

# Evolution of *Campylobacter* in a ‘persistently’ colonised human host

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# Background

A source attribution study identified a patient that has been recurrently excreting *Campylobacter* for 7 years.

Sequence typing determined that all isolates previously collected from the patient belonged to the same strain, *Campylobacter jejuni* ST45.

# Possible reasons for continued *Campylobacter* excretion:

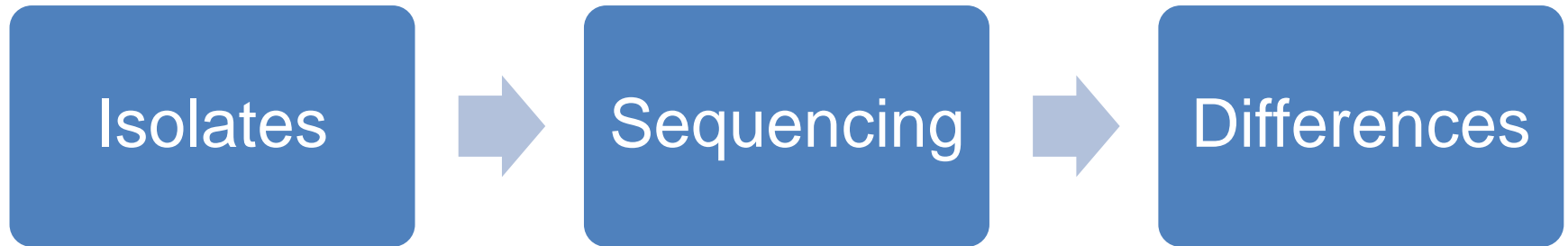
- Continued colonisation by the same *C. jejuni* ST45 strain.
- Exposure to *C. jejuni* ST45 from multiple sources.
- Persistent exposure to a single *C. jejuni* ST45 source.

# Aims

To determine the relatedness of *Campylobacter* isolates previously collected from a single patient.

To monitor *Campylobacter*'s phenotype and genotype in a long-term excreter.

# Whole genome sequencing



## ST45

- 6 isolates
- Isolated from faecal specimens from a single patient
- 2006-2013

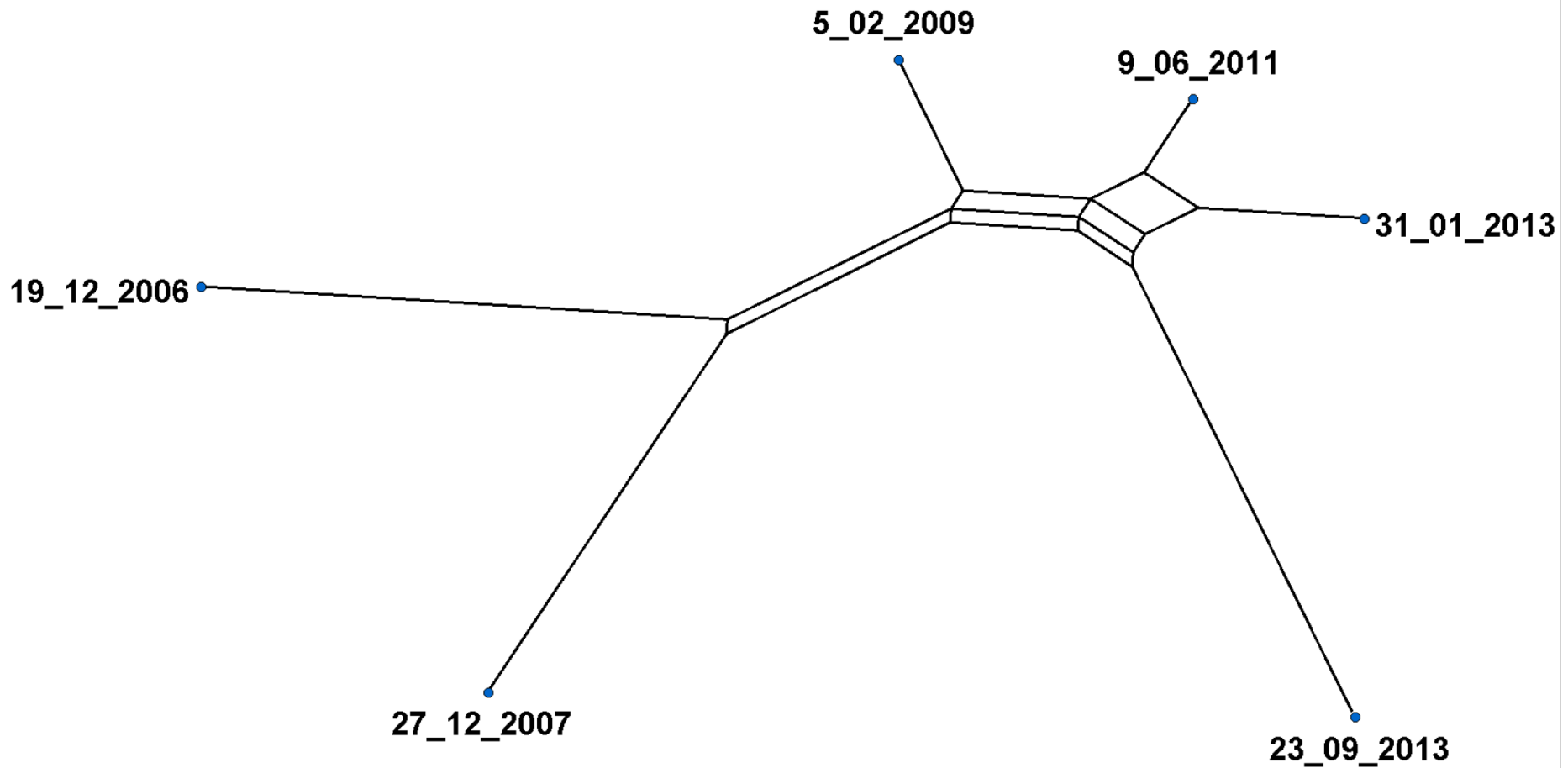
## Whole genome sequencing

- Illumina Miseq
- 300 base pair reads

## Single nucleotide polymorphisms

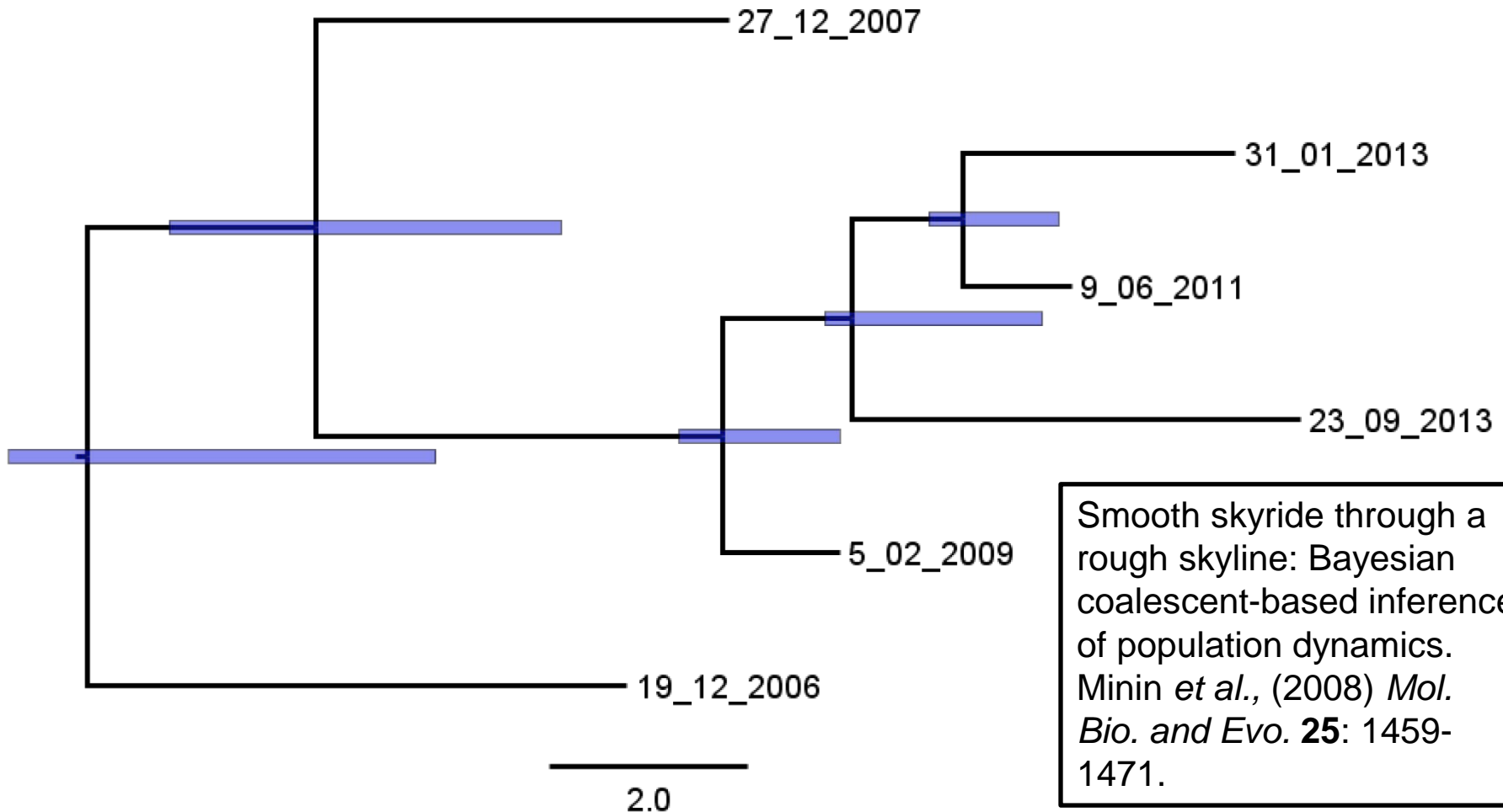
- SNPs
- Single bases that differ between isolates
- Snippy (v2.3) and kSNP (v3.0)
- *C. jejuni* str. 4031 as reference genome
- >10 read depth and >90% consensus

# NeighbourNet



NeighbourNet tree of 6 *Campylobacter jejuni* ST45 isolates  
(based on 170 core SNPs).

# Common ancestor

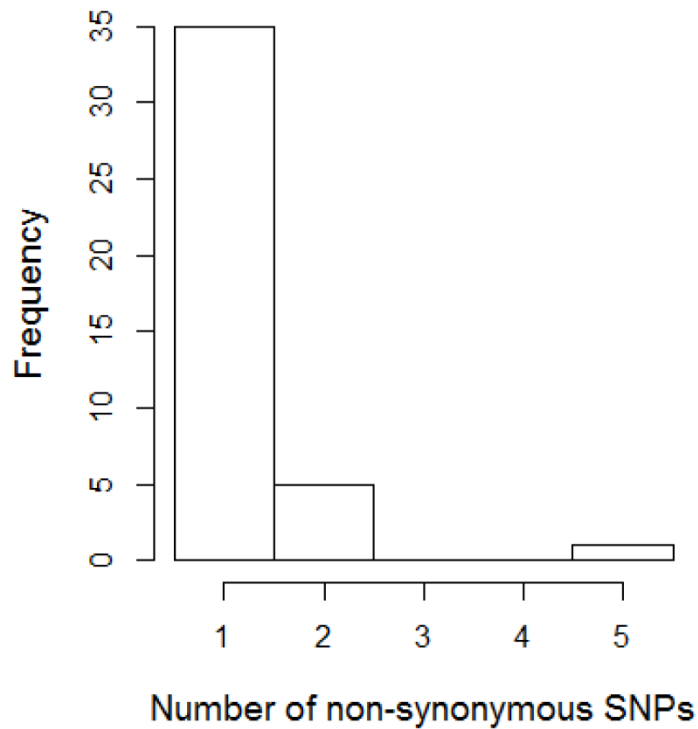


Maximum clade credibility tree of 6 *C. jejuni* ST45 isolates, using a GMRF skyline model in BEAST (based on 170 core SNPs).

Scale is the length of 2 years and blue bars represent the 95% HPD intervals for the timing of coalescent events.

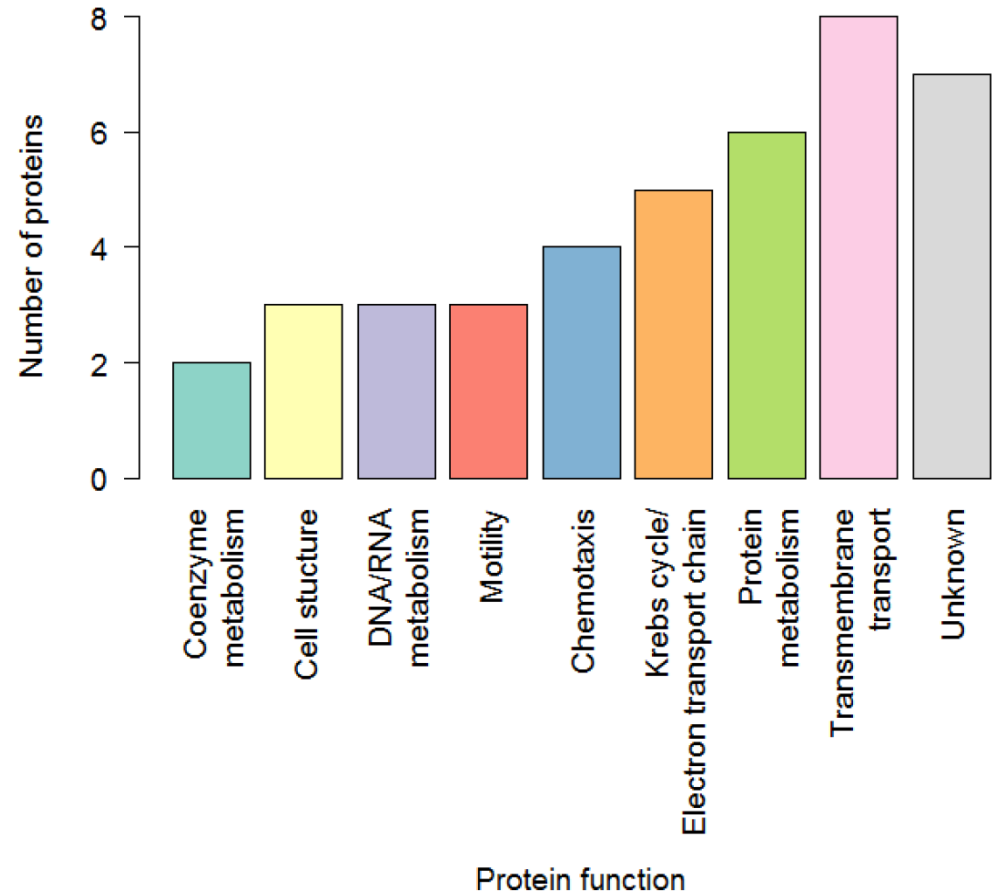
Table of SNP types

SNP	Number
Synonymous	120
Non-synonymous	50
Total	170



Histogram of the number of genes containing non-synonymous SNPs.

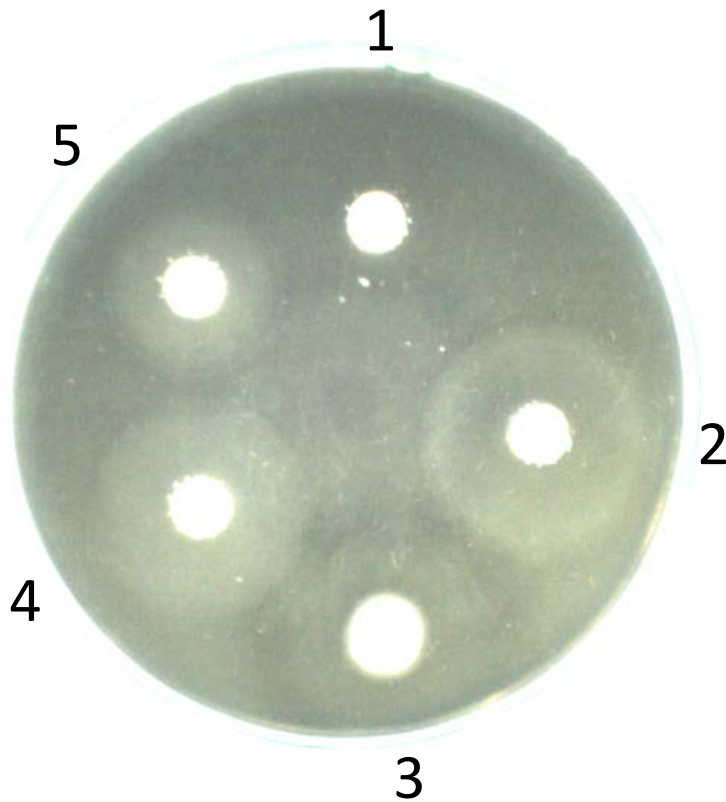
# Protein differences



Bar graph of proteins that differ between *C. jejuni* ST45 isolates and their functions, based on 50 non-synonymous SNPs. Total number of proteins that differ = 41.



# Chemotaxis

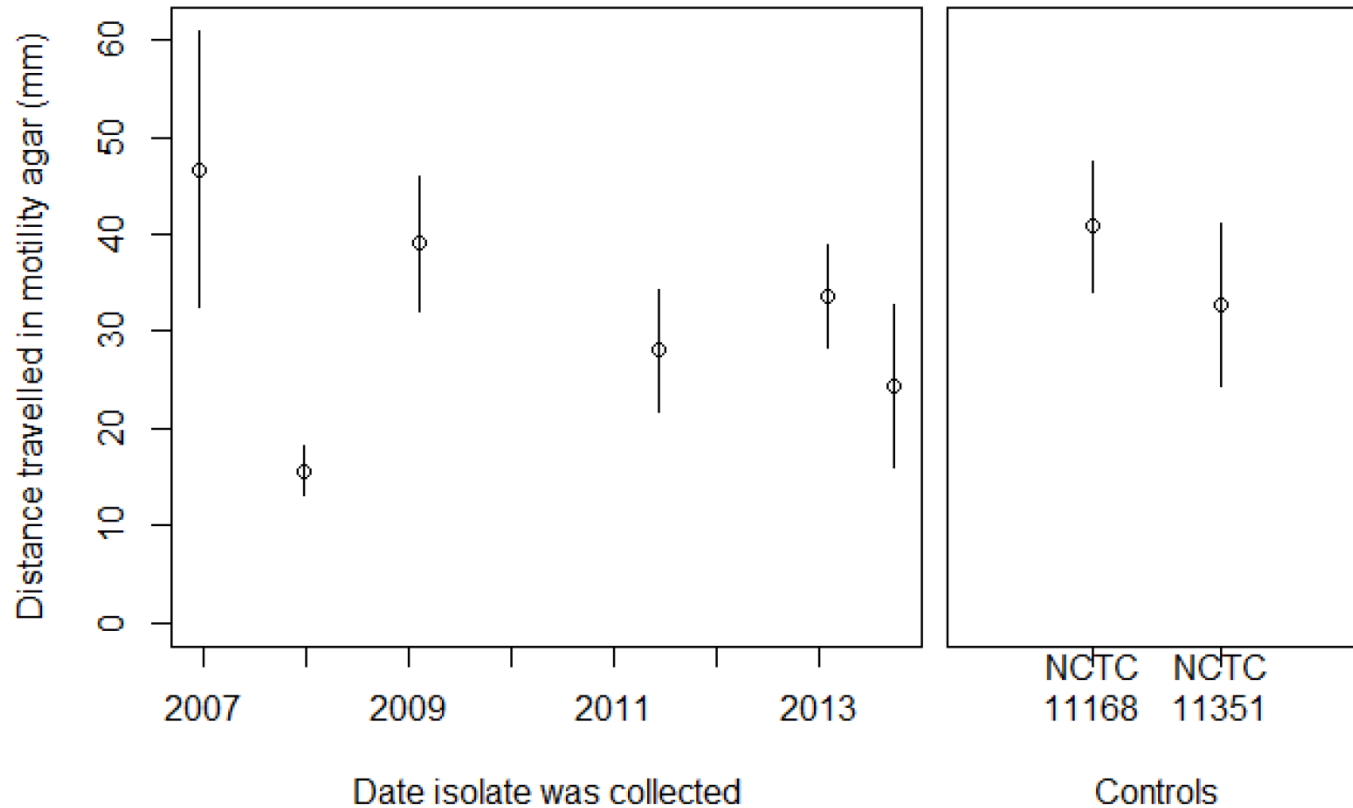
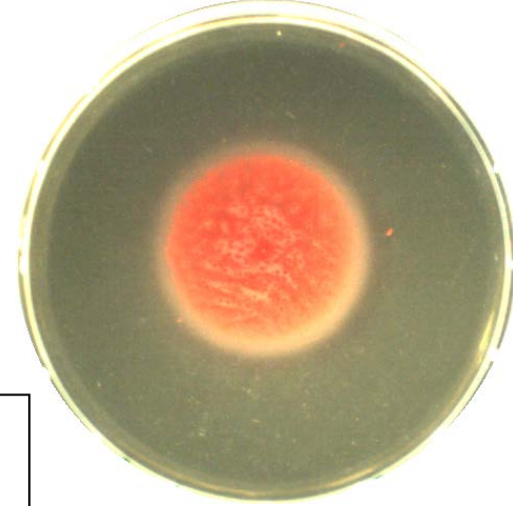


## Key:

0	No chemotaxis
-	Negative chemotaxis
+	Positive chemotaxis
1	PBS
2	Citrate
3	Deoxycholate
4	Pyruvate
5	L-serine

Isolate	1	2	3	4	5
19/12/2006	0	0	-	+	+
27/12/2007	0	0	-	+	+
5/02/2009	0	0	-	+	+
9/06/2011	0	0	-	+	+
31/01/2013	0	0	-	+	+
23/09/2013	0	0	-	+	+
NCTC 11351	0	0	-	+	+
NCTC 11168	0	+	-	+	+

# Motility



Time series of *Campylobacter* motility results  
(Error bars represent 95% confidence intervals).

# Antimicrobial susceptibility testing

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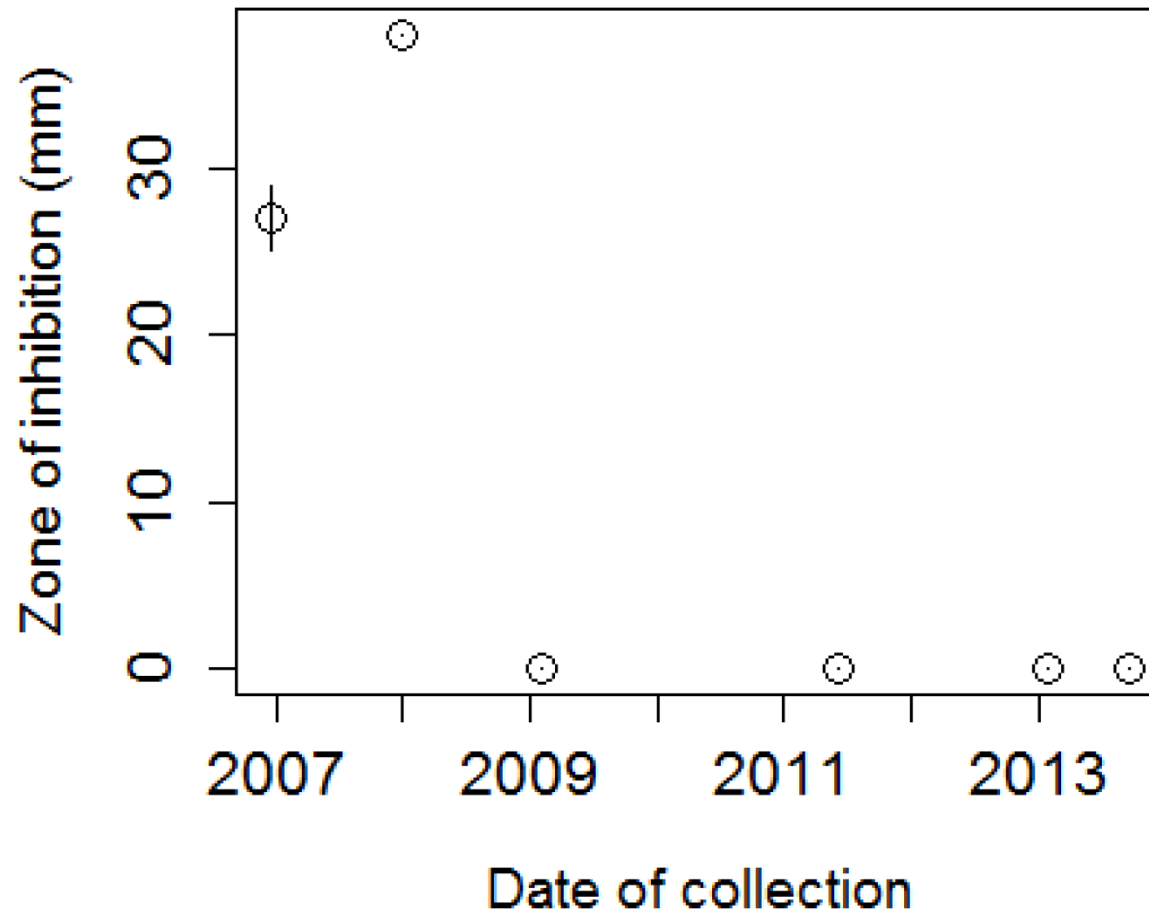
Isolate	AMX	CHL	CIP	ERY	NA	TET
19/12/2006	S	S	R	R	R	S
27/12/2007	S	S	R	R	R	S
5/02/2009	R	S	R	R	R	S
9/06/2011	R	S	R	R	R	S
31/01/2013	R	S	HR	R	R	S
23/09/2013	R	S	HR	R	R	S

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## Key:

S	Susceptible	AMX	Amoxicillin	ERY	Erythromycin
R	Resistant	CHL	Chloramphenicol	NA	Nalidixic acid
HR	Highly resistant	CIP	Ciprofloxacin	TET	Tetracycline

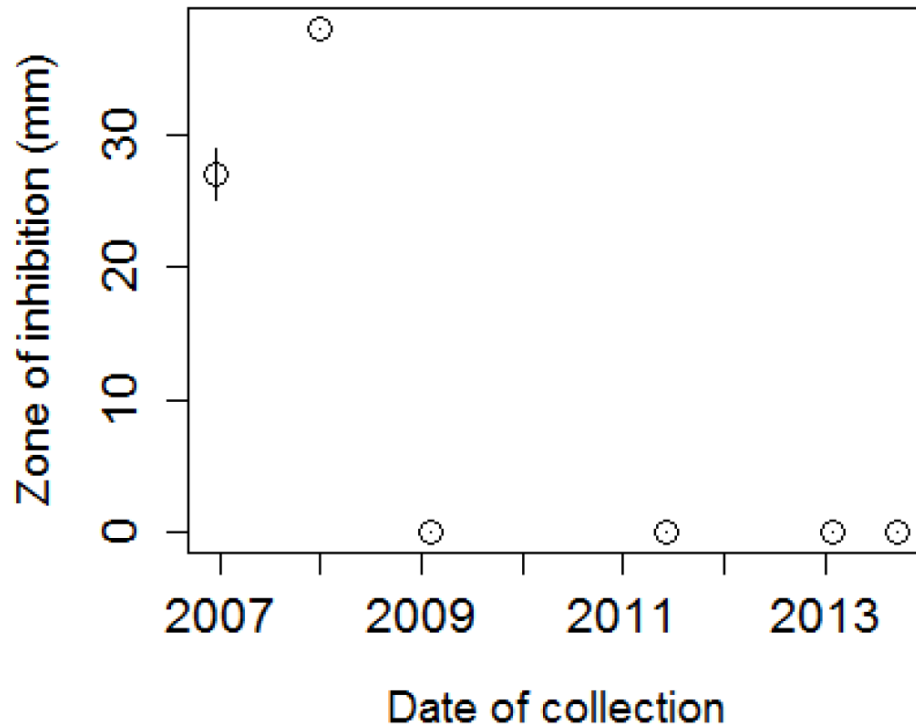
# Amoxicillin



Time series of *C. jejuni* ST45 amoxicillin disc diffusion results (error bars represent 95% confidence intervals).

# Amoxicillin resistance mechanism

A single nucleotide in the promoter region modulates the expression of the  $\beta$ -lactamase OXA-61 in *Campylobacter jejuni*.  
Zeng *et al.*, (2014) *J. of Anti. Chemo.* **69**: 1215-1223.



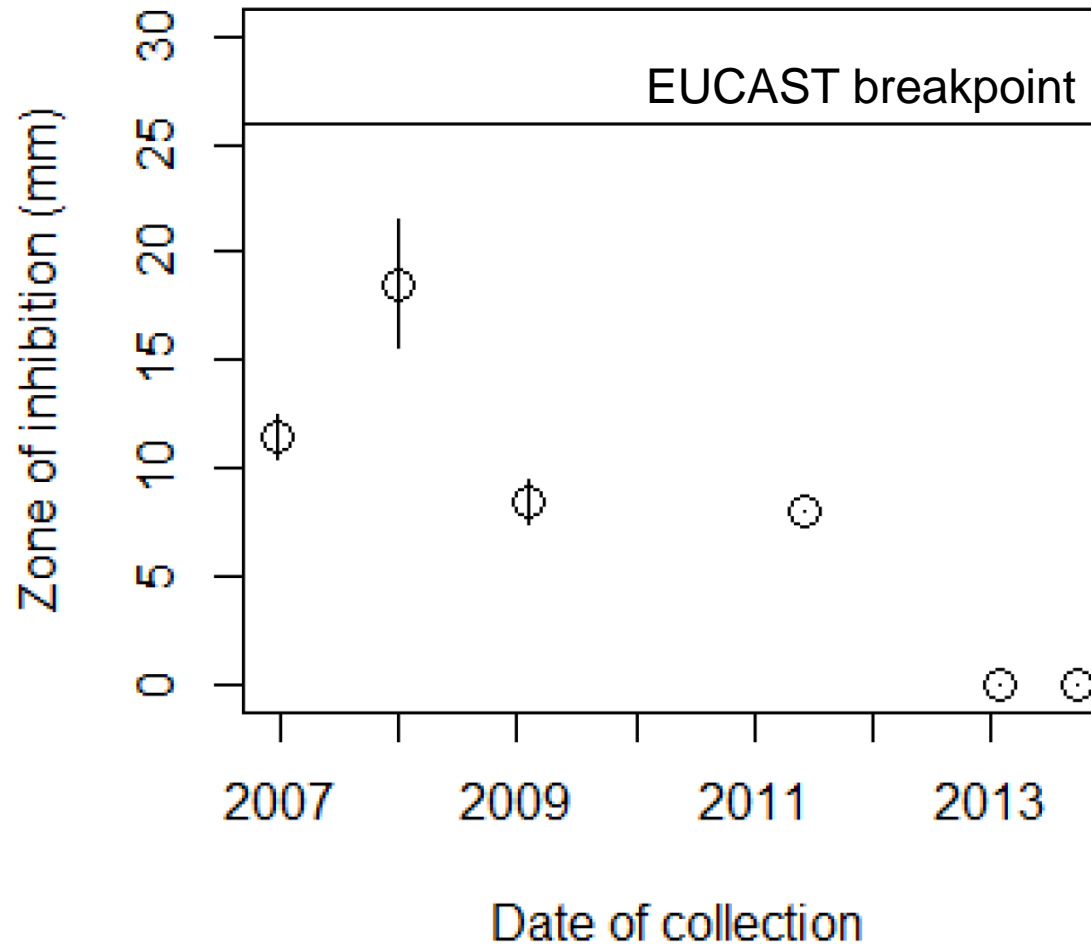
19/12/2006	A	T	T	T	A	A	A	G	A	A	A	T	A	A	A	G	C	T	T	T	A	A	A	A	G	T	A	T	T	T	T	G	T	T	T	A	A	A	T	T	A	T	T	T	A	A	A	T	A	G	A	A	A	G	A	T	A	T	T	T	T	A	T	G
27/12/2007	A	T	T	T	A	A	A	G	A	A	A	T	A	A	A	G	C	T	T	T	A	A	A	A	G	T	A	T	T	T	T	G	T	T	T	A	A	A	T	T	A	T	T	T	A	A	A	T	A	G	A	A	A	G	A	T	A	T	T	T	T	A	T	G
5/2/2009	A	T	T	T	A	A	A	T	A	A	A	T	A	A	A	G	C	T	T	T	A	A	A	A	G	T	A	T	T	T	T	G	T	T	T	A	A	A	T	T	A	T	T	T	A	A	A	T	A	G	A	A	A	G	A	T	A	T	T	T	T	A	T	G
9/6/2011	A	T	T	T	A	A	A	T	A	A	A	T	A	A	A	G	C	T	T	T	A	A	A	A	G	T	A	T	T	T	T	G	T	T	T	A	A	A	T	T	A	T	T	T	A	A	A	T	A	G	A	A	A	G	A	T	A	T	T	T	T	A	T	G
31/1/2013	A	T	T	T	A	A	A	T	A	A	A	T	A	A	A	G	C	T	T	T	A	A	A	A	G	T	A	T	T	T	T	G	T	T	T	A	A	A	T	T	A	T	T	T	A	A	A	T	A	G	A	A	A	G	A	T	A	T	T	T	T	A	T	G
23/9/2013	A	T	T	T	A	A	A	T	A	A	A	T	A	A	A	G	C	T	T	T	A	A	A	A	G	T	A	T	T	T	T	G	T	T	T	A	A	A	T	T	A	T	T	T	A	A	A	T	A	G	A	A	A	G	A	T	A	T	T	T	T	A	T	G

Promoter

Starting  
codon

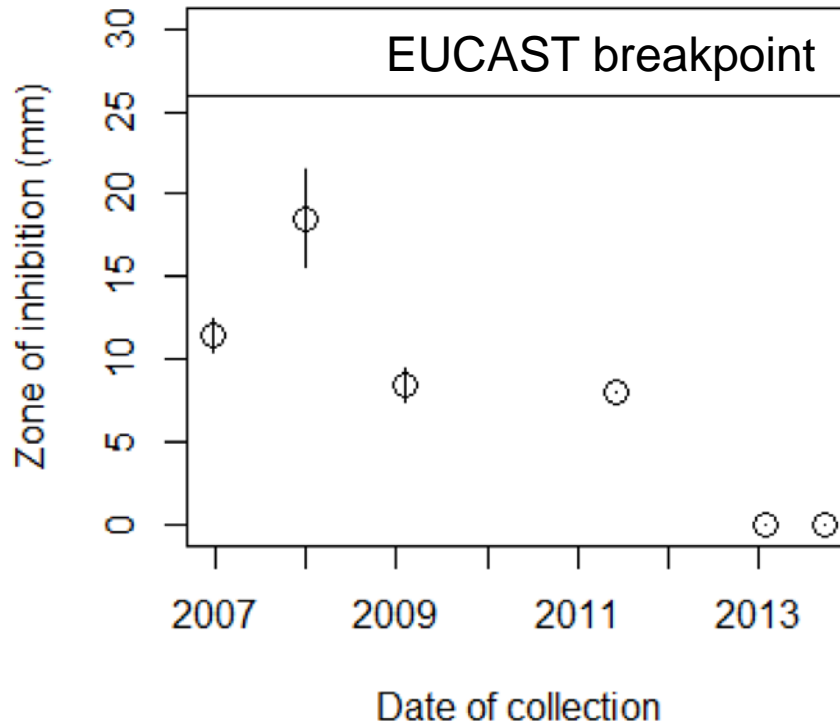
*Campylobacter jejuni* ST45 *bla*OXA-61 gene promoter alignment.

# Ciprofloxacin



Time series of *C. jejuni* ST45 ciprofloxacin disc diffusion results over time (error bars represent 95% confidence intervals).

# Ciprofloxacin resistance mechanism



Type II topoisomerase mutations in ciprofloxacin-resistant strains of *Pseudomonas aeruginosa*.  
Mouneimne, et al., (1999) *Anti. Agents and Chemo.* **43**: 62-66.

19/12/2006	DSAGGSAKQGRERSFQAAILPLRGKILNVEKARLDKILKSEQIQNMITAFGCGIGEDFDLSKLRVHKL
27/12/2007	DSAGGSAKQGRERSFQAAILPLRGKILNVEKARLDKILKSEQIQNMITAFGCGIGEDFDLSKLRVHKL
5/2/2009	DSAGGSAKQGRERSFQAAILPLRGKILNVEKARLDKILKSEQIQNMITAFGCGIGEDFDLSKLRVHKL
9/6/2011	DSAGGSAKQGRERSFQAAILPLRGKILNVEKARLDKILKSEQIQNMITAFGCGIGEDFDLSKLRVHKL
31/1/2013	DSAGGSAKQGRERSFQAAILPLRGKILNVEKARLDKILKFEQIQNMITAFGCGIGEDFDLSKLRVHKL
23/9/2013	DSAGGSAKQGRERSFQAAILPLRGKILNVEKARLDKILKFEQIQNMITAFGCGIGEDFDLSKLRVHKL

Amino acid 460

*Campylobacter jejuni* ST45 GyrB amino acid alignment.

# Conclusions

Gives insight into the evolution of *Campylobacter* within a continually excreting human host.

Provides evidence of *Campylobacter* phenotypic changes by the accumulation of SNPs.



# Future directions

Determine if the onset of antibiotic resistance coincides with antibiotic therapy.

Monitor *Campylobacter* excretion regularly over a year and identify factors associated with excretion, e.g. microbiome changes, inflammation and immune markers.

Identify potential sources of *C. jejuni* ST45.

# Acknowledgements

New Zealand Genomics Limited (NZGL)

Medlab Central, Palmerston North

PhD supervisors

IVABS post-graduate fund

Tui Shadbolt, Ministry of Primary Industries

Craig Thornley, Regional Public Health