD*)	***	9.8	1.5†	***	8.1	2	***	7.9	2†	***	4.6	2.67
JW0912 (Δ*ompF*)	***	6.7	2.33	***	8.4	5	***	4.4	3.3	***	4.5	3†

Microbiology. 2017 Nov 17. doi: 10.1099/mic.0.000573.

Herbicides alter selection at threshold concentrations

Most resistant genotype always wins.



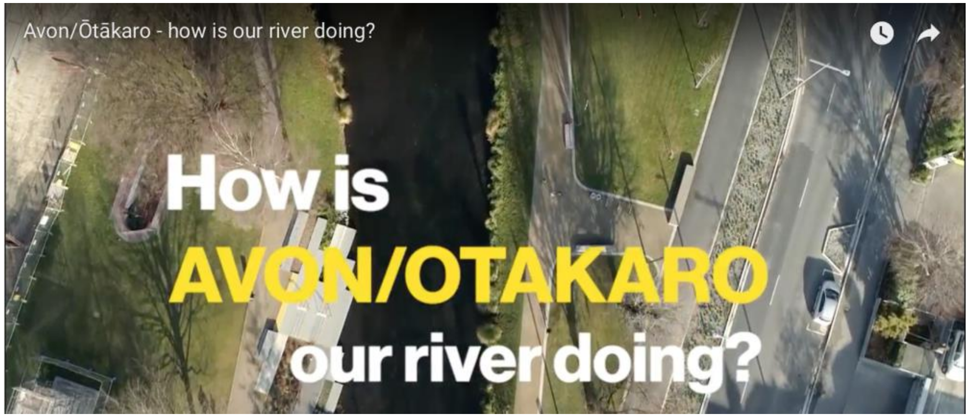
PeerJ. 2018 Oct 12;6:e5801. doi: 10.7717/peerj.5801. eCollection 2018.

Rural Convergence

Agricultural use

- >80% of antibiotic administered is excreted
- >MIC concentrations found in manure

Future work



Continue sampling over time, over longer range of Avon River.

Test potential link between agrichemical use and resistance.

In collaboration with Brent Gilpin (ESR Christchurch) and Amy Osborne (UC), complete WGS genotyping.

Characterise potential for resistance HGT.

Along with Matt Stott (UC), expand coverage region to nation wide.

Continue to work with ecologists to look for remediation strategies.

What does it matter?

Important wherever pathogenic bacteria -

1. are simultaneously exposed to herbicides and antibiotics.
2. resistant bacteria are transported to potential hosts.

Table 2.5 – Herbicide Recommended Application Rates.

Herbicide	Recommended Application Rate
2,4-D	33,080
Kamba	415 – 2,200
Roundup	2,664 – 87,912

Recommended application rates for three commercial herbicide formulations, 2,4-D Amine 800 WSG (Agpro, Auckland, NZ), Kamba^{500} (Nufarm, Otahuhu, NZ), and Roundup Weed Killer (Monsanto, Australia). Concentrations are given in ppm ae.