Metagenomic analysis and culture-based methods to examine the prevalence and distribution of antimicrobial resistance on two New Zealand dairy farms

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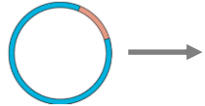
Transmission of antimicrobial resistant bacteria

- Misuse and over-use of antimicrobials in both human and animal health have been suggested as the main drivers of resistance.

- Other factors, such as heavy metal, biocide or disinfectant use may co-select for antimicrobial resistance (AMR).

(Collis *et al.*, 2018)

ESBL- and AmpC-producing *Enterobacteriaceae*

Characteristics of ESBL and AmpC beta-lactamase enzymes

Enzymes	Spectrum of resistance	Transmissibility
ESBL	Penicillins, monobactams, first- and third-generation cephalosporins	Plasmid-mediated
AmpC	Penicillins, monobactams, first-, second- and third-generation cephalosporins and cephamycins	Chromosomally encoded or plasmid-mediated

(Rubin & Pitout, 2014)

- Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) listed as "critical" on the World Health Organization "Pathogen Priority List".

AMR in agricultural environments globally

- NZ is a comparatively low user of antimicrobials in food-producing animals (Hillerton *et al*., 2017, Hillerton *et al*., 2021).

- AMR in food-producing animals is a growing consumer and regulator concern.
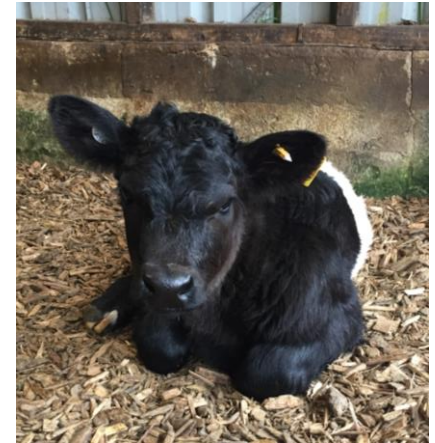
Hypothesis

Antimicrobial use influences the incidence of AMR in the dairy farm environment.

Research objectives

1) Investigate the prevalence of AmpC and ESBL-producing *E. coli* from two NZ dairy farm environments.

2) Determine the abundance of antimicrobial resistance genes (ARGs).

3) Assess the impact of systemic antimicrobial therapy on the bovine faecal microbiome.

Sample collection: Longitudinal study

October 2018 → Monthly sample collection → **December 2019**
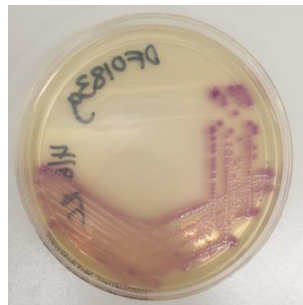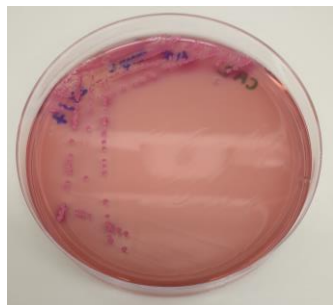
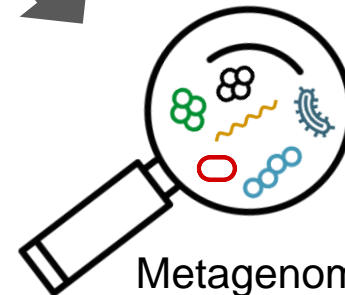

Bulk tank milk

Effluent

Soil

Pooled faeces

Culture-dependent
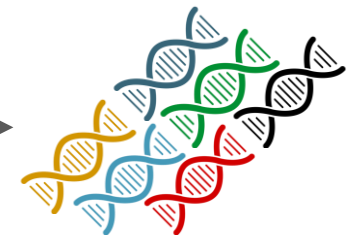
Culture-independent

Screening on selective agar → AmpC- and ESBL-producing *Enterobacteriaceae*

Metagenomic sample → Metagenomic DNA

Sequencing
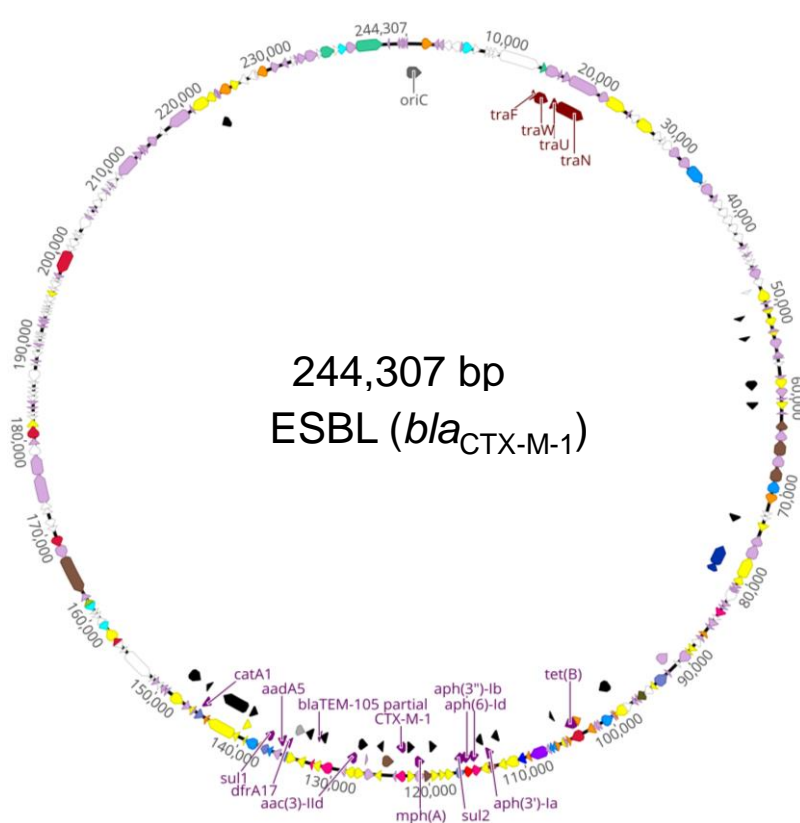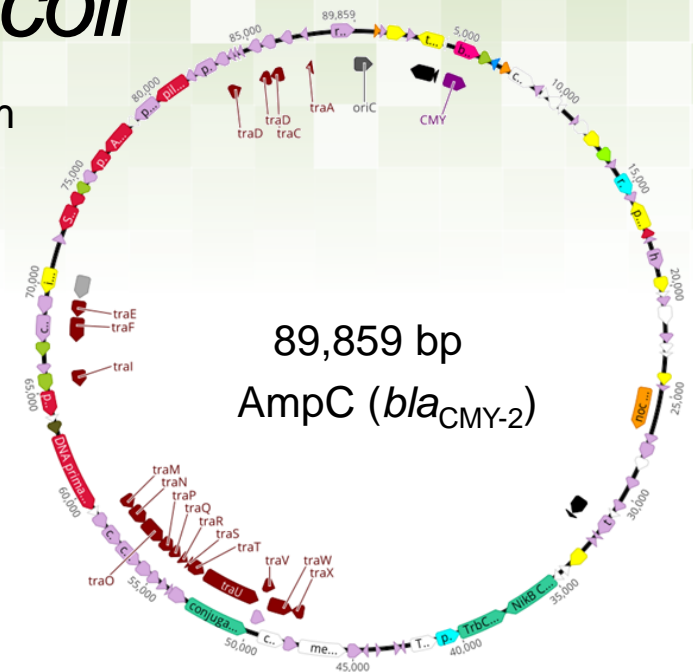
= 11 billion DNA sequence reads

Sample level prevalence of AmpC and ESBL-producing *E. coli*

- Farm dairy effluent, faeces, bulk tank milk and soil enrichments plated on selective agar.

- Sample level prevalence of ESBL-producing *E. coli* was low (n=3 isolates).

- All AmpC producers were plasmid-mediated from Dairy 4 (n=26) compared to 2 of 20 (10%) from Dairy 1.

| Farm	Sample type	ESBL-producing *E. coli*		AmpC-producing *E. coli*	
		No. samples (%)	No. isolates	No. samples (%)	No. isolates
Dairy 1	FDE	0/13	0	5/13 (38.46%)	14
	Faeces	0/60	0	2/60 (3.33%)	6
	Bulk tank milk	0/13	0	0/13	0
	Soil	0/15	0	0/15	0
Dairy 4	FDE	1/15 (6.67%)	2	1/15 (6.67%)	4
	Faeces	1/60 (1.67%)	1	5/60 (8.33%)	22
	Bulk tank milk	0/13	0	0/13	0
	Soil	0/15	0	0/15	0

AmpC and ESBL-positive *E. coli*

- Isolated from a composite faecal sample on Dairy 4 farm

- High resolution analysis: Illumina and Nanopore sequencing

- Plasmid-mediated AmpC bla_{CMY-2} gene and ESBL $bla_{CTX-M-1}$ gene

- WGS identified multiple ARGs

89,859 bp
AmpC (bla_{CMY-2})

244,307 bp
ESBL ($bla_{CTX-M-1}$)

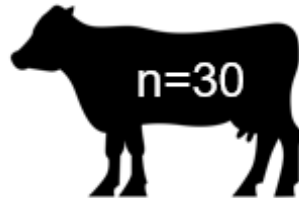| Genes	Class
aac(3)-IId *aadA5* *aph(3")-Ib* *aph(3')-Ia* *aph(6)-Id*	Aminoglycoside
tet(B)	Tetracycline
sul1 *sul2*	Sulfonamide
catA1	Phenicol
$bla_{CTX-M-1}$	Beta-lactam
dfrA17	Trimethoprim
mph(A)	Macrolide

Shotgun metagenomics: Resistome analysis
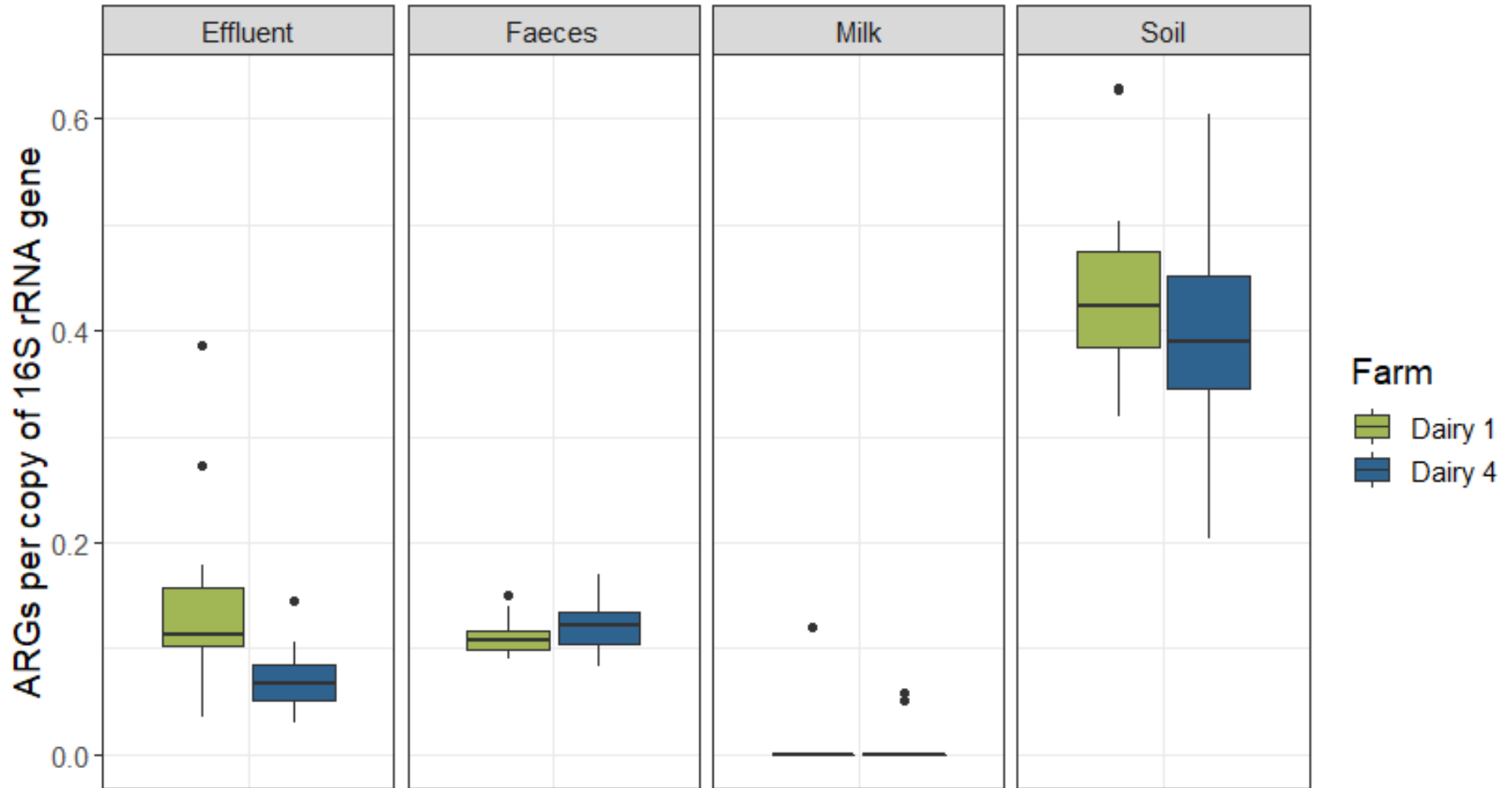
Sample collection: Microbiome study

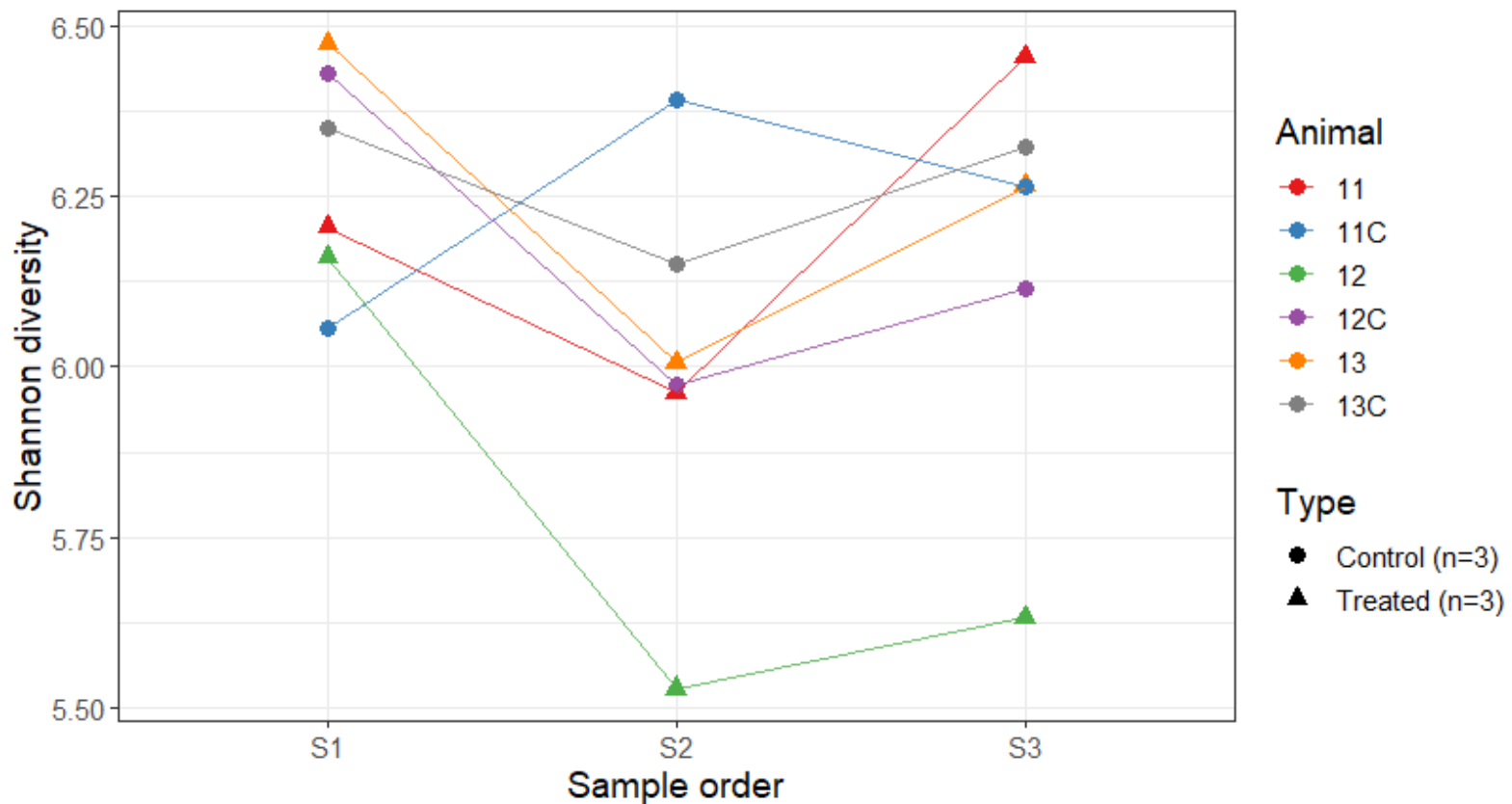01 July 2019   Opportunistic sampling   30 September 2020

Treated   Control

n=30   n=24

S1   **S2**   **S3**

Cows sampled directly before receiving systemic antimicrobial treatment

Cows sampled during treatment (median 1 day after 1st treatment)

Sampled post-treatment when the cow re-entered the milking herd (median 6 days)
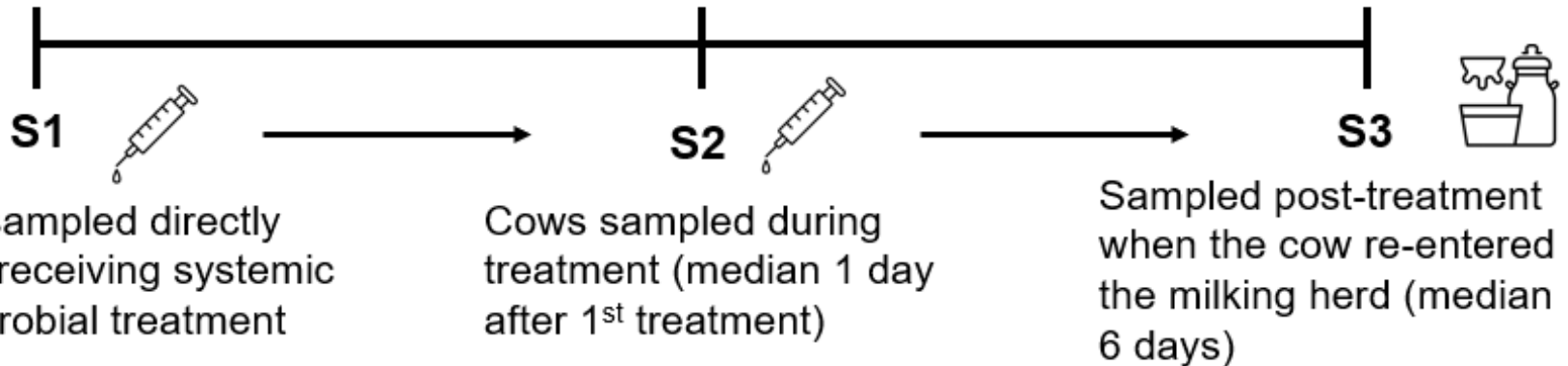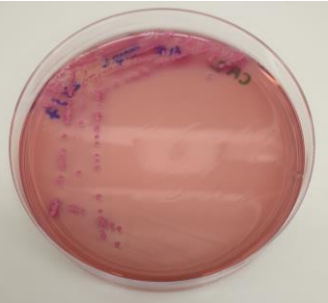
Case study: Ceftiofur treated cows

- Six individual animals
- Shannon diversity calculated from 16S V3-V4 amplicon sequencing
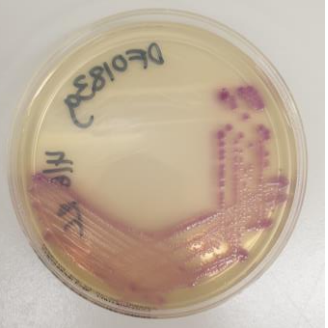- High diversity in the faecal samples

Culture-based enrichment: AMR emergence

Enrichments plated on selective agar:
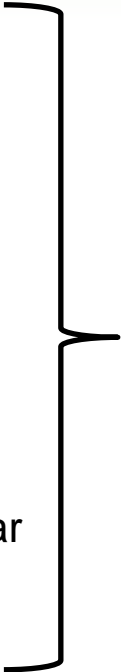
- Ceftiofur treated cows (n=3)
- Control cows (n=3)

MC agar +
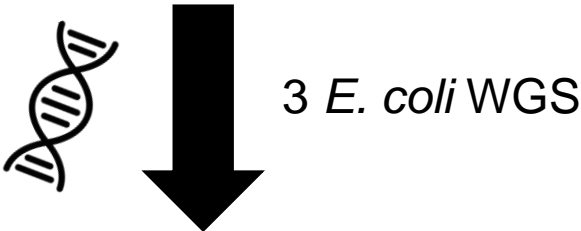1µg/ml CTX

MC agar +
1µg/ml CAZ

ESBL
CHROMagar

MacConkey
(MC) agar

Plasmid-mediated AmpC-producing *E. coli*
(bla_{CMY-2}) isolates from one treated cow (cow 12):

- Pre-treatment (n=0)
- During treatment (n=6)
- Post-treatment (n=4)

3 *E. coli* WGS

Isolate	Contigs	Genome size (bp)	GC (%)	Depth	N50	ST	Serotype	Plasmids
DG079c	112	4,880,186	50.3	120	110,495	5514	O160:H32	IncFIB_1, IncI1_1α
DG079h	308	4,789,972	50.3	82	37,402	5514	O160:H32	IncFIB_1, IncI1_1α
DG082f	128	4,875,060	50.8	89	91,090	5514	O160:H32	IncFIB_1, IncI1_1α

CTX: Cefotaxime; CAZ: Ceftazidime

Conclusions

- The sample level prevalence of ESBL-producing *E. coli* was low on the two NZ dairy farms.

- The number of ARGs per copy of 16S rRNA gene in the two NZ dairy farm environments was low compared to overseas studies. The highest abundance was in soil, however farm dairy effluent had the most diverse range of ARGs.

- Systemic antimicrobial therapy reduced the bacterial diversity in the bovine faecal microbiome during treatment ($p = 0.0029$).

- Plasmid-mediated AmpC-producing *E. coli* were isolated in cow faeces during and post-treatment with ceftiofur, but were absent pre-treatment.

Acknowledgements

References

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Shotgun metagenomics: Resistome analysis