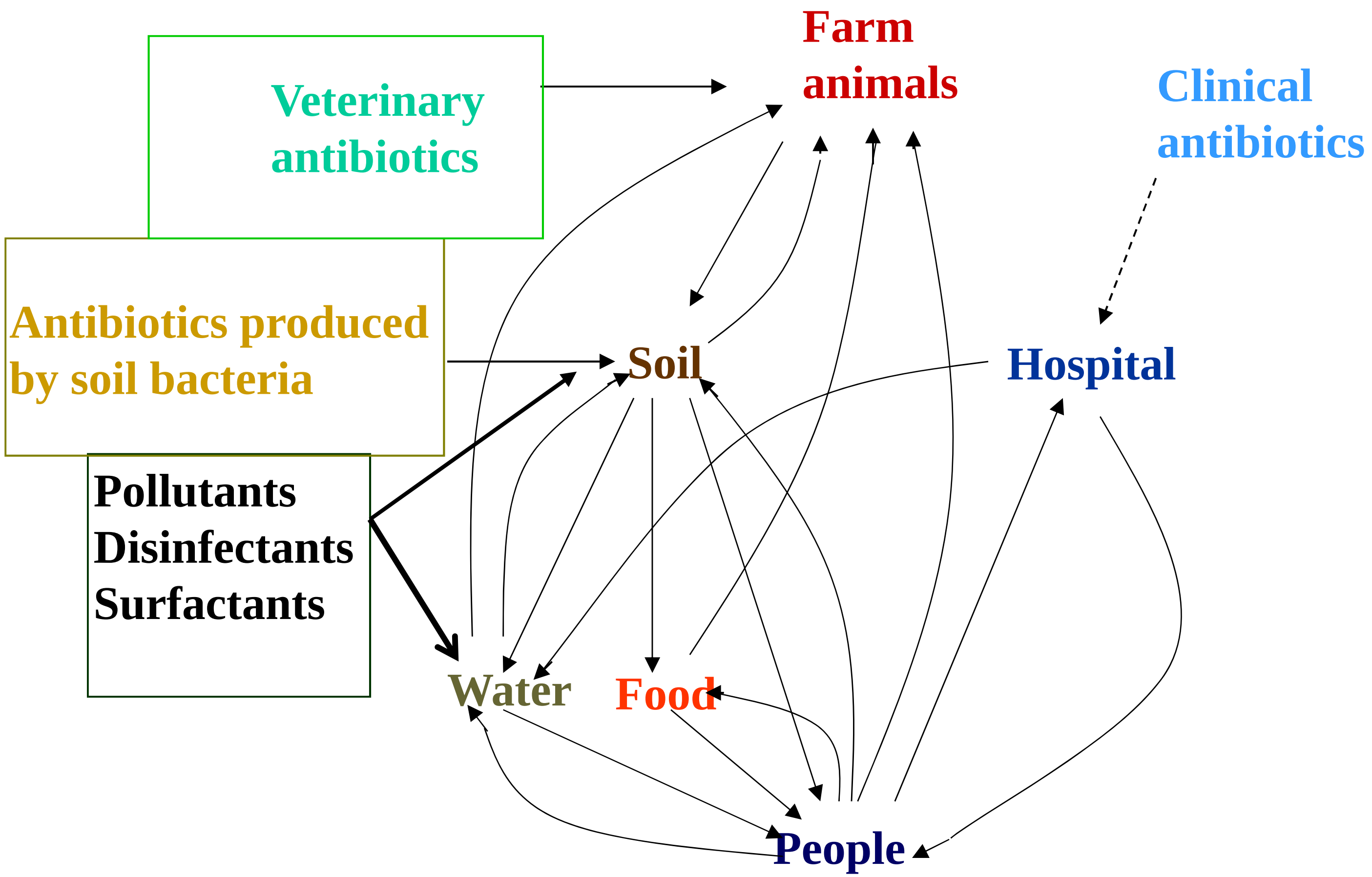
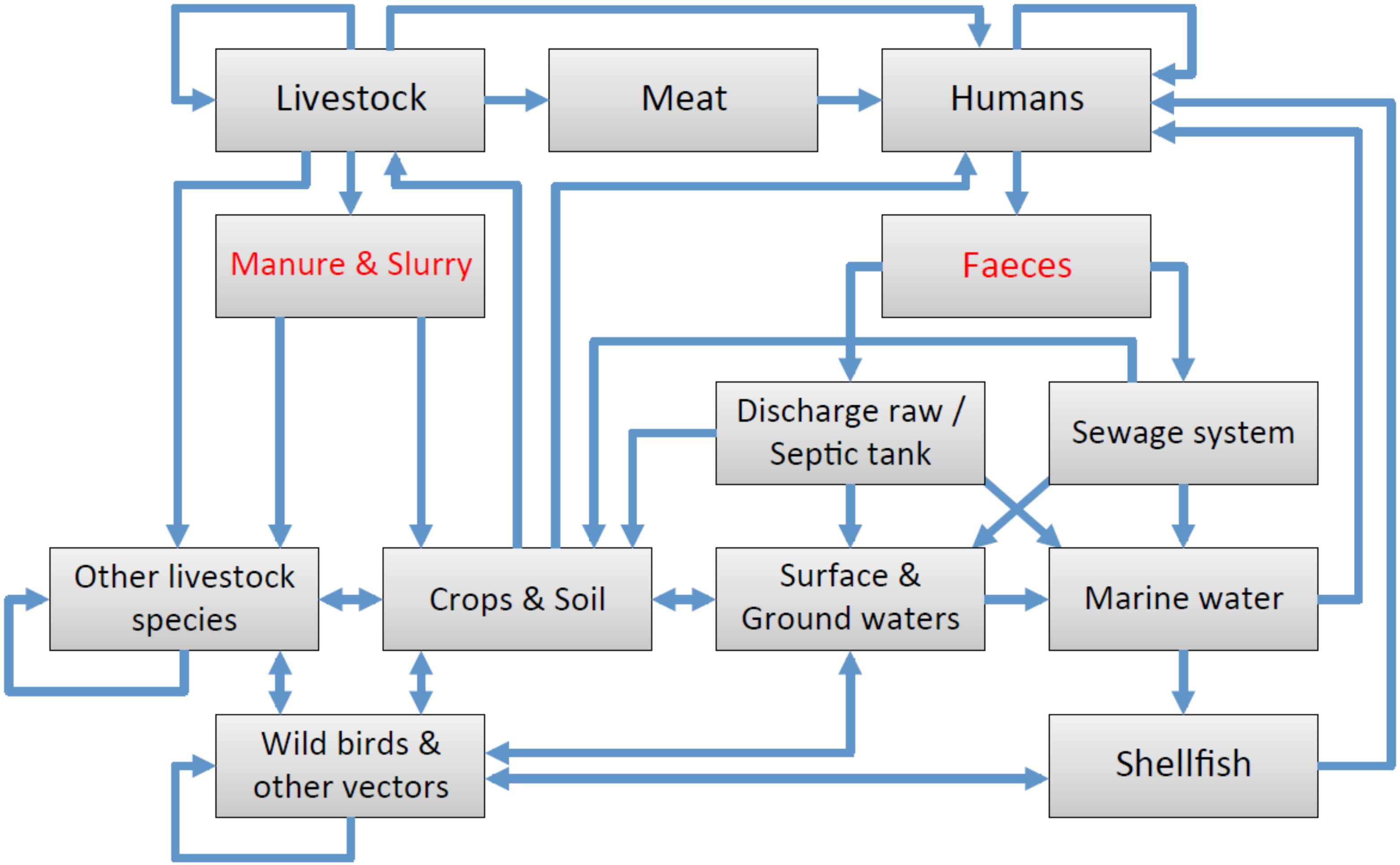
The environment as a reservoir of antibiotic resistance

THE UNIVERSITY OF WARWICK

Flow of antibiotic resistance genes, antibiotics and pathogens in the environment



The connectivity of potential sources of antibiotic-resistant bacteria



Studying the horizontal gene pool: Antibiotic resistance
Prevalence, selection and transfer

- Environmental survey: Isolation and PCR
- Comparative genomics- virulogenome, islands, gene clusters
- Metagenomics- expression screening
- Gene capture: mobilomes
    - Integrons -gene cassettes- gene capture
    - Transposons
    - Plasmids- exogenous isolation – host capture
    - Phage

Occurrence of antibiotics in the natural environment, fish, crops and drinking water from published studies

Antibiotic class	General behaviour	Sewage sludge	River water	Groundwater	Drinking water	Fish	Soil	Crops	Example compounds monitored
Chloramphenicol	impersistent/ mobile	-	√	X	-	-	-	-	-
2,4-diaminopyridines	persistent/ immobile	√	√	X	X	-	√	√	trimethoprim
Fluoroquinolones	persistent/ immobile	√	√	X	X	-	√	-	ciprofloxacin, norfloxacin, ofloxacin
β-lactams	impersistent mobile	-	X	X	X	-	-	-	amoxicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V
Macrolides	slightly persistent/ slightly mobile	√	√	X	-	-	-	-	azithromycin, clarithromycin, lincomycin, roxithromycin, spyramycin, tylosin
Sulfonamides	persistent/ mobile	√	√	√	X	-	√	√	sulfamethoxazole, sulfadiazine, sulfamerazine, sulfamethazine, sulfapyridine
Tetracyclines	persistent/ immobile	-	√	X	X	√	√	√	chlortetracycline, doxycycline, oxytetracycline, tetracycline

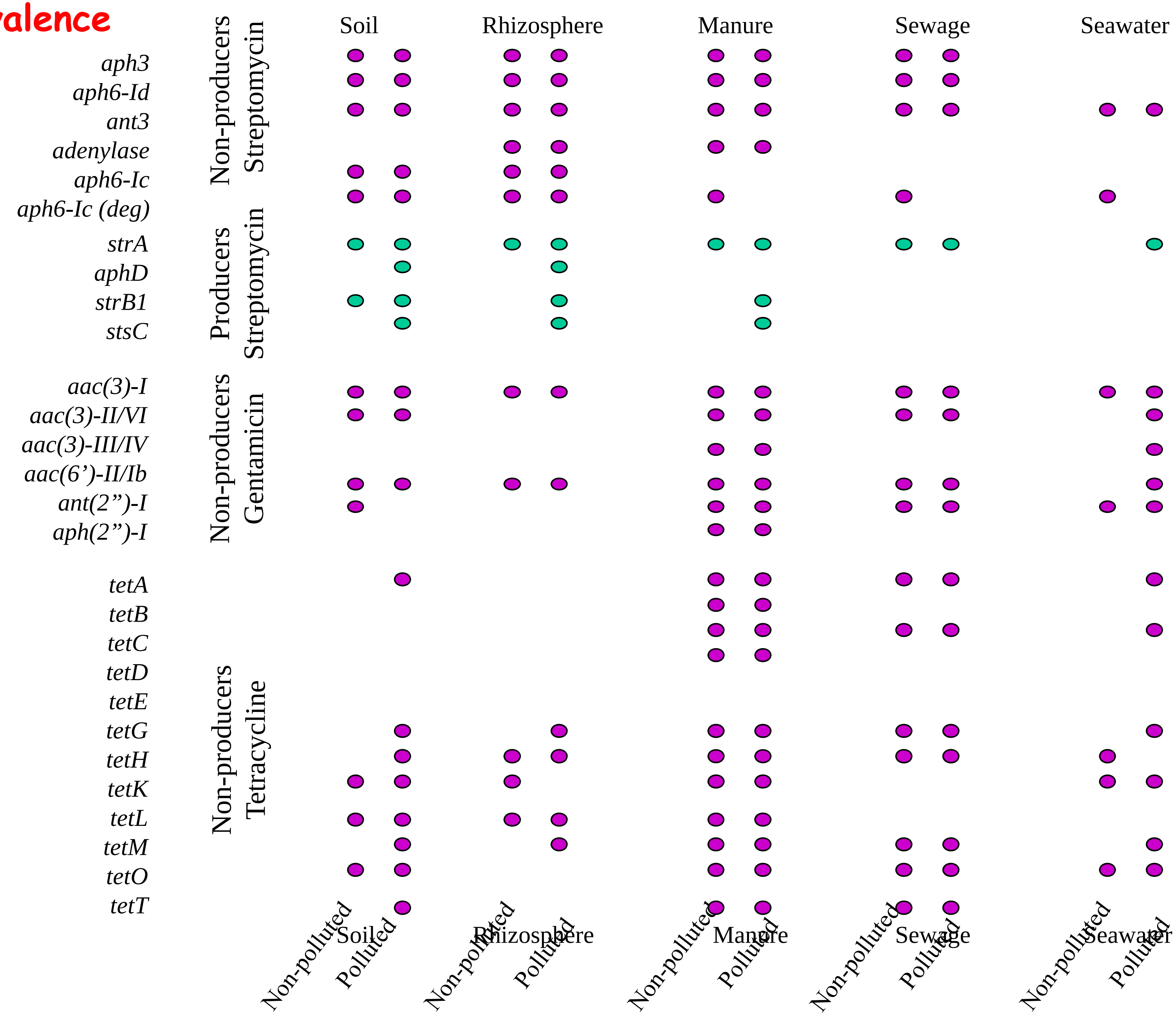
A tick means that it has been monitored for and detected and a cross means that it has been monitored for and not detected. No entry means that no monitoring has been done yet (Alistair Boxall)

Antibiotics in soil-biocontrol

Producer	Antibiotic	Antagonist	Environment
Pseudomonas fluorescens	phenazine 27-43ng/g +/- mutants	*Gae. graminis*	Wheat rhizosphere, Thomashow et al to date
S. hygroscopicus	geldanamycin 88 µg/g	*Rhiz. Solani*	Pea rhizosphere, Rothrock & Gottlieb1984,
S. griseoviridis	X	*Fusarium*	Ornamentals, Mycostop 2002
Ps. fluorescens	oomycin A reporter gene	*Pythium*	Cotton rhizosphere, Howie & Suslow 1991
Ps. putida	pseudobactin +/- mutants 3.5×10^{-10} M/g	*Fusarium*	Carnation rhizosphere, Lemancean et al 1992; Barley rhizosphere, Buyer et al 1993
Ps aureofaciens	Phenazine	*Pythium*	Bean, Seveno et al 2001
Streptomyces rochei	Streptothricin	*Gae. graminis*	Wheat rhizosphere, Watyam & Wellington 2005

Reservoirs of antibiotic resistance genes in diverse environments: RESERVOIR survey

Prevalence



Antibiotics in the farm environment:

Prevalence

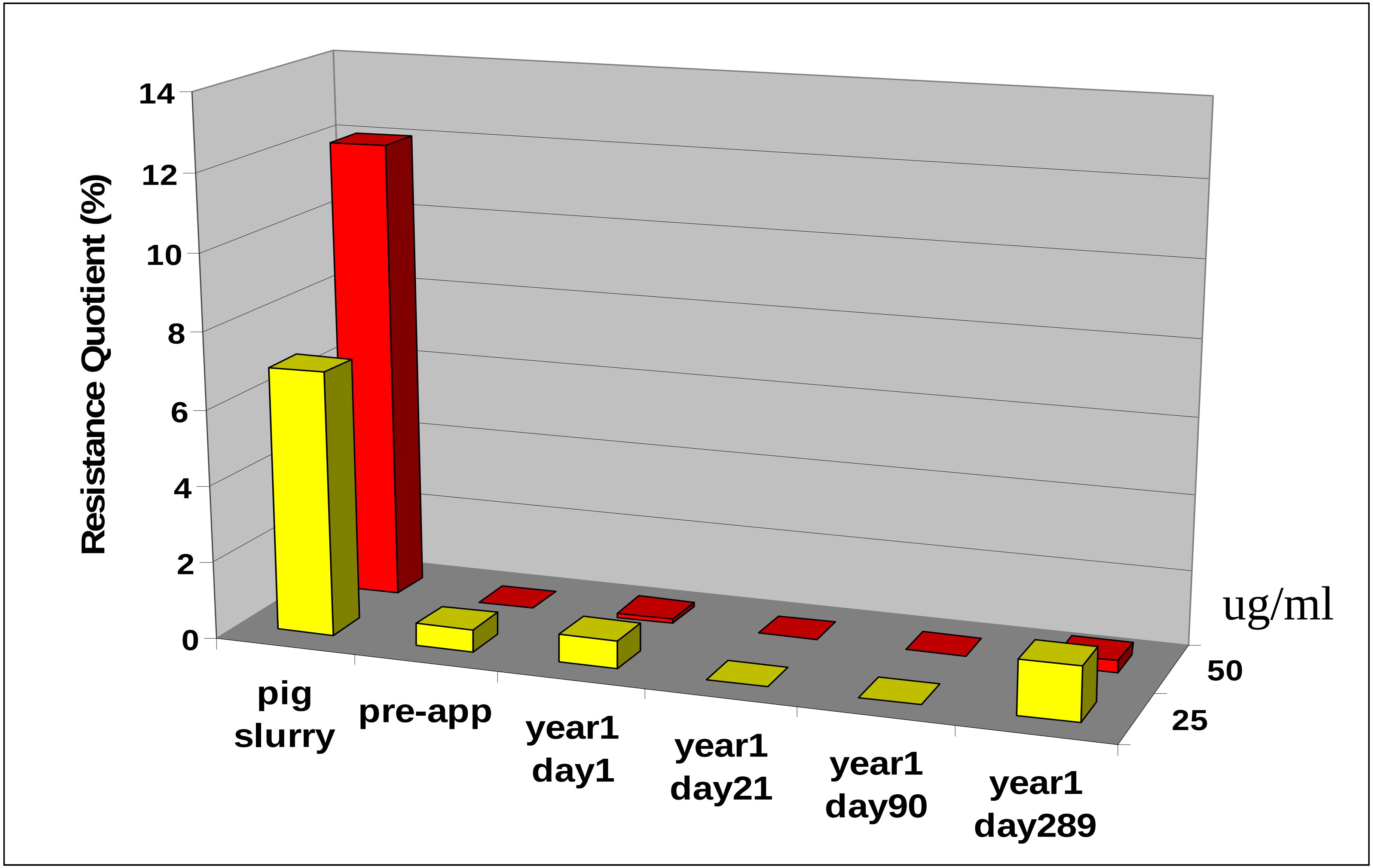
- Cranfield University EU Grant to assess the environmental fate and effects of veterinary medicines

- A field study, in Lincolnshire, was established in which slurry, from **tylosine** fed pigs, was applied to fields using a broadcast spreader.

- Slurry was spiked with **oxytetracycline**(OTC and **sulphachloropyridazine**, SCP) preceding field application.

Collaboration with Alistair Boxall, University of York

RQ values for scp over year 1



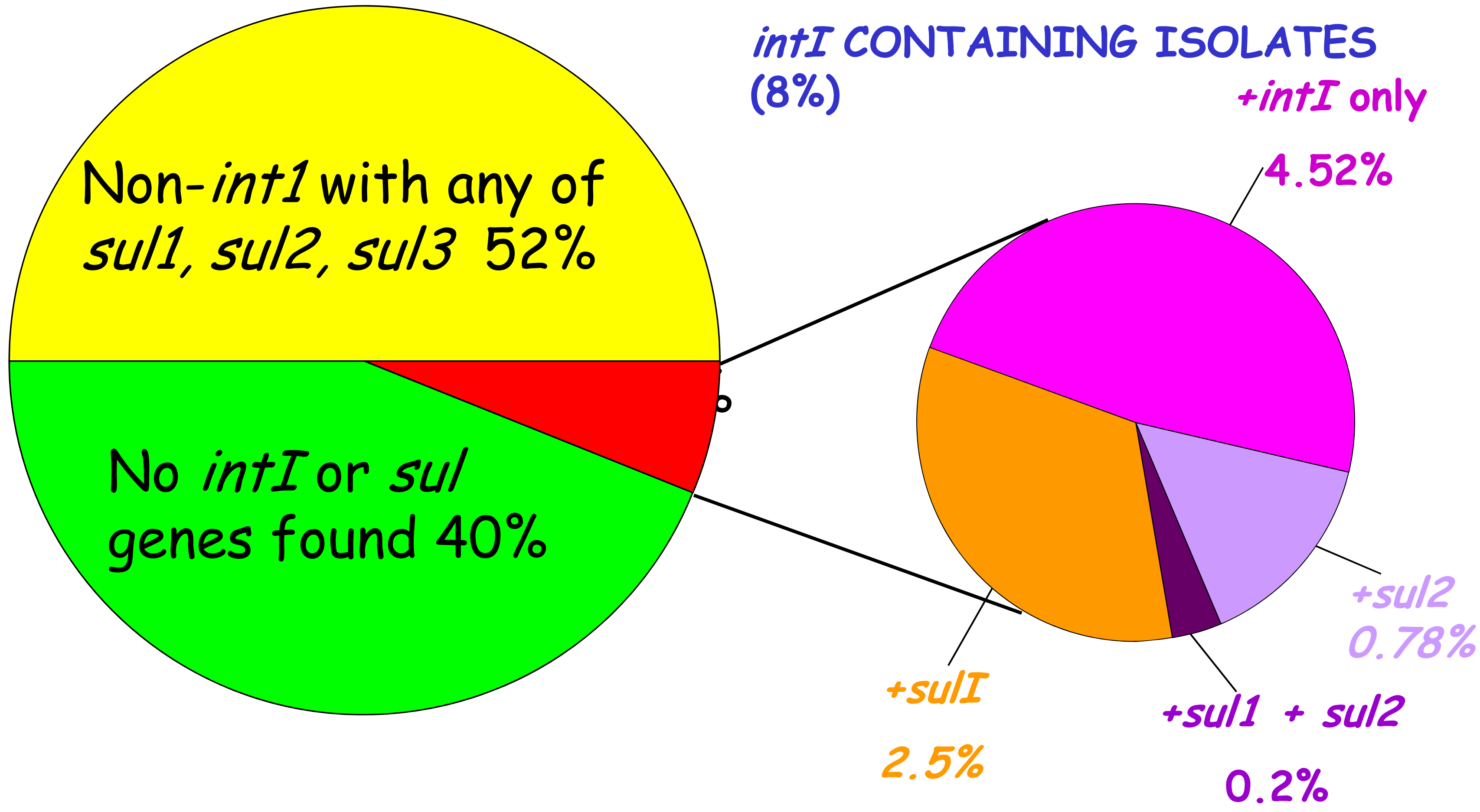
PCR SCREENING

•531 bacterial isolates were collected from soil samples taken over the two year period of the study

•Screened for *int1*, *sulI* ,*sulII*, *sul3* and *ermB* genes

- **Phenotypic resistance to SCP was significantly higher in isolates from pig slurry and postapplication soil than in those from preapplication soil**
- **Of 531 isolates, 23% carried *sul1*, 18% *sul2*, and 9% *sul3* only. 2% contained all three *sul* genes**
- **Only 8% of *sul1*-positive isolates carried the *intI1* gene.**
- **Sulfonamide-resistant pathogens, including *Shigella flexneri*, *Aerococcus* spp., and *Acinetobacter baumannii*, were identified in slurry-amended soil and soil leachate**
- **Sulfonamide resistance in *Psychrobacter*, *Enterococcus*, and *Bacillus* spp. was reported for the first time**
- **Study provides the first description of the genotypes *sul1*, *sul2*, and *sul3* outside the *Enterobacteriaceae* and in the soil environment**

ALL 531 ISOLATES, isolated +/- antibiotics



* No isolates contained *intI* + *sul3* genes

BACTERIAL COUNTS

•Resistance seen over two year study period maybe due to;

1. Survival of enteric bacteria
2. Transfer of resistance to the indigenous population

•Pig slurry contains many highly resistant bacteria.

•Resistance to SCP observed up to 50µg ml^{-1} at year1 day 289, despite lack of selective pressure, SCP conc. decreased rapidly in soil

- Resistance seen is patchy-maybe due to characteristics of clay soil
- Resistance to tylosin constant throughout both years

Byrne-Bailey KG, Gaze WH, Zhang L, Kay P, Boxall A, Hawkey PM, Wellington EM. (2011). Integron prevalence and diversity in manured soil. Appl Environ Microbiol. 77, 684-7.

Bailey-Byrne, K.G., Gaze, W.H., Kay, P; Boxall, A .B. A., Hawkey P.M. and Wellington E. M. H. (2009). Prevalence of sulfonamide resistance genes in bacterial isolates from manured agricultural soils and pig slurry in the United Kingdom. Antimicrob.Agents Chemotherap. 53, 696-702.

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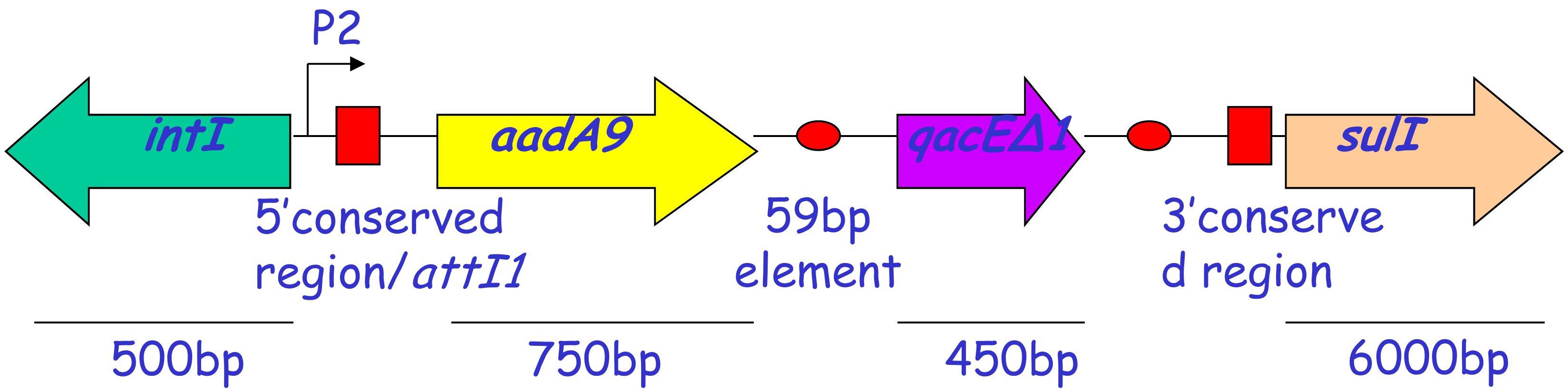
A1 Administrator, 09/12/2004

Schematic diagram of class 1 integron from *Arthrobacter aritaii* (strain C361), putatively carried on a transferable plasmid

Transfer



attI1=integrase binding site

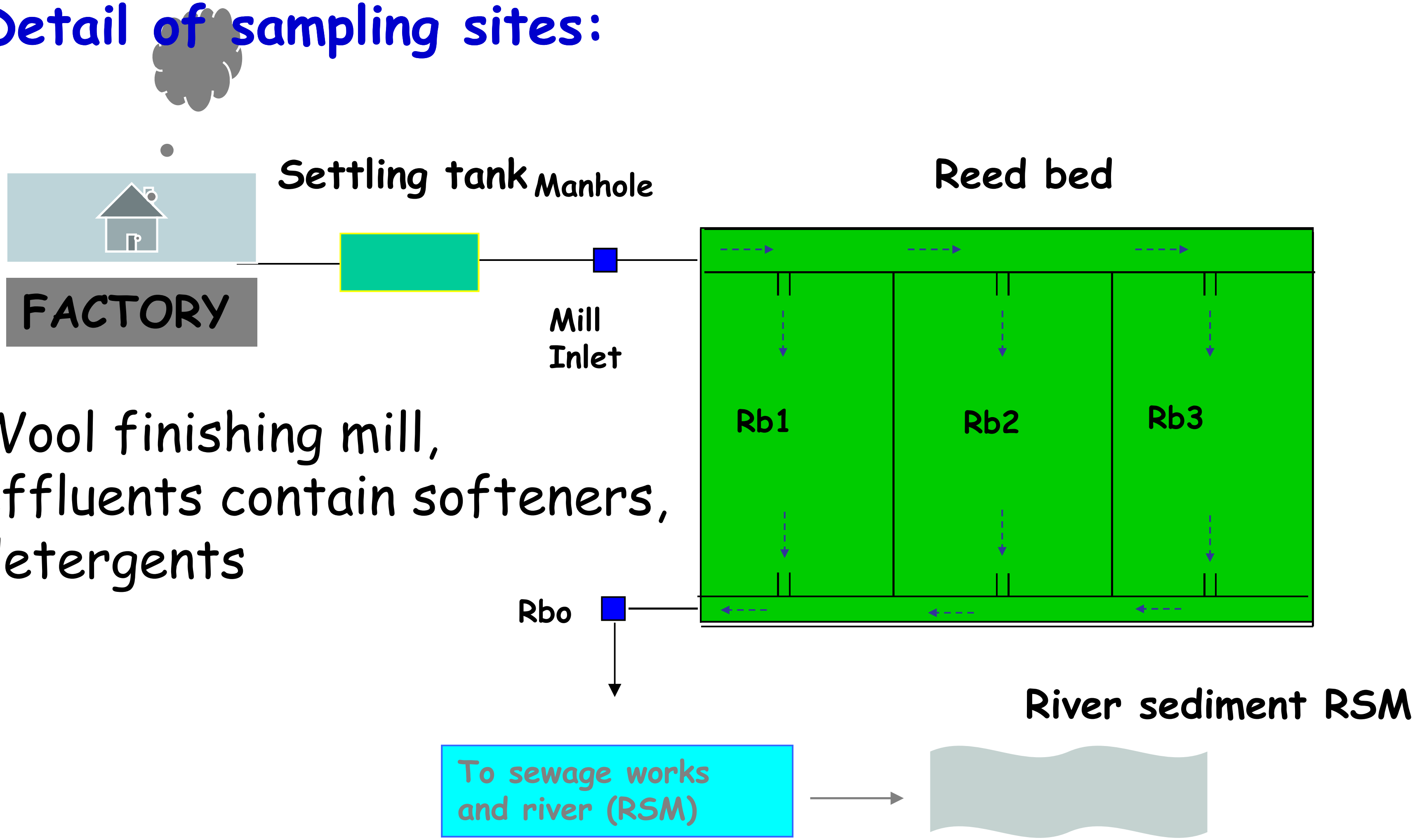
aadA9=streptomycin/spectinomycin resistance gene, 99% blast homology with aadA9 from *Corynebacterium glutamicum*

qacEΔ1=quaternary ammonium compound resistance gene, 98% blast homology with *E.coli*

sulI = sulphonamide resistance gene, 98% blast homology with *Salmonella enterica*

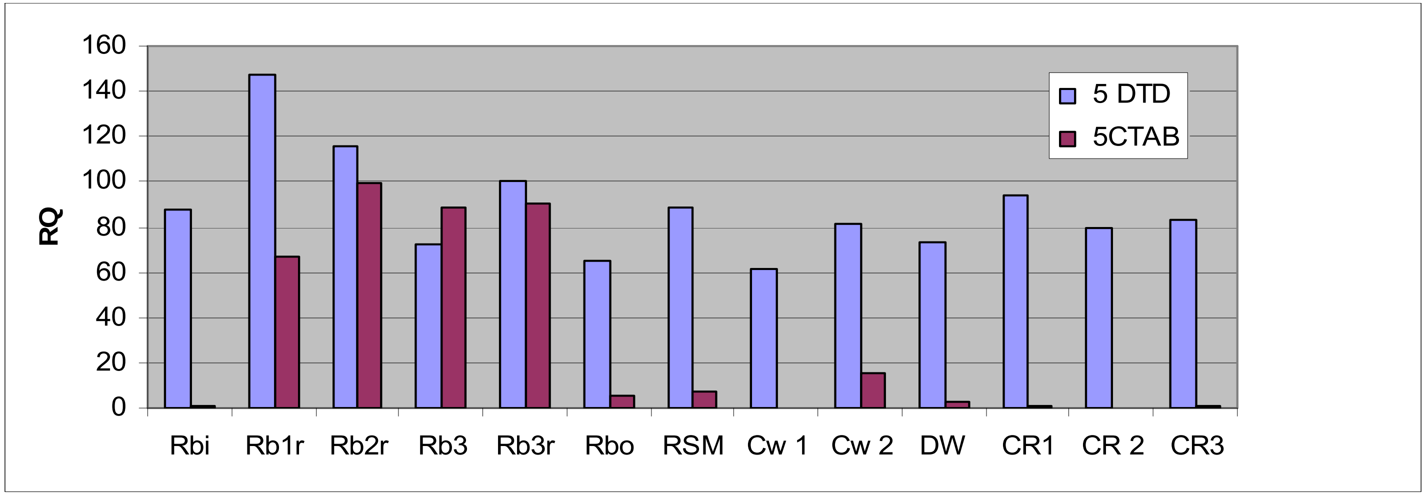
Selection: Impact of pollutants on antibiotic resistance gene pools

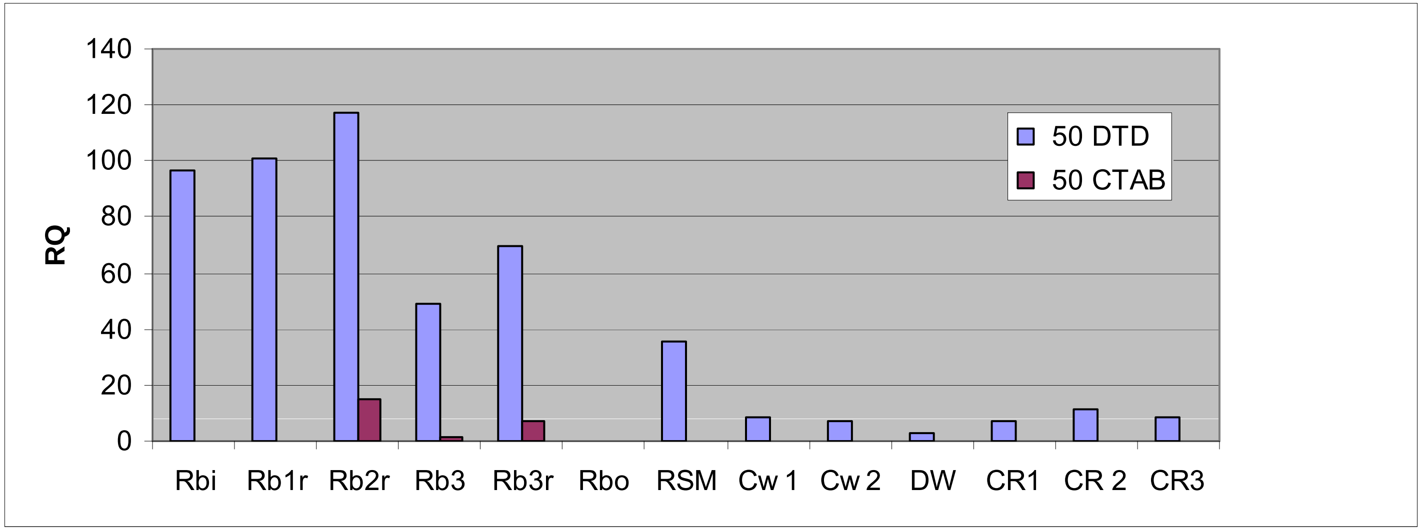
Detail of sampling sites:



Wool finishing mill,
effluents contain softeners,
detergents

Resistance quotients to detergents



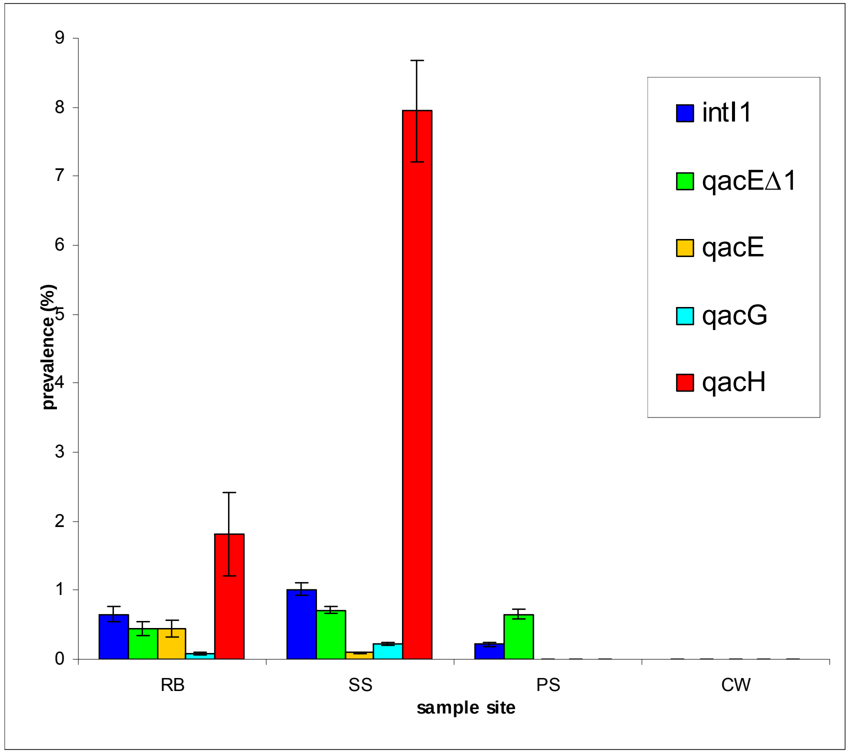


High levels of resistance to DTDMAC at 50µg/ml. Lower levels of resistance to CTAB, particularly in river water (RSM) where no resistance was observed.

Frequency of *intI* genes 10% reed beds & 0% elsewhere

Gaze W. H, Abdouslam N., Hawkey P. M. and Wellington E. M. H. (2005). Incidence of class 1 integrons in a quaternary ammonium compound (QAC) polluted environment. Antimicrob.Agents Chemotherap. 49, 1802-1807.

Molecular prevalence of class 1 integrons (*intI1*)



RB, sediment from reed bed used to remediate textile mill effluent with high QAC concentrations but no antibiotic residues. SS, fully digested sewage sludge containing QAC and antibiotic residues. PS, pig slurry from tylosin fed pigs amended with oxytetracycline and sulfachloropyridazine. CW, fallowed Cotswold soils with no history of sludge or slurry amendment.

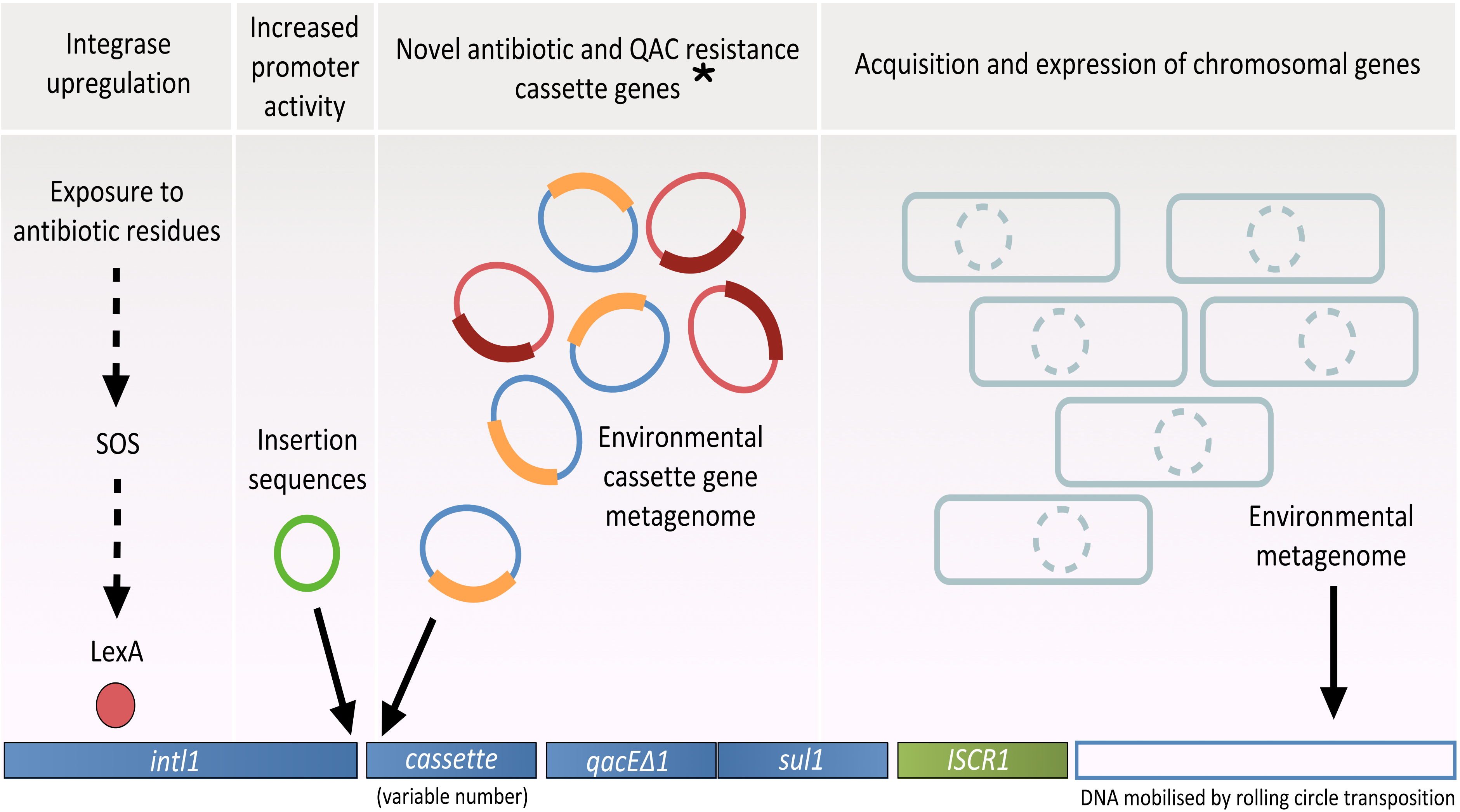
integrons (*intI1*) green bars; integron associated *qac* genes *qacEΔ1,* yellow bars and *qacE,* red bars; illustrating strong selective pressure for integrons in the absence of antibiotic residues

>1.5 x 10^{19} bacteria carrying mobile genetic elements capable of conferring antibiotic resistance being added to UK soil each year

Under strong QAC selection IS elements were maintained in the 5' region of class 1 intergons where they acted as powerful promoters for gene cassette expression

Gaze, W. H., Zhang, L., Abdouslam, N. A., Hawkey,P. M., Brown, H., Davis, S., Kay, P., Boxall, A. M. and Wellington, E. M., (2011). Impacts of anthropogenic activity on the ecology of class 1 integrons and integron associated genes in the environment. ISME J. 5, 1253-61

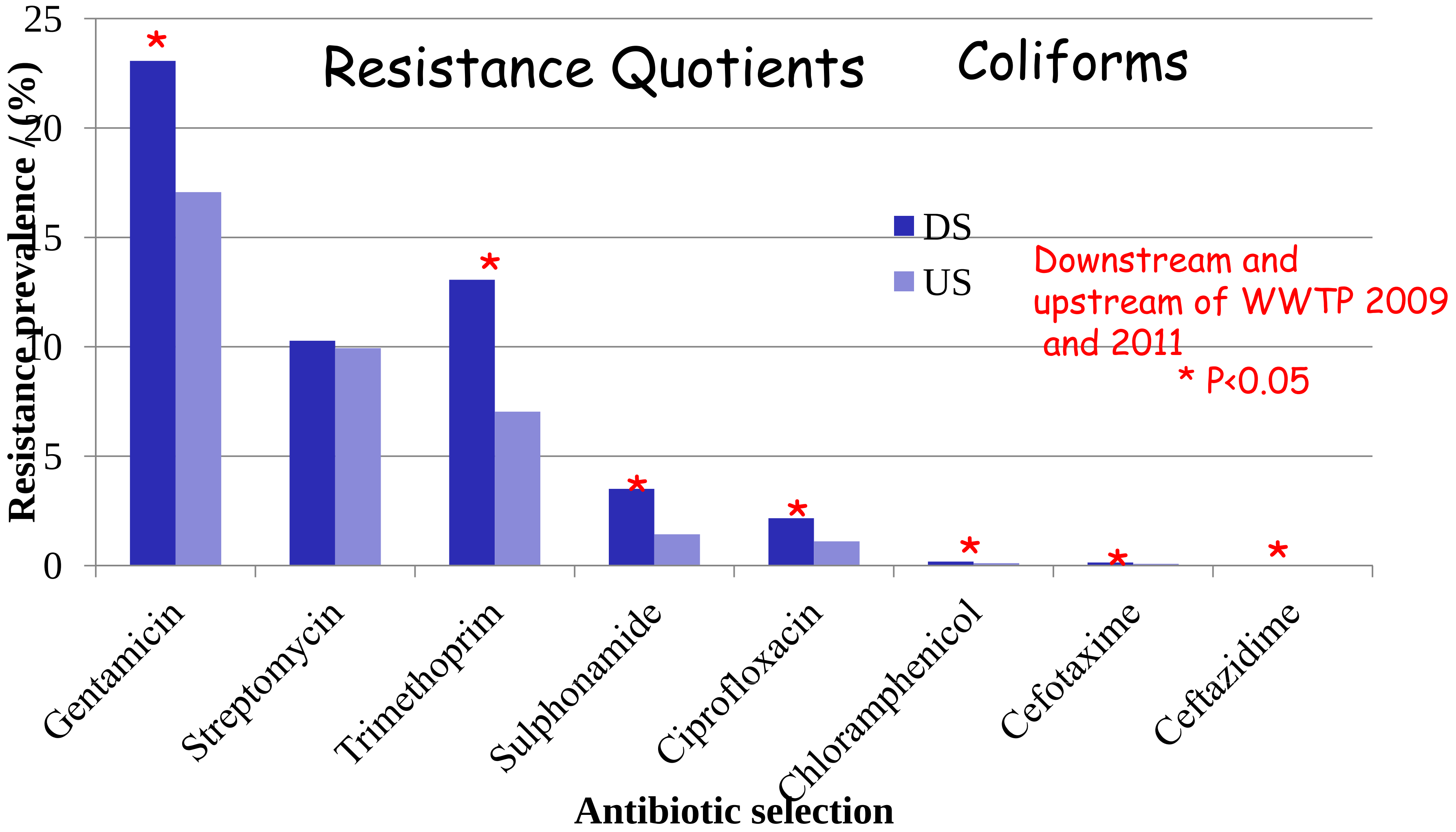
Integrons are genetic elements capable of integration and excision of resistance genes



*Antibiotic resistance genes (blue circles) and detergent / biocide resistance genes (red circles)

Flow of AMR genes into the rivers: Waste Water treatment plants

Hotspot for Horizontal Gene Transfer (HGT) as waste received from various sources



4.48×10^5 coliforms / g DS 2.07×10^5 coliforms / g US

Amos et al., 2012

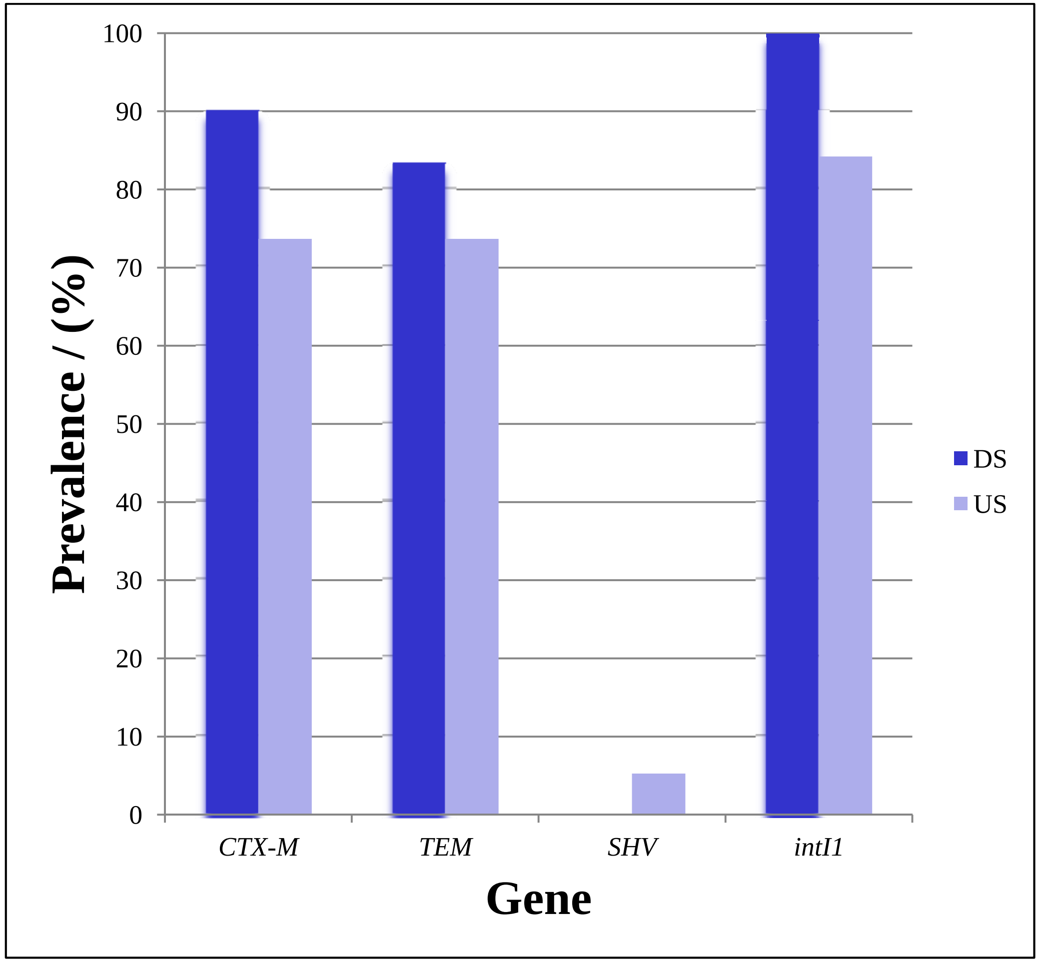
3GC gene analysis

A subset of *E. coli* and other *Enterobacteriaceae* were taken from 2011 samples for further analysis

708 CTX-M carrying presumptive coliforms / g DS

141 CTX-M carrying presumptive coliforms / g US

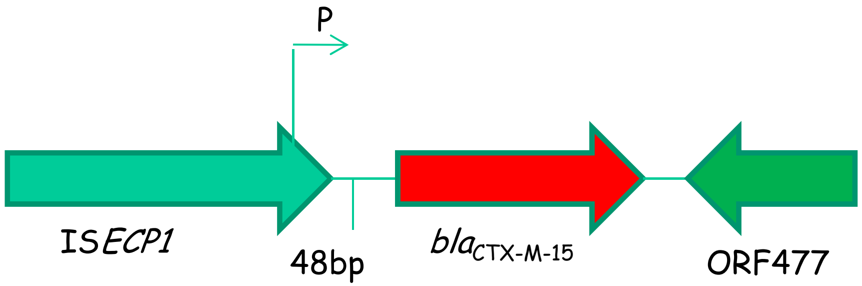
Sequencing of CTX-M revealed all belonged to the genotype CTX-M-15



Amos et al., 2012

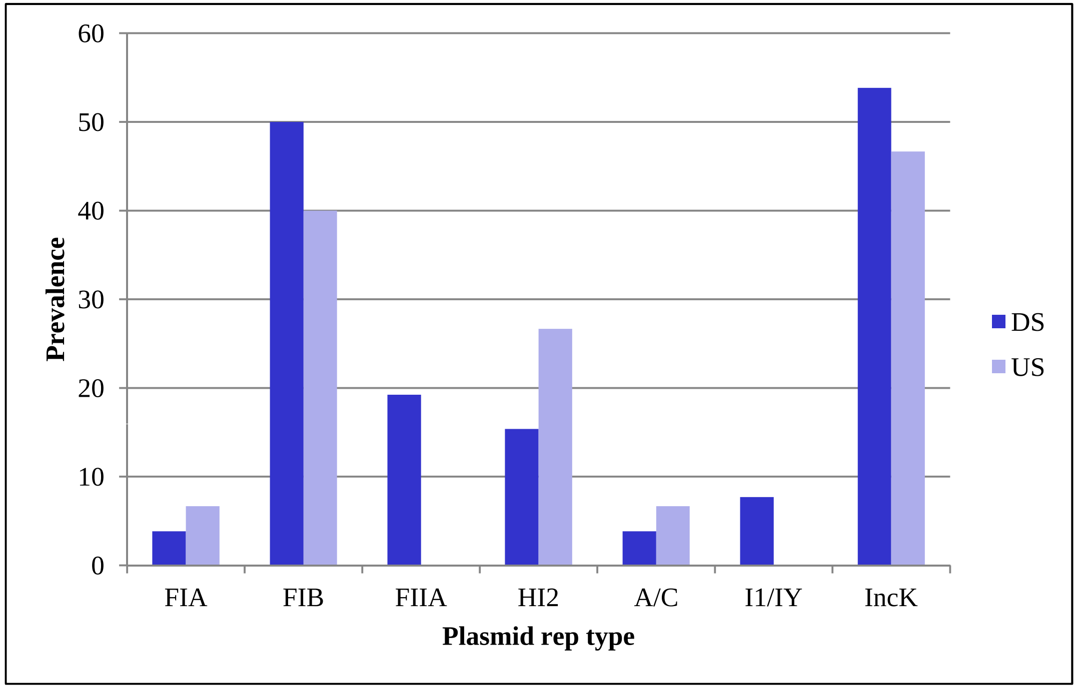
CTX-M-genetic context

CTX-M-15 was carried on 13 genetic contexts, including the 'international genetic context'



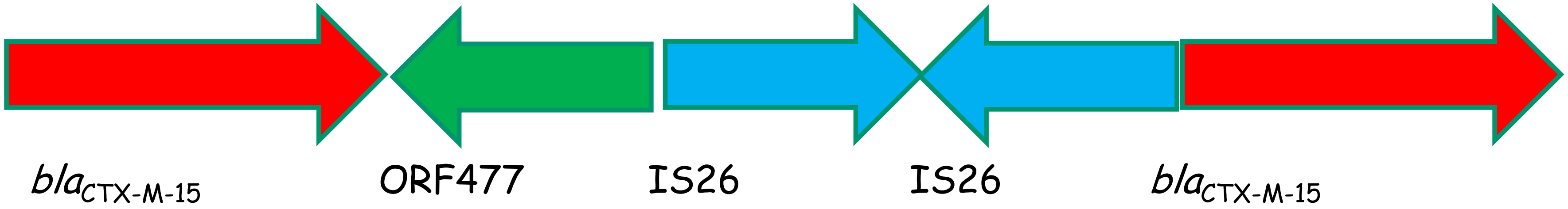
Eleven were novel, 8 were found DS, 3 were found US and 2 were found DS and US, simultaneously

Different genetic contexts were carried on different plasmids and one genetic context could be seen on multiple plasmids



Amos et al., 2012

Mobilisation of CTX-M-15



One DS *E. coli* carrying FIA, one DS *E. coli* carrying HI_2.
One US *E. coli* carrying FIB and HI_2.
One DS *C. Freundii* carrying FIB + K and one DS *C. Freundii* carrying FIB and I1/IY

CTX-M-15 is carried throughout a wide range of genetic contexts and plasmids

Contexts were seen in human pathogens, including several novel genetic contexts

The environment may mobilise CTX-M-15 between plasmids and species and WWTP effluent may drive this process

Amos et al., 2012

CONCLUSIONS

- Both resistance genes and integrons recovered in bacteria indigenous to soil and water environments
- Resistance genes prevalent in apparent absence of selection; co- selection can explain this
- Enteric bacteria survive long periods in soil and water, can transfer genes to both G+ and G- indigenous bacteria
- Integrons and ESBLs present in uncultured bacteria in soil and water- reservoir for antibiotic resistance uncharacterised to date
- Pollutants, sewage, WWTP effluent and antibiotics select for mobilome which provide a mechanism for horizontal gene transfer of resistance genes

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