Ministry for Primary Industries
Manatū Ahu Matua

Risk Profile

The Emergence of *Campylobacter jejuni* ST 6964 in Poultry in New Zealand and its Associated Antimicrobial Resistance

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Scientific Interpretive Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers

Risk Profile: The Emergence of *Campylobacter jejuni* ST 6964 in poultry in New Zealand and its associated antimicrobial resistance

Background

A sequence of findings from studies on *Campylobacter* isolates from poultry and human cases indicated the emergence of a poultry-host adapted strain, ST 6964, with the capability of causing human infections severe enough to require medical treatment.

The purpose of a risk profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions, and take further action if necessary. The food/hazard combination addressed by this risk profile is *Campylobacter jejuni* ST 6964 (ST 6964) and its associated antimicrobial resistance (AMR) in poultry in New Zealand.

This risk profile was commissioned to address the following risk management questions:

- What are the potential public health and food safety risks associated with the emergence of *Campylobacter* ST 6964 in New Zealand?
- What are the significant data gaps which, if filled, would allow a more comprehensive assessment of public health risks attributable to the emergence of *Campylobacter* ST 6964 and its associated antimicrobial resistance in New Zealand?

The risk profile consolidates information gathered from key informant interviews, review of available (mainly MPI) scientific data and reports, as well as a rapid evidence assessment of the scientific literature.

Risk Profile

Data on resistance of human *Campylobacter* isolates is limited. While fluoroquinolone and tetracycline resistance seems to be associated with ST 6964, to date no resistance of the strain to erythromycin, the drug of choice for human treatment has been observed. At this time, despite the emergence of this resistant strain, no change in the disease burden of human campylobacteriosis has been identified in New Zealand.

Current findings indicate a low selective pressure for AMR in the New Zealand poultry industry. Good food safety and hygiene practices are the most important control measures to ensure the reduction in transmission of both resistant and susceptible *Campylobacter* from contaminated poultry meat to people.

The appraisal of risk was limited by the fact that detections of ST 6964 and assessment of their AMR had not been the main focus of the studies to date, but rather incidental findings. The public health impact of ST 6964 and its associated emergence is yet to be fully understood and it is not known whether and how the ST and its associated AMR will continue to evolve and spread.

Various data gaps were identified in the Risk Profile:

- Absence of representative sampling of *Campylobacter jejuni* isolates from human cases and poultry to gain an understanding of the prevalence of AMR and the predominance of ST 6964
- Epidemiological and genetic assessment of the link between ST 6964 and the observed antimicrobial resistance emergence in isolates of poultry and human origin
- Assessment of transmission routes including in particular the importance of foodborne transmission of ST 6964 and its associated AMR into the human population
- Investigation of the presence of ST 6964 in other animal species in New Zealand, including AMR testing of detected isolates
- Factors leading to increased selection, maintenance and dissemination of ST 6964
- Improved understanding of the virulence and clinical manifestation of ST 6964 in humans, and in particular those that display AMR.

Given the present evidence, no conclusions can be drawn as to what has driven the emergence of ST 6964 and its associated AMR in New Zealand. No discernible effect on public health has been demonstrated at this time.

Risk Profile

The Emergence of *Campylobacter jejuni* ST 6964 in Poultry in New Zealand and its Associated Antimicrobial Resistance

Prepared for
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1 Executive Summary

The purpose of a risk profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions, and take further action if necessary. Risk profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management.

The food/hazard combination addressed by this risk profile is *Campylobacter jejuni* ST 6964 (referred to as *Campylobacter* ST 6964) and its associated antimicrobial resistance in poultry in New Zealand. This risk profile has been commissioned in order to address the following specific risk management questions:

- What are the potential public health and food safety risks associated with the emergence of *Campylobacter* ST 6964 in New Zealand?
- What are the significant data gaps which, if filled, would provide greater certainty of risk attributable to the emergence of *Campylobacter* ST 6964 and its associated antimicrobial resistance (AMR) in New Zealand?

A sequence of findings from independent studies on *Campylobacter* isolates from poultry and human cases indicate the emergence of a poultry-host adapted strain (i.e. ST 6964) with the capability of causing human infections severe enough to require medical treatment. Currently available exposure data suggest a high degree of association between the observed recent events both in poultry and human cases. A high level of fluoroquinolone resistance seems associated with the ST (up to 19%), although to date no resistance of the strain to erythromycin, the drug of choice for human treatment has been observed. Presently the assessment of risk is hampered, as in most studies to date, detection of ST 6964 and assessment of its AMR were not the main focus of the work, but rather incidental findings. Given the current evidence, no conclusions can be drawn as to what has driven the emergence of ST 6964 and its associated AMR in New Zealand, although possible hypotheses include travel- or trade-associated imports. Further the public health impact of ST 6964 and its associated emergence is yet to be fully understood and it is not known how the ST and its associated AMR will continue to evolve and spread.

Industry controls for *Campylobacter* contamination in poultry are in place in New Zealand and any interventions that aim to control contamination will indirectly affect resistance elements on poultry meat and impact on AMR dissemination. Specific control measures for AMR in *Campylobacter* in poultry are currently limited, relying on general measures such as surveillance, kitchen hygiene, and occupational safety as well as focussing on lowering the frequency and levels of contamination. At this time good food safety and hygiene practices are the most important control measures to ensure the reduction in transmission of both resistant and susceptible *Campylobacter* from contaminated meat to people.

The data gaps identified in this Risk Profile are:

- Representative and parallel sampling of isolates from human cases and poultry to gain an understanding of the prevalence of AMR and the pre-dominance of ST 6964.
- Epidemiological and genetic assessment of the link between ST 6964 and the observed antimicrobial resistance emergence in isolates of poultry and human origin. It is critical for this to be done on representative samples. Ideally this would involve evolutionary analysis.

- Assessment of transmission routes from poultry to humans (and vice-versa) including in particular the importance of foodborne transmission of ST 6964 and its associated AMR into the human population.
- Factors leading to increased selection, maintenance and dissemination of ST 6964.
- Investigation of the presence of ST 6964 in other animal species in New Zealand, including AMR testing of detected isolates.
- Improved understanding of the virulence and clinical manifestation of ST 6964 in humans, and in particular those that display AMR.

2 Statement of Purpose

The purpose of a risk profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk profiles are part of the Risk Management Framework[1] (RMF) approach taken by the Ministry for Primary Industries (MPI).

The RMF ensures that MPI's strategic direction is developed based on scientific evidence, and includes the following benefits:

- It establishes food control systems, which achieve acceptable levels of consumer protection.
- It ensures that regulatory decisions are in proportion to the health risks involved.
- It allows innovation and flexibility in applying regulatory control measures.
- It supports the industry by facilitating technical advice and helping to develop tools that manage food safety risks.

The RMF consists of a four-step process, as shown in Figure 1.

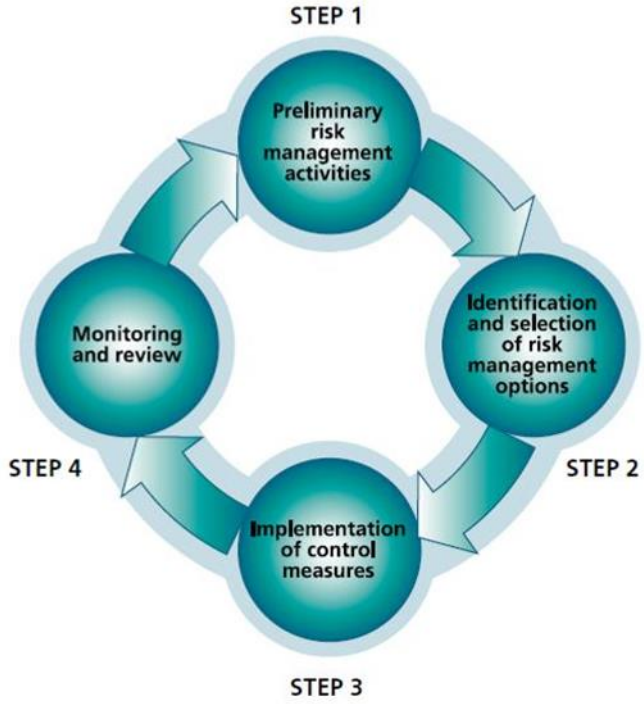


Figure 1: *The four steps of the Risk Management Framework.*

Step 1 in the RMF includes a number of tasks:

- Identification of food safety issues;
- Risk profiling;
- Establishing broad risk management goals;
- Deciding on the need for a risk assessment;
- If needed, setting risk assessment policy and commissioning of the risk assessment;

[1] http://www.foodsafety.govt.nz/science-risk/about-us/food-safety-science-group/rmf.htm

- Considering the results of the risk assessment; and
- Ranking and prioritisation of the food safety issue for risk management action.

Risk profiling is one component of Preliminary Risk Management Activities, the initial step of MPI's RMF framework. Risk managers might use risk profiles to guide identification and selection of different risk management options. This is of particular relevance where rapid action is needed, where sufficient scientific information for action is not available or where embarking on a full risk assessment is considered impractical.

To ensure a standardised approach, risk profiles are organised as much as possible as they would be for a conventional qualitative risk assessment, as defined by the Codex Alimentarius Commission[2] (CAC), the international body established by the Food and Agriculture Organisation (FAO) of the United Nations and the World Health Organisation (WHO) for setting food standards.

This risk profile considers *Campylobacter* ST 6964 in poultry in New Zealand and its associated AMR. It does not consider other poultry products such as eggs or food products from other animal species. This risk profile is the first MPI assessment considering this food-hazard combination.

MPI has recognised *Campylobacter* as one of the three most important foodborne pathogens in New Zealand. The organisation has taken a strategic approach to *Campylobacter* risk and has in the past commissioned a range of preliminary risk evaluation activities, including risk profiling, to better understand the public health and food safety risks associated with *Campylobacter* contamination of food.

In 2014, *Campylobacter* ST 6964, previously not described in New Zealand, was reported as having been identified in *Campylobacter* spp. cultured from New Zealand retail poultry, broiler chickens and human stools. In addition these samples were found to demonstrate AMR.

This risk profile has been commissioned in order to address the following specific risk management questions:

- What are the potential public health and food safety risks associated with the emergence of *Campylobacter* ST 6964 in New Zealand?
- What are the significant data gaps which, if filled, would provide greater certainty of the human health risk attributable to the emergence of *Campylobacter* ST 6964 in New Zealand?

To answer these risk management questions, this risk profile:

- Describes the emergence of *Campylobacter* ST 6964 in New Zealand.
- Identifies, accumulates and critically reviews the findings of any surveys/studies describing the strain and its AMR prevalence.
- Briefly describes the emergence of other *Campylobacter* STs in New Zealand.
- Places the New Zealand finding in an international context both with respect to the ST emergence and the AMR findings.

This assessment consolidates information gathered from key informant interviews, review of available data and reports as well as a rapid evidence assessment of the scientific literature.

[2] http://www.codexalimentarius.org/codex-home/en/

3 Hazard and Food

3.1 HAZARD

3.1.1 *Campylobacter*

Campylobacter jejuni is one of the most frequent causes of bacterial gastroenteritis in humans (Cody *et al.,* 2012). As outlined by Lake *et al.,* (2013) *Campylobacter* spp. are non-sporulating, Gram-negative bacteria that appear as slender, spirally curved rods under the microscope. Optimum growth of *Campylobacter* is at 42°C, ranging from 30.5 to 45°C. *Campylobacter* spp. are comparatively slow growing even under optimum conditions with optimum growth at a_w= 0.997[3]. There are many species of *Campylobacter* but the evidence suggests that in New Zealand two species, *C. jejuni* and *C. coli*, are of major significance to public health. Seasonal patterns in *Campylobacter* infections in humans have been identified in many temperate countries, including New Zealand, and are associated with a peak of human cases in summer (Friedrich *et al.*, 2015).

In New Zealand, a country with a historically high rate of campylobacteriosis notifications, results from molecular-based surveillance in a sentinel site founded in 2005 provided strong evidence that a large proportion of human cases were linked to poultry meat consumption (Muellner *et al.,* 2009). These findings stimulated the implementation of regulatory and voluntary control strategies along the poultry supply chain (Muellner *et al.,* 2013). They were announced in 2007 and fully implemented in 2008 (NZFSA, 2008), resulting in a 50% reduction in human disease incidence in 2008 compared with the previous high disease levels reported from 2002 to 2006 (Muellner, 2011). Active risk management of *Campylobacter* is on-going (MPI, 2013).

Overall typing technologies have been instrumental in enhancing epidemiological investigations of *Campylobacter* spp. (Muellner *et al.,* 2013; Taboada *et al.,* 2013). The recent risk profile on *Campylobacter* spp. in poultry (Lake *et al.,* 2014) provides a high-level overview of typing approaches for *Campylobacter* spp. Multi-locus-sequence typing (MLST) in particular has been instrumental in the elucidation of risk factors and for source attribution studies both in New Zealand (e.g. Muellner *et al.,* 2009; Muellner *et al.,* 2011) and overseas (e.g. Strachan *et al.,* 2009; Bessel *et al.,* 2012). In MLST, sequence types are grouped into clonal complexes (CC) by their similarity to a central allelic profile (genotype); sometimes also referred to as sequence type complexes (Dingle, 2001). As a potential alternative to MLST and other subtyping approaches currently being utilised, Multiplex Ligation-Dependent Probe Amplification–Binary Typing (Mbit) has recently been established in New Zealand (Cornelius *et al.,* 2014) as a same-day subtyping approach with short turn-over time and less need for specialist equipment. A further method, whole genome sequencing (WGS), provides the highest level of discriminatory power for epidemiological subtyping (Taboada *et al.,* 2013). Given the largely sporadic nature of *Campylobacter* spp. the ability to deploy WGS analysis in large-scale surveillance for the bacterium and its utility are yet to be confirmed. Further, the expertise and tools required for rapid analysis preclude the use of WGS data for routine subtyping. However, laboratory-based and in-silico WGS-based methods could be designed under a common framework to achieve optimal epidemiological and phylogenetic performance (Taboada *et al.,* 2013).

Antimicrobial resistance is known to be present in several pathogens including major foodborne zoonotic pathogens such as *Salmonella*, *Campylobacter* and *Escherichia coli*, with large variation reported from different countries (Garcia-Migura *et al.,* 2014). Detection of AMR is commonly achieved via phenotypic susceptibility testing (such as the disc diffusion

[3] Pathogen Data Sheet *Campylobacter*; available at: http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter-Organism_Causes.pdf

test) or via measuring the presence or absence of known resistance genes. Aspects of antimicrobial susceptibility such as the preferred choice of cut-off value (e.g. epidemiological cut-off (ECOFF)) or on what constitutes a resistant pathogen (e.g. therapeutic resistance only) are still not universally defined (Garcia-Migura *et al.,* 2014); however guidelines are available e.g. on the interpretation of results.

Use of antimicrobials in animals can select for resistance in animal pathogens, but can also cause resistance in pathogens that can be transmitted to humans via the food chain (e.g. foodborne *E. coli*) which can then exchange resistance genes with other pathogens causing human disease (PHAC, 2015). Evidence is available that directly links the use of antimicrobials in animals to resistant bacteria in humans, for example the observed association between the emergence of AMR in humans and the use of quinolone in food-producing animals (Alfredson *et al.,* 2007; Rushton *et al.,* 2014). In summary (from Alfredson *et al.,* 2007) *Campylobacter* spp. are capable of the genetic mechanisms for natural transformation and conjugation, indicating that acquired antibiotic resistance genes can rapidly be transferred between strains.

AMR in *Campylobacter* spp. has been identified against several classes of antimicrobial drugs. Typical tetracycline resistance is mediated by ribosomal protection proteins (RPP). These proteins are located in a self-transmissible plasmid and include *Tet(O)* and *Tet(M)*, which bind to the ribosome and prevent protein synthesis. The prevalence of plasmids in human isolates has been reported to be 13–52%, with the majority being resistance plasmids. Plasmid-mediated resistance can be exchanged between bacterial cells via horizontal gene transfer (in this case conjugation) (Alfredson *et al.,* 2007).

Resistance to the quinolone nalidixic acid and the fluoroquinolones ciprofloxacin and enrofloxacin appear to be primarily acquired through the acquisition of mutations in the DNA gyrase and DNA topoisomerase IV genes, with resistance in *Campylobacter* spp. primarily attributed to mutation in the *gyrA* gene. Macrolide resistance in *Campylobacter* spp. has been attributed to multiple mechanisms including point mutation or methylation of the 23S rRNA gene, hydrolysis of the drug, and efflux pump as well nucleotide mutations in the petidyl transferase region of the genome. Resistance against aminoglycoside, β-lactam antibiotics, sulphonamides and multidrug resistance are also known in *Campylobacter* spp.

3.1.2 Rapid evidence assessment

Methods

A rapid evidence assessment (REA) methodology was used to identify resources pertinent to the emergence of *Campylobacter* ST 6964 and its AMR, and to identify and evaluate recent scientific literature on the global emergence of other *Campylobacter* MLST sequence types and their associated AMRs. REAs are a form of knowledge synthesis in which components of the systematic review process are simplified in order to produce key information in a timely manner (Khangura, 2014). In the health sector rapid assessments are gaining popularity as a means of supporting efficient evidence-based decision-making (Featherstone, 2015) particularly in response to urgent or emergent needs (Khangura, 2014). This structured approach was chosen to gain a first overview of the density and quality of evidence on the emergence of ST 6964 and its associated AMR.

Search 1: To identify information on *Campylobacter* ST 6964, a scanning search of the scientific literature databases *Web of Science*[4], *Scopus*[5] and *PubMed*[6] was conducted using the following Boolean query: (*'Campylobacter'* AND 'ST 6964'). The search included all fields with no restrictions on date or publication language. To cover unpublished work, the grey literature was investigated through a Google web search using the core search term described above.

Search 2: To identify recent scientific literature pertaining to other *Campylobacter* sequence types and their associated AMRs, peer-reviewed publications in the scientific databases *Web of Science* and *PubMed* were interrogated using the following search queries:

1. To identify information on other STs the Boolean query ((*'Campylobacter'*) AND ('MLST' OR 'sequence type' OR 'ST' OR 'CC' OR 'clonal complex')) was run.

2. To identify publications specific to the link between ST or CC and AMR, the sub-query ((*'Campylobacter'*) AND ('MLST' OR 'sequence type' OR 'ST' OR 'CC' OR 'clonal complex') AND ('AMR' OR 'resist*')) was run. Through the use of wildcards (*) articles containing any variation of the search term were identified.

All articles published in the last 15 years (2000 and later) were included. This was considered sufficient as a MLST scheme for *Campylobacter* was first published in 2001 (Dingle *et al.*, 2001). The *Web of Science* query was restricted to a title search while the *PubMed* query was restricted to a title/abstract search. Only English-language publications were considered. A complete list of all articles retrieved and assessed by the described protocols is available on request.

Following retrieval, data from both searches were pooled and any duplicate references were removed. A first-level screening of abstracts was undertaken to identify articles of likely relevance to the risk profile. The retained references were then reviewed in full.

Results

Search 1, for information on the emergence of *Campylobacter* ST 6964, returned no records from any of the databases interrogated. Search 2, of recent literature on the emergence of other *Campylobacter* sequence types and their associated AMRs, returned a total of 404 publications, 152 from *Web of Science* and 252 from *PubMed*. Of these 116 were duplicates, bringing the combined total of unique articles included in the first screening to 288. Following assessment of all abstracts a total of 37 articles were identified as providing information of potential relevance to the risk profile and were reviewed in full. Of the 37 articles, 36 provided information of direct relevance to the risk profile and informed this assessment.

3.1.3 *Campylobacter* ST 6964

Campylobacter ST 6964 belongs to CC 354. According to the *PubMLST* database[7] CC 354 includes a total of 160 different STs reported from 32 different countries. Isolates from this CC have been found in a wide variety of samples, including those from humans, environmental water, chickens, ruminants and dogs. Other than ST 6964, five different STs of CC 354 have previously been reported in New Zealand, originating from four different

[4] http://wokinfo.com
[5] http://www.elsevier.com/solutions/scopus
[6] http://www.ncbi.nlm.nih.gov/pubmed/
[7] http://pubmlst.org/campylobacter/; accessed on 14 September 2015

species sources (Table 1). *PubMLST* did not state details of the samples from which the strains were isolated e.g. if from chicken were they meat or caecal samples.

Table 1: Previous isolations of multilocus sequence typing (MLST) sequence types (ST) belonging to clonal complex (CC) 354 recorded in the *PubMLST* database from New Zealand.

Year	Source	ST
2005	Chicken	3721
2007	Human stool	3784
2011	Cattle	5654
2011	Sheep	5656
2011	Cattle	5656

A MLST profile detected in a *Campylobacter* isolate from human stool in China is currently the only recorded ST 6964 isolation in the *PubMLST* database. The profile was submitted in September 2014 by the Center for Disease Control and Prevention, Beijing; however the sampling date could have been much earlier. Results from molecular typing and susceptibility testing of *C. jejuni* from North China have been published, including authors from China's Centre for Disease Control and Prevention. While this work does not report the occurrence of ST 6964 resistance to nalidixic acid, levofloxacin and ciprofloxacin resistance was observed in 100% (44/44) of tested isolates (Zhang *et al.,* 2010). However, in another study resistance levels to fluoroquinolones ranged from 13–16%, with resistance levels increasing up to 45% for specific CCs (Xin *et al.,* 2010). In addition, STs from CC 354 have recently been reported from China (Zhang *et al.*, 2015) with ST 354 being reported as the dominant strain from chicken and food in Eastern China.

The REA did not retrieve any additional published information that made specific reference to ST 6964.

3.1.4 Emergence of *Campylobacter* STs

Campylobacter ST strains (e.g. 474) have previously emerged in New Zealand, spread throughout the poultry flock, spilled over into humans and then declined in prevalence (Muellner *et al.,* 2013; Muellner *et al.,* 2011). The emergence of ST 474 in New Zealand may be explained by the country's geographical isolation and its uniquely structured poultry industry (Müllner, 2010). A distinctive molecular epidemiology of *C. jejuni* in the New Zealand poultry industry was discovered in the country's sentinel surveillance site (Müllner, 2010). Sequencing of ST 474 revealed a high rate of recombination and it was hypothesised that the ability to uptake and insert naked DNA into the *C. jejuni* genome could be an important mechanism for population-level adaptation to fluctuating environments, such as those encountered during transmission between different host species (Biggs *et al.,* 2011). Further studies concluded that the genetic uniqueness of ST 474 might also have arisen due to the geographic isolation of New Zealand, the structure of the poultry industry and an absence of exchange of sequence types, which might typically occur through international trade of fresh poultry meat (Mohan, 2011). Novel CCs have previously been identified in New Zealand (Carter *et al.,* 2009) along with a wide geographical distribution of internationally rare *Campylobacter* clones (McTavish *et al.,* 2008).

In general *Campylobacter* is found to show substantial genetic diversity (e.g. Kärenlampi *et al.,* 2007; Guyard-Nicodeme *et al.,* 2015) and strains have previously also emerged in other host

species, such as ST 8 in ruminants in the United States (US) (Sahin *et al.,* 2012). Host-association is commonly observed, such as the occurrence of ST 3272, which is infrequently detected in other hosts, in organically farmed laying hens in Finland (Kovanen *et al.,* 2014). Many commonalities exist between different studies and internationally common STs (e.g. ST 21 complex and ST 45 (Guyard-Nicodeme *et al.,* 2015)) have been reported from different countries. Higher rates of overseas travel have been reported in patients from the United Kingdom (UK) infected with previously unassigned strains (Bessel *et al.,* 2012). Husbandry practices associated with intensive agriculture have previously been associated with generating a reservoir of human disease-associated *Campylobacter* lineages (Colles *et al.,* 2011).

While many studies report snapshots of the prevalence of *Campylobacter* and specific STs and CCs (e.g. Kovanen, 2014) the detection of temporal trends and true emergence relies on the availability of longitudinal studies that can monitor chronological developments. The clonal distribution of *Campylobacter* in a 10-year study in Finland showed that the otherwise uncommon ST 677, was frequently isolated during the seasonal May–August peaks from the blood of severely infected human patients. This accounted for 64% of isolations in these summer months (Feodoroff *et al.,* 2010).

Sequence type 4526 was reported to be widespread in Japan in 2005–2006 and a follow-up study in 2010–2011 revealed continued widespread presence of both ST 4526 and the closely related ST 4523 (Asakura *et al.,* 2013). In addition, the authors reported increases in *in vivo* fitness and suggested that microevolution of the pathogen in poultry could have enabled these STs to become widespread. Further studies on the genomic evolution of ST 4526 in cattle poultry and human isolates concluded that a combination of molecular features might have been responsible for the clonal thriving of this ST in Japan (Asakura *et al.,* 2012).

In a single site in the UK, clonal complexes of *Campylobacter* isolates from human samples exhibited a changing incidence and differences in seasonality over a 6-year period with substantial year-to-year variation and increases of relative incidence for specific CCs (Cody *et al.,* 2012). Studies in Belgium have revealed the presence of a clonal group (including nine STs) of *Campylobacter*, comprising 22% of all isolates, originating from five different companies and isolated over seven sampling months (Habib *et al.,* 2009).

3.1.5 Emergence of antimicrobial resistance in other *Campylobacter* STs

Several surveys have combined MLST analysis and susceptibility testing. Most of these studies are very recent (14/21 articles retrieved were published in 2012–2015) and most concentrate on isolates from humans and poultry. Furthermore, poultry sampling is commonly focused on chicken meat or faecal samples, with the origin of the isolate not always clearly described. An overview of findings to date is presented in Table A1 (see Appendix). While these studies combined MLST and susceptibility testing, several centred on describing the frequencies of detections and did not comprehensively investigate the link between ST and AMR. However, several studies did assess this link and reported significant correlations between certain MLST CC's and AMR (e.g. Cody *et al.,* 2012 (who described an association between ST 354 and ciprofloxacin resistance in human isolates); Guyard-Nicodeme *et al.,* 2015) while some reported no association (e.g. Stone *et al.,* 2013). A lack of significant association could be explained by a deficiency of power due to a small sample size.

Emergence of ciprofloxacin resistance in *Campylobacter* from 3% to 38% over a period of 17 years has been observed in the UK, and specific clonal complexes could be linked to ciprofloxacin sensitivity or resistance (Cody *et al.,* 2012). Chicken-associated *Campylobacter*

isolates have shown widespread acquisition of AMR by relatively distantly-related lineages, both through independent mutation events and horizontal gene transfer, with subsequent expansion and clonal propagation of these resistant lineages (Wimalarathna *et al.,* 2013). Proliferation of resistant lineages is likely occurring in farmed chicken, as *Campylobacter* is not known to multiply outside of the host and humans are rarely long-term carriers of the pathogen (Wimalarathna *et al.,* 2013). Although resistance in human pathogens is often attributed to incomplete or inappropriate use of antimicrobials, for *Campylobacter* this explanation is likely insufficient because most human cases are self-limiting and not treated with antibiotics. There is little opportunity for resistance to proliferate in humans due to limited human-to-human transmission and hence identification of reservoirs acquiring AMR in *Campylobacter* is required (Wimalarathna *et al.,* 2013).

It has been reported that the acquisition of specific AMR increases pathogen fitness and ability to colonise (Asakura *et al.,* 2013). Susceptibility testing of the persisting clones ST 4526 and ST 4523 in Japan in both 2005–2006 and 2010–2011 showed resistance to nalidixic acid and fluoroquinolone in these sequence types to be high on both sampling occasions (100%; 6/6 ST 4526 and 1/1 ST 4523 on both occasions). However, the two strains differed in their resistance to tetracycline as a result of the presence of the plasmid-mediated *Tet(O)* gene in ST 4526 (Asakura *et al.,* 2013). Evolutionary analysis concluded there was a close lineage between the two strains.

Previous studies suggest a complex acquisition pathway for ciprofloxacin resistance, whose distribution may be influenced by the food industry (Cody *et al.,* 2012). The study by Habib *et al.* (2009) identified a high prevalence of resistance of CC 21 to tetracycline and ciprofloxacin (53% and 48%) while at the same time pan-susceptibility of CC 45, a CC that is more ubiquitous and less associated with poultry (Sopwith *et al.,* 2008), was high (90%). Evidence for clonal association (e.g. Habib *et al.,* 2009; Cody *et al.,* 2012; Wimalarathna *et al.,* 2013) and spread (Kovac *et al.,* 2014) of resistance in *Campylobacter* is also available.

The link between AMR and *Campylobacter* MLST does not seem limited to isolates originating from poultry and humans. The emergence of ST 8 in ruminants in the US (Sahin *et al.,* 2012) has been further explored by Wu *et al.* (2014) and this latter investigation revealed not just an increase in predominance of the sequence type over time (66% vs. 91%) but also increased resistance to tetracycline (19% vs. 100%). Given the absence of specific virulence properties essential for ovine abortion in *C. jejuni* the authors hypothesised that the rapid expansion of the clone in affected sheep in the United States was due to selection pressure independent of the host. Further the presence of tetracycline resistance in the clone suggests that veterinary use of this antimicrobial may have provided the required selection pressure.

3.2 FOOD

3.2.1 Description of the food

This risk profile specifically considers poultry and poultry products. The term poultry refers to commercially produced chickens (*Gallus gallus*), turkeys and ducks. Poultry products include whole poultry, poultry portions, raw value-added products (e.g. marinated or crumbed portions), packaged ready-to-eat products (e.g. cooked and smoked products) and ready-to-eat poultry products served by the food service industry.

The water activity (a_w) of poultry meat is around 0.98–0.99. The pH of chicken breast muscle is 5.7– 5.9, while that of leg muscle is 6.4–6.7. Both poultry muscle and skin provide ideal

substrates for the growth (and survival) of a wide variety of microorganisms (ICMSF, 2005). The shelf life of refrigerated (3.5–4°C) raw poultry has been reported as 12–13 days for untreated carcasses and 14 days for acidified sodium chloride treated carcasses (Sexton *et al.*, 2006).

3.2.2 Poultry sources in New Zealand

According to data from the Poultry Industry Association of New Zealand (PIANZ), approximately 190,000 tonnes of poultry meat were produced in 2014.[8] Annual production of broiler chicken meat was between140,000 and 160,000 tonnes per year from 2004–2010, with a gradual increase observed over recent years (Figure 2).

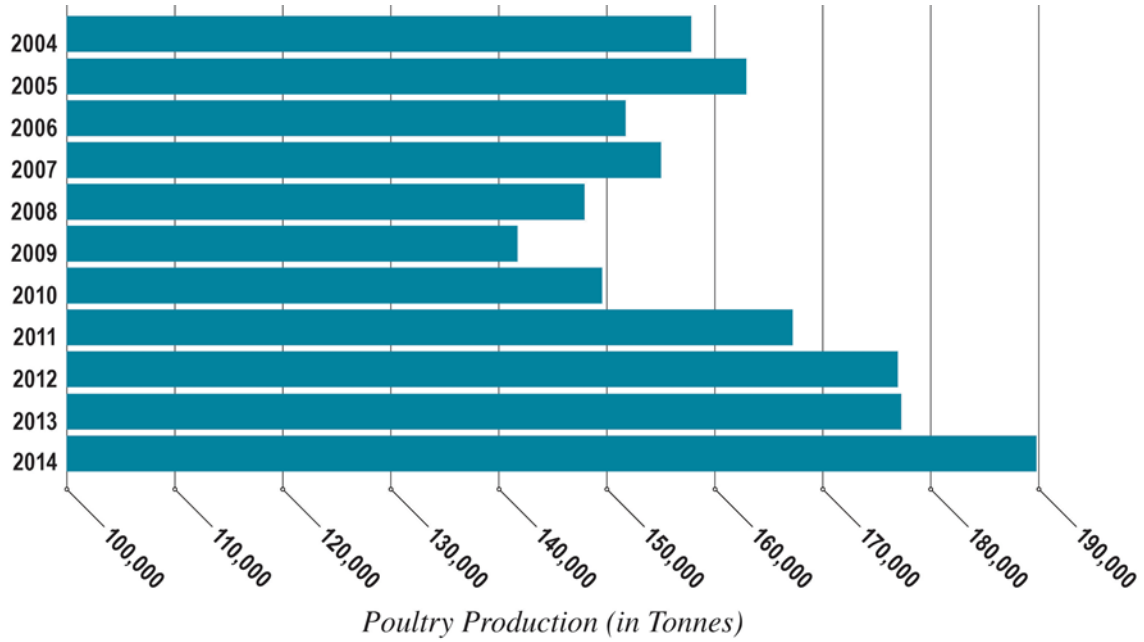


Figure 2: *New Zealand poultry meat production, 2004–2014 (data from PIANZ[1]).*

The majority of New Zealand broilers are raised in barns. Statistics New Zealand reported a total of 263 broiler farms across 14 regions in New Zealand in 2014[9]. This number is likely to increase, given that resource consent was granted for a further 22 farms in a single region in 2015[10]. A 2006 overview of broiler farming in New Zealand reported holding capacities of between 50,000 and 200,000 birds per farm (Hudson *et al.*, 2008), with a reported average shed capacity of 25,000 birds (Lake *et al.*, 2008).

Approximately half of New Zealand chicken is purchased and consumed by domestic households, with the remaining 50% entering the food service industry (Lake *et al.*, 2013). Figures from 2006 indicate that the majority of chicken is purchased as fresh, chilled product (79% vs. 21% frozen; Lake *et al.*, 2013).

Raw chicken imports are currently not permitted in New Zealand, for biosecurity reasons. The importation of specified cooked poultry meat products for human consumption from

[8] http://pianz.org.nz/industry-information/industry-statistics/poultry-production/meat-chicken-production
[9] http://nzdotstat.stats.govt.nz/wbos/Index.aspx?DataSetCode=TABLECODE7601
[10] http://www.stuff.co.nz/business/farming/agribusiness/68410224/Taranaki-economy-could-see-sunny-side-up-of-chicken-farms

Australia, and of turkey meat and meat products from approved countries, are permitted in accordance with MPI import health standards[11]. The major source of imported poultry products is Thailand, followed by the US and Australia (Lake *et al.*, 2013).

Historically only a small proportion of New Zealand poultry products were exported, mainly to Australia and the Pacific Islands. However, there is evidence of an increase in total poultry exports, with the 4,000 tonnes exported in 2009 increasing to 8,000 tonnes in 2012 (Lake *et al.*, 2013). In 2014 10% of domestically produced poultry were exported, which included export into the Gulf States.[12]

3.2.3 Structure of the New Zealand poultry industry

The New Zealand poultry industry has a unique structure, with an almost exclusive focus on the domestic market, and no importation of raw, fresh or frozen poultry products permitted due to biosecurity risks. Further 95% of poultry meat consumed is chicken meat (Muellner *et al.*, 2010). New Zealand production is very cohesive, with four major companies producing 99% of poultry meat consumed. Production within each of the major companies is vertically integrated, with all aspects of meat production including feed production, breeding, processing and value adding controlled by the company.

Two major livestock breeding companies import fertile breeding eggs for hatching every 1–2 years from both the US and the UK (Scotland)[9]. Imported great-grandparent eggs are air freighted in sealed containers and transported directly to company-owned quarantine farms where they are hatched. Quarantine farms are under MPI supervision. Hatched grandparent birds are then transported to company-owned breeding farms where they produce parent stock. Parent stock and their offspring eggs are hatched in hatcheries and raised as broilers on grow-out farms.

Poultry supply lines and product flow are described in Figure 3. While there are some common production elements between the major producers, no elements were identified as being common to all companies. For instance, in terms of feed production Major Producers 2 and 3 obtain feed from Mill B, whilst Major Producer 1 obtains feed from Mill A, and Producer 4 from Mill C. In terms of breeding stock, Major Producers 1 and 3 obtain chicks from Breeder A, whilst Producers 2 and 4 obtain chicks from Breeders 2 and 3, respectively. All three Major Producers obtain fertile breeding eggs from Egg Supplier 1 whereas Producer 4 obtains breeding eggs from Egg Supplier 2. Although staff is not shared across companies, there is some crossover of cleaning staff and contractors between two companies. In terms of product processing and distribution, one of the three major companies has a single processing plant that distributes nationwide, and one has multiple plants each serving more localised markets, whilst the remaining companies primarily distribute locally (Muellner *et al.*, 2010).

[11] http://mpi.govt.nz/importing/overview/import-health-standards/
[12] The information contained in this and the following paragraphs is based on personal communication by Kerry Mulqueen, PIANZ.

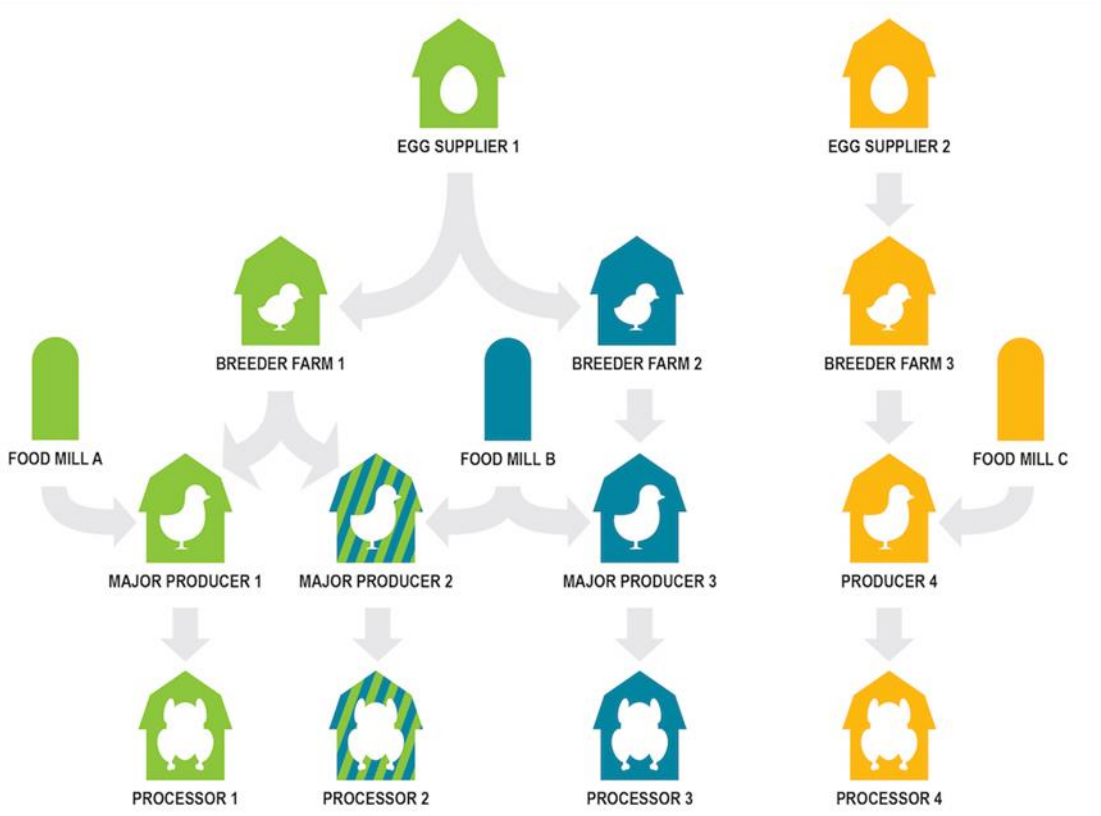


Figure 3: *Schematic overview of poultry supply lines and product flow within the New Zealand poultry industry (based on Lake et al., 2013 and information provided by PIANZ).*

3.2.4 Sources and pathways of contamination of the food by the hazard

A brief summary of potential contamination sources and factors that can affect the hazard before, during and after processing, extracted from Lake *et al.* (2013), is provided below. For a high-level overview of the behaviour of *Campylobacter* in poultry on-farm, during processing and during preparation, refer to the risk profile on *Campylobacter* in poultry by Lake *et al.* (2013). Although *Campylobacter* contamination of poultry is common, contamination of eggs with *Campylobacter* is considered a rare event (Sahin *et al.*, 2003).

The core body temperature of chickens is around 41–42°C, providing an ideal environment for survival and growth of *Campylobacter*. *Campylobacter* spp. populations can increase rapidly in the gut, leading to the excretion of large numbers of bacteria in faeces, providing a ready source of inoculation for other birds within the flock. The initial introduction of *Campylobacter* into broiler flocks has been attributed to multiple sources, including workers clothing, equipment, feed and water, flies and other insects, vertebrate pests, birds, other livestock, transport and crate contamination and poor cleaning and disinfection of sheds between flocks.

The primary processing of poultry in New Zealand follows a general sequence involving receiving, killing, bleeding, scalding, defeathering, washing, evisceration, chilling, weighing, grading and packaging. Studies have demonstrated that *Campylobacter* concentrations generally decrease following the scalding, chilling and washing steps, but may increase following defeathering or evisceration (e.g. Guerin *et al.*, 2010) where there is potential for carcasses to become contaminated with gut content. New Zealand processors typically use

scalding temperatures of 56–58°C for approximately 2 minutes followed by immersion chilling techniques.

Secondary processing of poultry can include portioning or cooking prior to packaging. Many New Zealand supermarkets have adopted leak-proof packaging for raw poultry products, although some still use the older method of packing chicken portions on trays wrapped in cling film. The inclusion of chicken skin in processed products such as sausages or burgers can increase the likelihood of *Campylobacter* presence in the raw product (Sampers *et al.*, 2008). Production of frozen poultry typically involves bagging and subsequent freezing of product using high-velocity freezers. Freezing has been shown to consistently decrease *Campylobacter* concentrations of chicken under a range of circumstances, although the extent of reduction is dependent on temperature and storage times (McIntyre, 2009).

Poultry has a relatively short shelf life compared with other meats. Although growth of *Campylobacter* is unlikely to occur during refrigerated storage, survival of *Campylobacter* is enhanced under refrigeration[13]. Standard cooking temperatures should be sufficient to destroy *Campylobacter*, as it is rapidly inactivated by temperatures of 55°C and greater. Cooking poultry to an internal temperature of 74°C will achieve at least a 7 $\log_{10}$ reduction in *Campylobacter* concentrations (Codex, 2011). As such, it is thought that risk of exposure through undercooking is low, with cross contamination being a more likely source of infection. Phillips *et al.* (2004) concluded that current evidence does not indicate that antibiotic-resistant strains are more refractory to cooking than susceptible strains. However resistance genes could still be transmitted even if the organisms carrying them are no longer viable. Further work is needed, and currently underway (ASMSF, 2014), to better understand the role of the food chain in AMR transmission.

3.2.5 Antimicrobial use in the New Zealand poultry industry

Data on national antibiotic sales have been formally collected in New Zealand since 2004, enabling the monitoring of trends in antimicrobial sales as a proxy for usage. The latest report is available in the form of the 2011–2014 sales analysis (MPI 2015; currently in draft form). This report is focused on antibiotic products used in horticulture, and those that are classed as restricted veterinary medicines (RVM) and used in animals. A total of 6,446 kg (active ingredient) of antimicrobials was sold in 2013/2014. As per information provided by PIANZ[14], no fluoroquinolones are registered for use in poultry in New Zealand. Tetracyclines are mostly used in breeder chickens to treat respiratory or digestive conditions, with poultry use of tetracycline estimated at approximately 100 kg in 2013–2014 (i.e. 1.6% of 17,448 kg sold over the same period for all species)[11]. No tetracyclines are used in broiler chicken production. Macrolides are not routinely used, but may be used in direct response to health issues in flocks, such as the 2011/2012 outbreak of femoral head avascular necrosis in broiler chickens. Zinc bacitracin, registered for treatment and prevention of necrotising enteritis due to *Clostridium perfringens* infection, is the most frequently used antimicrobial in broiler chickens.

Information provided by both the UK and US suppliers of eggs to the New Zealand industry indicate no antimicrobials are being used on eggs imported into New Zealand. Further it has been confirmed that no fluoroquinolones were used in the breeding stock of the UK supplier.

[13] http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter-Organism_Causes.pdf
[14] Personal communication: Kerry Mulqueen, Poultry Industry Association of New Zealand (PIANZ).

3.3 EXPOSURE

3.3.1 General exposure

Details of the prevalence and concentration of *Campylobacter* in New Zealand poultry products are described in detail in a recent risk profile on *C. jejuni* and *C. coli* in poultry by Lake *et al.,* (2013).

In summary, although the concentration of *Campylobacter* on poultry in New Zealand has declined since interventions were introduced in 2006–2007 (Sears *et al.,* 2011; Muellner *et al.,* 2013), there is still a high probability (>50%) that poultry purchased by consumers will contain *Campylobacter* (Lake *et al.,* 2014). Recent results from the Manawatu Surveillance Site in New Zealand reported that 84.7% of poultry samples were contaminated with *Campylobacter* and 35% of human cases were attributed to poultry (Marshall *et al.,* 2015).

To date, the rate of contamination of New Zealand food with AMR pathogens is believed to be low, based on the findings from surveys and comparative studies (e.g. Pleydell *et al.,* 2011; Heffernan *et al.,* 2011; Cornelius *et al.,* 2014). The year-long baseline survey carried out by MPI in 2009–2010 (Heffernan *et al.,* 2011), was aligned with the National Microbial Database and focused on AMR to important and commonly used antibiotics for *E. coli*, *Enterococcus*, *Campylobacter* and *Salmonella* found in freshly dressed carcasses of calves, pigs and broiler poultry from New Zealand abattoirs and processing plants. Resistance was found to be uncommon in *Campylobacter*. Among the *C. jejuni* isolates, 91.8% and 95.9% of those from very young calves and poultry, respectively, were susceptible to all antimicrobials tested. Ciprofloxacin and nalidixic acid resistance, which is conferred by the same mechanism, was significantly higher among *C. jejuni* from poultry processed in the South Island compared with that processed in the North Island. When compared with available information from other countries, *C. jejuni* isolated from New Zealand poultry were less frequently resistant than Danish isolates to ciprofloxacin (2.7% vs. 13.3%), nalidixic acid (2.7% vs. 13.3%) and tetracycline (0.3% vs. 12.0%). Compared with retail chicken breast meat in the US in 2008 *C. jejuni* from New Zealand poultry were found to be less resistant to ciprofloxacin (2.7% vs. 14.6%), nalidixic acid (2.7% vs. 14.6%) and tetracycline (0.3% vs. 49.9%). Results from a pilot surveillance programme of AMR in bacteria of animal origin in Australia showed that none of the *Campylobacter* spp. isolated from chickens exhibited resistance to gentamicin, ciprofloxacin or nalidixic acid, but 19.7% of isolates were tetracycline-resistant and 9.8% were erythromycin-resistant (DAFF, 2007; similar findings by Barlow *et al.,* 2008).

As described above, New Zealand Import Health Standards (IHS) do not permit importation of raw chicken into the country, and IHS are in place to manage risks associated with the importation of cooked poultry products and turkey meat. Pre-cooked poultry is rarely contaminated by *Campylobacter* (Lake *et al.,* 2013) and hence it is unlikely that the prevalence or concentration of the pathogen in processed imported product is high. No formal information is available on the prevalence of AMR in poultry products imported into New Zealand. Given the expected low prevalence and concentration in the pre-cooked product that is imported, if AMR *Campylobacter* were present on imported products, prevalence and concentration would likely also be very low, however this is yet to be substantiated by targeted studies.

3.3.2 Emergence of ST 6964 and its associated antimicrobial resistance in New Zealand

In 2014, ST 6964 was first detected at the Sentinel Surveillance Site in the Manawatu region of New Zealand. Further characterisation of the ST and its associated AMR has been provided by work completed by mEpiLab (Massey University), PIANZ and Environmental Science and Research Limited (ESR). The following section aims to provide an overview of available findings to date, and in part builds on preliminary evidence from on-going studies. Methodologies of relevance to this risk profile are briefly explained. Details are described in the referenced publications and reports and are not repeated in this risk profile.

Completed studies

2005/2006 PIANZ chicken study

In order to provide baseline data on AMR expressed by Gram-negative bacteria isolated from retail poultry carcasses in New Zealand in 2005 and 2006 (Mullner *et al.,* 2009a; Müllner *et al.*, 2010), a total of 193 *Campylobacter* isolates from 193 chicken carcasses were tested by disc diffusion sensitivity testing for a panel of six antibiotics (Pleydell *et al.,* 2010). This included erythromycin, ciprofloxacin, enrofloxacin, nalidixic acid, chloramphenicol and tetracycline. Almost all *Campylobacter* isolates (192/193; 99%) were fully susceptible to all of the drugs that were tested, the only exception being resistant to erythromycin. Levels of resistance in Gram-negative bacteria recovered from poultry in New Zealand were found to be amongst the lowest in the world (Pleydell *et al.,* 2010). The finding of low levels of AMR in this survey was later supported by the 2009–2010 baseline survey of AMR in bacteria from selected New Zealand foods (Heffernan *et al.,* 2011).

2014 PIANZ chicken study

In a repeat of the poultry baseline study from 2005–2006, 199 *Campylobacter* isolates from 123 chicken carcases/pieces sampled in 2014 were tested using the same methodology as described above. Fifteen isolates from eight chickens were resistant to both ciprofloxacin and tetracycline. The eight chickens were from three different companies and the *Campylobacter* were isolated between May and November 2014. All of the resistant isolates were identified as ST 6964 by MLST typing (results unpublished).

2014 Manawatu Sentinel Surveillance Site

Simultaneous collection of human samples and retail poultry samples has been occurring since 2005 (Müllner *et al.,* 2010; Muellner *et al.,* 2013). In 2014, a total of 239 human samples were submitted to Massey University's mEpiLab, of which 204 were considered to be primary samples (residing within the Manawatu region, and the first sample from an individual case acquired through the routine surveillance system). Of the 204 primary samples, 157 were successfully cultured and 153 samples were sequence typed, yielding 153 MLST allelic profiles with 45 different STs isolated (Marshall *et al.,* 2015).

Six retail poultry samples were taken per month (n=72), of which 61 (84.7%) were *Campylobacter*-positive. Prevalence ranged from 80–95% for individual poultry suppliers. A total of 107 isolates from poultry were successfully MLST typed from 58 of the 61 positive samples, with the most prevalent ST being ST 45. A new sequence type, ST 6964, was observed on carcasses from poultry suppliers B and C. In addition ST 6964 was found on chicken pieces from supplier A (collected as part of a separate study[15]). A total of 11 poultry isolates were typed as ST 6964. The same ST was isolated from three human cases between

[15] PhD project (mEpiLab; ongoing) *Molecular epidemiological studies of human campylobacteriosis in New Zealand between 2005 and 2014.*

May and November 2014. Antimicrobial sensitivity testing of two of the three human isolates revealed resistance to ciprofloxacin, nalidixic acid, enrofloxacin and tetracycline. No resistance testing has been done on the non-ST 6964 human isolates in this study.

2014/2015 Human cases in Auckland

In November 2014, the Antibiotic Reference Laboratory at ESR was alerted to a possible cluster of fluoroquinolone-resistant *C. jejuni* in South Auckland. Testing on these samples was performed for a variety of reasons (e.g. severe infection or travel history). A preliminary study was subsequently undertaken in conjunction with LabTests[16] Auckland in February 2015. One hundred consecutive isolates of *C. jejuni* underwent antimicrobial susceptibility testing using the disc diffusion technique (see ESR (2015) for details). Of the 100 isolates, 30 were resistant to fluoroquinolones, representing a significant increase compared to historical patterns of resistance. Of these 30 fluoroquinolone-resistant isolates, 23 isolates (77 %) were also resistant to tetracycline (results unpublished).

Isolates tested:	Resistant to fluoroquinolones:	Resistant to both fluoroquinolones & tetracycline:
100	30 (30%)	23 (23%)

Studies currently underway

Work is currently underway at Massey University[17], PIANZ[18] and ESR[19], which upon completion might provide additional insight into ST 6964 and its associated AMR. Preliminary results (provided to the author between 15 August and 15 September 2015) from these studies are summarised below.

2015 PIANZ chicken study

In 2015 195 swabs from pooled (up to five) caecal contents from chickens being slaughtered for meat were collected at processing plants. The birds were from four different poultry suppliers (A, B, C and D) and were sampled both in the North and South Islands. Swabs were taken between the 25th and 29th of May 2015. The swabs were plated on mCCDA and mCCDA+cip+tet (ciprofloxacin 4 mg/litre, tetracycline 16 mg/litre) and incubated microaerobically at 42°C. Seventy-two swabs out of the 195 swabs (37%) produced colonies resembling *Campylobacter* on the mCCDA+cip+tet plates and hence were considered resistant to ciprofloxacin and tetracycline. One to two single colonies from each positive plate were subcultured, frozen and a DNA preparation made. At least one isolate from each positive plate was tested by PCR for the *hipO/ceu* genes for *C. jejuni/coli*. All 72 plates had at least one isolate that was *C. jejuni* by PCR. Subsequently, 71 isolates from 71 swabs were tested by MBiT, with one swab of the initial 72 missing for technical reasons. Seventy isolates had the same MBiT code (i.e. 124111), while one had the code 524111. Of the 71 isolates, 43 were from Company A, 18 from Company B and 10 were from Company D. None were isolated from Company C. No sample from the South Island grew any *Campylobacter* on the mCCDA+cip+tet plates so no isolates were sent for subtyping (Company A and D plants).

2015 PIANZ breeder chicken study

PIANZ is conducting surveys on an on-going basis as breeder flocks depopulate. During one such study ST 6964 was detected in a breeder flock, while other breeder flocks and also

[16] http://www.labtests.co.nz
[17] Personal communication, Prof Nigel French, mEpiLab, Massey University, Palmerston North.
[18] Personal communication, Kerry Mulqueen, PIANZ, Auckland, New Zealand.
[19] Personal communication, Dr Debbie Williamson, ESR Kenepuru, Porirua, New Zealand

grandparents tested have tested negative. No further information on the sampling strategy of this study is available at this time. In addition, swabs of pooled caecal material from breeder birds have been resistance-tested, starting in July 2015. As of 1 September 2015, 15 completed culture results were available with eight samples considered antibiotic-resistant to ciprofloxacin and tetracycline, based on susceptibility testing with mCCDA+cip+tet plates.

2015 Whole genome sequencing

Eight isolates (from the 8 positive chickens) from the 2014 PIANZ chicken study, 34 isolates (from 34 swabs) from the 2015 PIANZ Chicken Study, one chicken-related isolate provided by one of the chicken processing companies, and four human isolates (confirmed ST 6964) have been whole-genome sequenced and analysis of the sequences is currently in progress.

2015 Manawatu Sentinel Surveillance Site

At the Manawatu Sentinel Surveillance Site, as of 25 August 2015 a total number of 65 human case swabs have been received by mEpiLab to the end of June 2015. Forty-three of these samples were culture-positive and MLST typing results were available for 32 isolates, including human cases up until June 2015. Two human cases of ST 6964 were detected in February (n-=1) and May (n=1) respectively.

Poultry sampling currently consists of six samples per month. As at 25 August 2015, samples taken prior to July 2015 had typing results available. A total of 36 samples were cultured of which five were culture negative (presumably not *C. jejuni* or *C. coli*) and 31 samples were culture positive. Thirty samples had typing results available, with 11 samples testing positive for ST 6964 (36.7%).

Detections by supplier, sample and month were as follows:

- Company A – May (n=2), June (n=1)
- Company B – January (n=1), February (n=1), June (n=2)
- Companies C&D (pooled) – March (n=2), April (n=2)

No susceptibility testing has been performed on the samples to date.

2015 Ministry of Health study on AMR in *C. jejuni*

A targeted period-prevalence survey from sentinel community laboratories New Zealand was conducted by ESR to (I) further investigate AMR patterns in *C. jejuni*, specifically to tetracycline and ciprofloxacin, and (ii) establish whether fluoroquinolone resistance in human isolates of *C. jejuni* is due to one or several *C. jejuni* clones (see ESR (2015) for details). Analysis on MLST data and genetic determinants of resistance is still on going, and further data will be collected from those laboratories from which only a small number of isolates were initially received.

As of 11 September 2015, a total of 238 viable, non-duplicate isolates, originating from faecal samples and referred as *C. jejuni*, were collected from five New Zealand laboratories between May and August 2015. All 238 isolates were susceptible to erythromycin. The geographic source of the isolates, and ciprofloxacin and tetracycline resistance are illustrated in Figure 5.

The study found a fluoroquinolone resistance rate of 19%, which was a considerable increase on previously reported rates of fluoroquinolone resistance in *C. jejuni* (ESR, 2015). A total of 88.9% (40/45) of the ciprofloxacin resistant isolates were also tetracycline resistant. Ciprofloxacin resistance and tetracycline resistance was significantly higher ($p \leq 0.05$) among *C. jejuni* obtained from North Island District Health Boards (DHBs) compared with isolates referred from the South Island DHBs. Only 2.1% (n=5) and 1.3% (n=3) of the isolates had

mono-resistance to ciprofloxacin and tetracycline, respectively. Notably all ciprofloxacin-resistant isolates from Northland DHB, the three Auckland DHBs, and Bay of Plenty and Lakes DHBs were also tetracycline resistant.

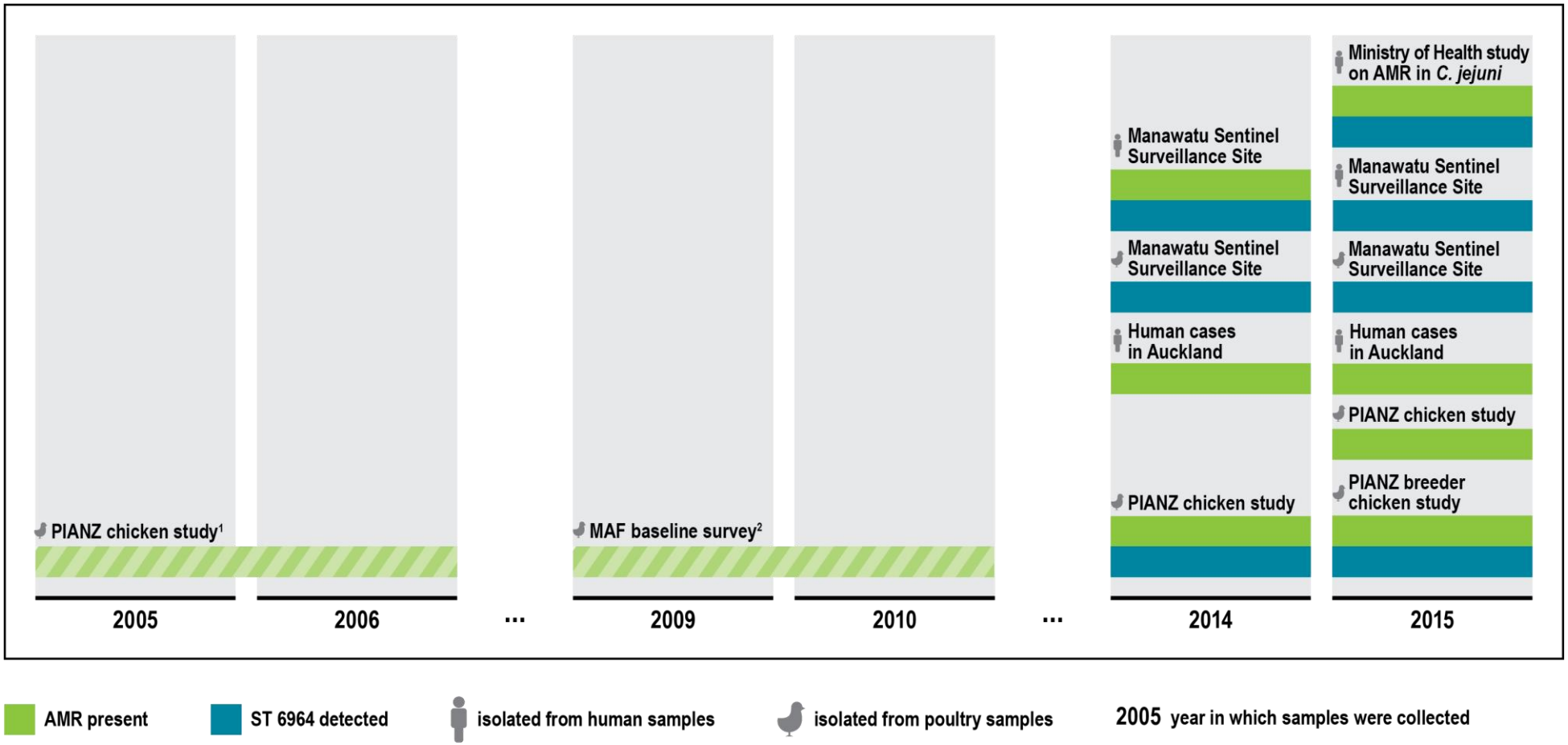
A subset of 60 of the 238 isolates underwent MLST analysis. These were selected with the aim of having representative samples of both ciprofloxacin resistant and susceptible isolates. Twenty-four MLST types were identified among the 60 isolates, with ST 6964 being by far the most dominant type accounting for 37% of isolates (22/60). The geographical distribution of the typed isolates is described in Table 2. Twenty-nine of the isolates that were typed were resistant to both ciprofloxacin and tetracycline (48%; 29/60), and the majority of these isolates (76%; 22/29) were ST 6964. The 31 remaining isolates were either fully susceptible (n=25; 16 STs), tetracycline resistant only (n=2; 1 ST) or ciprofloxacin resistant only (n=4; 4 STs). Notably in this study all confirmed ST 6964 were resistant to both ciprofloxacin and tetracycline.

Table 2: Geographical distribution of the 60 human isolates typed in the 2015 MoH study on antimicrobial resistance (AMR) in *C. jejuni.*

Region	Isolates typed	ST 6964	Other STs (n)
Canterbury	3	0	ST 2345 (1); ST 3528 (1); ST 331 (1)
Capital and Coast	7	5	ST190 (1); ST 354 (1)
Combined Auckland	21	8	ST 354 (1); ST 474 (1); ST 48 (2); ST 486 (2); ST 50 (2); ST 53 (1); ST 4053 (1); ST4056 (1); Novel (2)
Combined Wellington	14	6	ST 1726 (1); ST 2345 (1); ST 2350 (1); ST 257 (1); ST 48 (3); Novel (1)
Lakes	1	1	NA
Nelson Marlborough	1	0	ST 2256 (1)
Northland	2	1	ST 50 (1)
Southern	11	1	ST 190 (1); ST 1911 (1); ST 2343 (1); ST2345 (2); ST 48 (1); ST 50 (1); ST 520 (1); ST 7323 (1); ST 1707 (1)
Total	**60**	**22**	**38**

Exposure: Timeline and spatial distribution

Figures 4 and 5 present a timeline of current evidence on the occurrence of ST 6964 and its associated AMR in New Zealand. Different methodologies and sampling procedures have resulted in substantial diversity of findings and only a few studies have conducted MLST and susceptibility testing in parallel. Regardless, frequent occurrence of ST 6964 and a high proportion of resistance (to fluoroquinolones and tetracycline) in *C. jejuni* are consistently observed in the 2014/2015 studies.



[1]Only 1 isolate of 193 found have resistance to antimicrobials (erthromycin).
[2]Very low levels of resistance detected i.e. ciprofloxacin 2.7%, nalidixic acid 2.7%, tetracycline 0.3%.

Figure 4: *Timeline illustrating the detection of ST 6964 and associated resistance in different studies in New Zealand 2005–2015.*

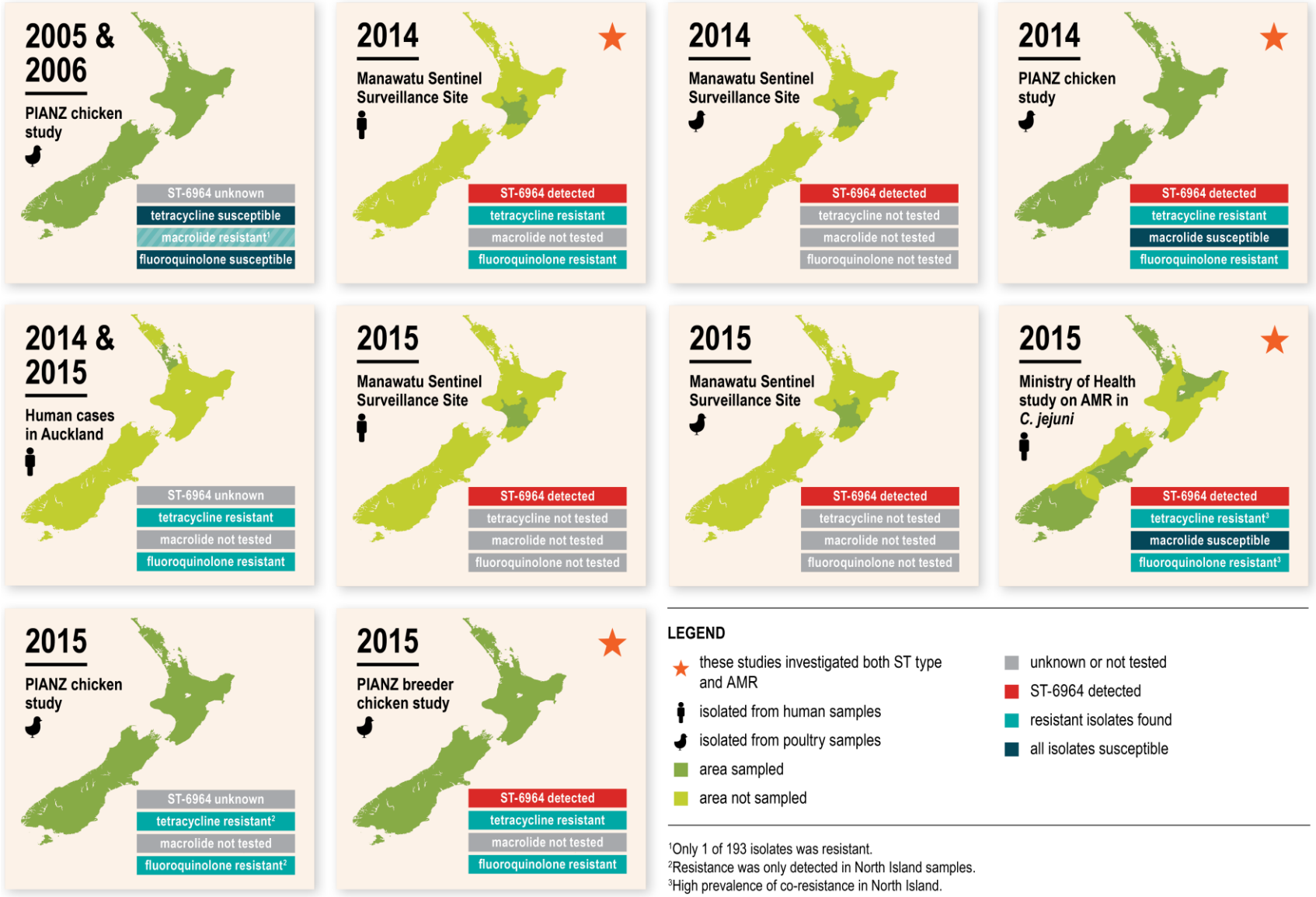


Figure 5: *Maps illustrating the spatial distribution of detections of ST 6964 and associated antimicrobial resistance in different studies in New Zealand 2005–2015.*

3.3.3 International context of the emergence of ST 6964 in New Zealand

The emergence of previously unknown STs and CCs in *Campylobacter* in both humans and animals has been reported on numerous occasions (e.g. Asakura *et al.,* 2012; Sahin *et al.,* 2012; Guyard-Nicodeme *et al.,* 2015). Findings from previous studies suggest that the global diversity of *Campylobacter* isolates responsible for human disease remains incompletely understood (Cody *et al.,* 2012). Many novel STs are reported to *PubMLST* as surveys are reporting snapshots of the pathogen population through MLST. However, the epidemiological significance of low prevalence novel types is decreasing as reporting to the repository increases, in particular given that in most populations few species in a sample are abundant but most are rare (Hughes *et al.,* 2001).

Campylobacter AMR in poultry is internationally well recognised (Alfredson *et al.,* 2007), with estimates of the prevalence of resistance in different countries ranging widely, for example from 23% in the US to 71.4% in rural India and 97.9% in China (Cody *et al.,* 2012). High-levels of AMR have also been reported in the Netherlands, Spain, Germany and Taiwan (Alfredson *et al.,* 2007).

Quinolone and tetracycline are the most widely used veterinary antimicrobials in Eastern Asia and resistance to nalidixic acid, ciprofloxacin and tetracycline is frequently reported (Cha *et al.,* 2014). A different picture has been painted by a study in Finland (Olkkola *et al.,* 2015) where prevalence in broilers was the lowest amongst all sources, with the low resistance to fluoroquinolones and tetracycline attributed to the minimal use of these antimicrobials in Finnish poultry. In this and other studies (Habib *et al.,* 2009; Wirz *et al.,* 2010), resistance to quinolone and tetracycline in *Campylobacter* was found primarily in rare rather than common STs, but this link requires further investigation.

Notably, the only other ST 6964 reported upon was an isolate profile from China that was deposited in the *PubMLST* databases in September 2014. It is worth highlighting that reporting to this public repository is voluntary and thereby potentially incomplete and biased (Muellner *et al.,* 2015); the ST could have been detected in other countries and in other samples and it is not known whether the ST is rarely or commonly observed in Chinese studies. ST 354, another representative from CC 354, has also been previously identified in China and was reported to be the dominant ST in chickens and food (Zhang *et al.,* 2015).

A link between the ST 354 complex, to which ST 6964 belongs, and ciprofloxacin resistance has been reported in human isolates from the UK and also in other studies (Cody *et al.,* 2012). The case of ST 8 in ruminants in the US also suggests that use of tetracyclines could have facilitated selection of this highly pathogenic clone; however the clonal population explosion seen for this ST is likely not due to selection for resistance alone and other factors might be contributing (Wu *et al.,* 2014).

Variation of approaches between studies is likely, including differences in methodologies, sample size and approaches used to define AMR; use of different breakpoints to define resistant strains; use of AMR pheno- vs. genotyping; and use of different panels of antimicrobials which can be tested against. This limits international comparison of antimicrobial susceptibility testing and assessment of the presence of genes associated with antimicrobial resistance; however, this does not apply to *C. jejuni* subtyping as MLST typing does provide excellent international comparability. It is one of the advantages of MLST typing that it has established unified nomenclature that facilitates integration and comparisons of findings from different sources (Habib *et al.,* 2009).

In summary, the international context provides evidence for other AMR-associated emergence events in *Campylobacter* strains in other countries, and strengthens the assumption that the observed emergence of ST 6964 is not an unprecedented event.

3.3.4 Brief overview of consumption

In 2009 New Zealanders consumed 136,728 tonnes of poultry meat, constituting 35.8% of total meat consumption, making poultry the most consumed meat nationally[20]. The proportions of fresh, frozen and cooked chicken consumed by New Zealanders are approximately 63%, 23% and 14%, respectively (Lake *et al.*, 2013).

The following information describes the types of poultry consumed by New Zealanders and the most common methods of cooking used. This information is derived from Lake *et al.* (2013), based on combined data from the 1997 National Nutrition Survey, the 2002 Children's National Nutrition Survey (MoH 2013), and the 2008–2009 Adult Nutrition Survey (Russel, 2009).

According to the 1997 National Nutrition Survey, the percentage of adult New Zealanders consuming chicken on any given day ranged from 12.2% to 27.5%, while consumption of other poultry types was reportedly negligible. The more recent 2008–2009 New Zealand Adult Nutrition Survey confirmed that poultry remains a frequently consumed food, with only 6.6% of those surveyed reporting no chicken consumption in the preceding four weeks.

Among New Zealand adults, the most frequently consumed poultry portion type was breast (28%) followed by drumstick (11.4%) light meat (white meat, most likely of breast origin; 11.4%), leg (9.8%), thigh (9.1%) and wing (8.2%), with 10.2% of overall servings described as 'Chicken, KFC'. Data on cooking methods revealed baking/roasting to be the most common method (39.2% of servings), followed by frying (12.5%), stewing/braising (12.3%), and grilling/barbecuing (8.9%), with cooking method not specified for 16.7% of servings. Among New Zealand children (aged 5–15 years), most commonly consumed portion types were drumstick (25.9%), followed by breast (19.9%), wing (10.7%), light meat (8.8%), thigh (7.1%) and leg (6.7%), with only 4.2% reported as 'Chicken, KFC'. The most common cooking methods were baking/roasting (44.4% of servings), followed by frying (15.7%), stewing/braising (10.1%) and grilling/barbecuing (10.1%).

A risk model for *Campylobacter* in the New Zealand poultry food chain identified barbeque and microwave cooking methods as having greater potential to result in undercooking, and therefore an increased risk of *Campylobacter* persistence after cooking (Lake *et al.*, 2007).

3.3.5 Critical evaluation

In general, rates of AMR, including to fluoroquinolones, in *Campylobacter* have historically been considered low in New Zealand. To date, AMR data in human isolates of *Campylobacter* have been collected annually from diagnostic laboratories, and reported by the Antibiotic Reference Laboratory at ESR[21]. For the past 13 years, fluoroquinolone resistance in human isolates of *Campylobacter* spp. has been reported at a prevalence of <6%. In addition,

[20] http://www.pianz.org.nz/industry-information/industry-statistics/meat-consumption/meat-consumption-percentages
[21] Reports available at: https://surv.esr.cri.nz/antimicrobial/antimicrobial_resistance.php

a systematic survey of AMR in animal isolates of *Campylobacter* (from bobby calves and poultry), performed in 2009, found no erythromycin resistance in *C. jejuni*, and a fluoroquinolone resistance rate of only 2.3% across all samples (Heffernan *et al.*, 2011). The tetracycline resistance rate in poultry (n=295) was found to be 0.3% and resistance for fluoroquinolones was found to be very low for different antimicrobials of this class (ciprofloxacin = 2.7%; nalidixic acid = 2.7%). More recent systematic studies on AMR in animals and food in New Zealand are not publically available.

The MoH survey demonstrated a clear increase in fluoroquinolone resistance in isolates of *C. jejuni* from humans compared to historic data, largely driven by the emergence of a single clone, ST 6964 (ESR, 2015). While published assessments of the association between MLST and AMR seem to be focused on the link between CCs and AMR, there seems to be a strong link in the New Zealand situation between ST 6964 and the observed resistance pattern. The highly clonal nature of these co-resistant isolates, and the relatively rapid emergence in human populations in New Zealand (compared with historic rates of resistance), suggest a recent introduction from a common source (ESR 2015).

To date, only assessment of phenotypic antimicrobial susceptibility of ST 6964 in New Zealand has been done. Molecular level studies, such as the on-going WGS of selected isolates, will allow a more in-depth assessment of the genetic and evolutionary drivers of the clone's emergence, expansion and persistence in New Zealand as well as the transmission of strains between the human and poultry reservoirs. It is currently unclear what has driven the expansion of ST 6964 and the role that AMR plays in the process. Previous evidence suggests that the structure and evolution of *C. jejuni* populations are very complex (Guyard-Nicodeme *et al.*, 2015). Evolutionary studies might deliver some insight into how resistance was acquired by this ST, the molecular mechanism behind it as well as how this links to the clone's apparent predominance.

It is also unclear if ST 6964 has emerged in New Zealand or was introduced to the country via travel or trade. Due to the country's high biosecurity standards for animals and animal products and historically low prevalence of AMR in both human and animal populations and food supply, an introduction into the country via human travel or other products might be an alternative explanation. Resistance can pass in both directions between livestock and humans (Schmidt *et al.*, 2015) and reverse zoonotic disease transmission has been observed for many species (Messenger *et al.*, 2014). It is also worth highlighting that *Campylobacter* is a multi-host pathogen with the capability to amplify in many hosts (Woolhouse, 2002; Mullner *et al.*, 2009b).

Overall, our knowledge about *Campylobacter* ST 6964 in New Zealand is currently limited, however some evidence has emerged from previous and on-going studies. While the full scientific analysis and interpretation of on-going studies once they are completed might improve our understanding, none of the studies conducted to date were specifically designed to examine this ST and associated AMR issues, either in terms of prevalence, food safety risks or to inform particular risk management decisions. It would be of value to assess if in response to the emergence of ST 6964 over time a change in the relative frequency of STs and CCs is occurring, both in human cases as well as in the animal source

In the absence of longitudinal sampling and consistent subtyping and resistance testing, the link between the occurrence of ST 6964 and its associated AMR is difficult to quantify. Given current evidence it cannot be confirmed that all *C. jejuni* with the detected resistance profile are indeed ST 6964, and an understanding of the epidemiology of ST 6964 has yet to be gained.

Without an assessment of concordance between MBiT and MLST typing, MBiT typing results do not allow for inference of a likely MLST. However, MBiT is considered more discriminatory than MLST (Cornelius *et al.*, 2014) and in the 2015 PIANZ chicken study most isolates were identified as the same MBiT type, suggesting a predominant genotype.

No fluoroquinolones are registered for use in poultry in New Zealand and the information provided to date by the overseas egg suppliers also suggests no use. No information is available on the use of tetracyclines in breeding stock eggs for importation into New Zealand, however use of tetracyclines within New Zealand is limited to breeding flocks. This assessment of risk could be affected by "off-label" use of antimicrobials, i.e. the use of a veterinary medicine to treat an animal in a way that is not described on the registered label, Legal restrictions and the Code of Professional Conduct for veterinarians in New Zealand are in place to control such "off-label" use[22].

Overall the current evidence suggests a low selective pressure towards AMR in the New Zealand poultry industry, particularly when compared with other countries where use of fluoroquinolones and tetracyclines in poultry is widespread and has been linked to the development of resistance.

[22] http://www.vetcouncil.org.nz/CPC/VetMed/CPC_VetMedicines.php

4 Evaluation of Adverse Health Effects

4.1 HEALTH CONSEQUENCES

Campylobacteriosis is the most common form of bacterial gastroenteritis in New Zealand. In 2014 alone, there were 6,766 notified cases of campylobacteriosis in New Zealand, representing an incidence of 150.3 cases per 100,000 population (ESR, 2015). Health effects of *Campylobacter* infection have been described in detail in the risk profile by Lake *et al.,* (2013).

From a human health perspective options are reduced in the treatment of AMR resistant *Campylobacter* infections, requiring the use of more expensive and often toxic drugs. Even if an effective alternative treatment is available, this potential cost could be avoided (SafeFood, 2010). Additional concerns include prolonged illness and associated with this increased cost of isolation, increased risk of outbreaks and the additional risks to vulnerable patients with different underlying morbidities that might be exposed to AMR in a hospital setting (SafeFood, 2010). Longer durations of illness for patients infected with quinolone-resistant *Campylobacter* strains has been reported (e.g. Engberg *et al.,* 2004; Nelson *et al.,* 2004); however some studies could not detect differences in investigated clinical outcome parameters between patients infected with resistant and susceptible strains (Evans *et al.*, 2009; Wassenaar *et al.*, 2007). While existing evidence suggests that infection with resistant *Campylobacter* strains increases the risk of death there is still controversy about the magnitude of the public health impact of resistance in *Campylobacter* and studies are needed on excess mortality due to infection with resistant strains (WHO 2012).

Fluoroquinolones have been classified as of critical importance by the WHO and as "of very high importance to human medicine" by Health Canada's Veterinary Drugs Directorate (PHAC, 2015). Macrolides were considered the drug of choice for campylobacteriosis in a study of antimicrobial use and resistance in Canada, but in ~ 33% of human cases ciprofloxacin was prescribed (Agunos *et al.,* 2015). Tetracycline can also be used for human treatment, although this is uncommon (Alfredson *et al.,* 2007). High rates of fluoroquinolone resistance in *Campylobacter* in other geographic settings have precluded their use in many parts of the world (ESR, 2015).

The health consequences of AMR in general, i.e. the extra human health consequences of AMR as opposed to the health effects caused by susceptible organisms, have been summarised as follows (from SafeFood, 2010).

(i) Infections that would not otherwise have occurred if the organisms were not resistant.
This is primarily due to humans (and animals) taking antimicrobial medication being at increased risk of infection with resistant organisms.

(ii) Increased frequency of treatment failures.
A potential lack of therapeutic options can become a real concern, for example, the drug of choice in the treatment of children with invasive *Salmonella* spp. disease is ceftriaxone. However, in a child with a ceftriaxone-resistant strain of *Salmonella* spp. associated with the same organism in a local outbreak of salmonellosis in cattle, the preferred drug was not usable.

(iii) Increased severity of infection.
This has been illustrated by increased severity of resistant infections resulting in greater hospitalisation rates, greater case-fatality rates, increased risk of haematological infections as well as increased short-term risk of death.

Globally there is consensus that antimicrobial resistance poses a profound threat to human health with the indirect impact of antimicrobial resistance, extending beyond increased health risks and including many public health consequences with wide implications, for instance on development and economic losses (WHO, 2015).

4.2 NEW ZEALAND SITUATION

In New Zealand, AMR in *Campylobacter* spp. isolated from human patients has to date been considered low. Overall at present, for most human infections in New Zealand due to antibiotic-resistant bacteria, a relatively effective treatment is available, even if this treatment has disadvantages with regard to cost or convenience or adverse effects (Thomas *et al.*, 2014). In 2013, published AMR data from hospital and community laboratories[23] showed that 0.8% of *Campylobacter* isolates (of 238 tested) were considered resistant to erythromycin and 2.1% (of 236 tested) were considered resistant to fluoroquinolones, a similar rate to previous years such as the 0.8% and 2.0% percent resistance reported in 2011 for erythromycin and fluoroquinolone respectively. Previous studies on the differences in carriage of the antibiotic resistance genes in human isolates from Belgium and New Zealand showed that the two resistance genes *Tet(O)* and CJE1733 were more frequently observed in Belgian isolates than in New Zealand isolates (Cornelius *et al*., 2014). Of the 318 human *Campylobacter* isolates tested in New Zealand during 2009, none were resistant to erythromycin and 5 (1.6%) were resistant to fluoroquinolone (Heffernan *et al*., 2011). Despite historically low rates of resistance, Thomas *et al.* (2014) point out that there is a risk for untreatable infections due to completely drug-resistant bacteria becoming common in New Zealand.

During the seven years between 2005 and 2012, annual per capita antimicrobial consumption by community-based patients in New Zealand increased by 43%, an average annual increase of just over 6% (Thomas *et al.*, 2014). At the same time the general landscape of antimicrobial resistance is believed to have changed considerably over the last years, which is illustrated by the regent emergence of community-associated methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase-producing *Enterobacteriaceae* and multi-resistant *Neisseria gonorrhoeae* (Williamson *et al.*, 2015). Steadily rising rates of antimicrobial resistance, in a range of common bacterial pathogens, are described as a major threat to human health in New Zealand with a high level of antimicrobial consumption linked to this threat (Thomas *et al.*, 2014). The same publication also reported an increase in resistance to ciprofloxacin in *Neisseria gonorrhoeae* from 6% in 2002 to 40.6% in 2012. A similar increase of gonococcal quinolone resistance has also been reported from other countries including the Netherlands, the UK and the US, where for at risk-groups prevalence of ciprofloxacin resistance was recorded to be as high as 42.5% (Goldstein *et al*., 2012).

4.3 ADVERSE HEALTH EFFECTS SUMMARY

Infection with resistant bacteria can result in additional adverse health effects, when compared with infection with susceptible strains. These include infections that would not otherwise have occurred if the organisms were not resistant, increased frequency of treatment failures, as well

[23] https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/AR/National_AR_2013.pdf

as increased severity of infection. Antimicrobial resistance affects all areas of health, involves many sectors and has an impact on the whole of society (WHO, 2015).

5 Evaluation of Risk

5.1 EXISTING RISK ASSESSMENTS

Different approaches to attribution of *Campylobacter* infections in New Zealand have indicated that poultry as a source, and chicken consumption as a pathway, still represent the most important components of the epidemiology of the disease. Although the risk of campylobacteriosis from the consumption of poultry in New Zealand has declined considerably following risk management interventions by MPI and the poultry industry, poultry remains an important vehicle for infection with *Campylobacter* (Lake *et al*., 2013).

Antibiotic resistance is complex in nature and large data gaps exist, making informed scientific decisions on the use of antimicrobials in food animals challenging (SafeFood, 2010). AMR is interlinked between the human and animal populations and resistance can pass between both groups; however the degree to which antimicrobial use in farm animals poses a threat to human health has not yet been quantified (Woolhouse *et al.,* 2015). An important observation in this context is also that human-associated bacteria commonly stem from human clinical cases, often those with invasive infections, while most animal-associated bacteria collected in surveys stem from healthy animals or animal food products, which raises some questions regarding comparability. The contribution food makes to the problem of AMR in humans is also not known (ACMSF, 2014). Environmental contamination, such as the dissemination of resistance into the surroundings via wastewater or manure, also needs to be considered (Rushton, 2014).

Generally, *Campylobacter* spp isolated from pork and poultry are believed to have a higher and broader resistance to antibiotics than those isolated from other livestock species such as beef or lamb. This could be attributable to the intensive farming methods used in pig and poultry rearing, where mass medication is relatively common and high stocking levels could facilitate dissemination of pathogens and AMR genes between animals (SafeFood, 2010). AMR emergence is frequently observed, but for example it is unclear how resistance to fluoroquinolones emerged in *Campylobacter* from broilers in Canada, and if and how it spread from the western to central and eastern regions, or if it emerged independently (Agunos *et al*., 2014).

Different exposure routes have previously been proposed for emerging AMR (Safe Food, 2010), and are described as follows:

(i) Food-producing animals and the environment

Humans are exposed to or acquire AMR bacteria through selection pressure associated with prolonged use of antimicrobial drugs. Furthermore, humans could also be exposed through direct contact with animals and other humans that are colonised/infected with AMR bacteria. A lesser-recognised route however, is the food chain, which can contain AMR bacteria derived from food-producing animals or from cross-contamination during food processing. In the environment, AMR bacteria can enter the food chain through the contamination of ground and surface water, or from the spraying of food crops with contaminated water containing AMR bacteria derived from human and animal waste.

(ii) Food processing technologies

Food processing technologies are designed to reduce the risk of transmission of hazards, including bacteria, through the food chain. As bacteria become resistant, following the stress imposed by antibiotic selection, they can evolve and undergo

genetic changes, which make them more difficult to eliminate. This can in turn increase the likelihood of transmission through the food chain.

(iii) Use of sanitising agents and biocides in food production

Sanitizers are used in the food industry to eliminate contaminating bacteria that occur on food preparation surfaces or equipment in direct contact with food. Although sanitizers, biocides and antibiotics kill bacteria, there is increasing concern that resistance to these cleaning agents might be directly or indirectly linked to AMR. Concerns have been expressed in regard to the application of sanitizers and biocides in food processing and in domestic food preparation environments where they might promote the development and dissemination of AMR via the human food chain. There is a need to develop a better understanding of this relationship.

The persistence of resistant strains in the food supply might have an important impact on foodborne transmission to humans (Habib *et al*., 2009); however considerable uncertainty exists as to the role of transmission of AMR from animals to humans via food (ACMSF, 2014). Outbreaks of resistant infections associated with food animal sources have been reported (SafeFood, 2010), but the role of food in AMR in humans currently remains un-quantified. There is a need for research into transmission dynamics and possible interventions that could prevent human AMR infections from becoming established in food-animal populations (NCCID, 2009). The literature suggests that on-farm sources and operational factors could contribute to the spread of AMR *Campylobacter* (Agunos *et al*., 2014). The authors further concluded from a review of the literature that AMR *Campylobacter* can be disseminated within a flock through drinking water lines and could spread across farms via farm workers (wearing contaminated footwear). *Campylobacter* has been reported to be spread by chicken catching crews that travel from farm to farm, and to slaughter plants when birds were exposed to contaminated poultry equipment during transport (Agunos *et al*., 2014).

Assessment of risk is challenged by lack of harmonisation of methods to determine AMR. For example, several approaches are in use for susceptibility testing, and the application of clinical breakpoints (used for clinical purposes) versus epidemiological cut-off values (recommended for monitoring programmes) is subject to discussion. However, an understanding of resistance prevalence data is considered a starting-point for assessing the risk associated with AMR (WHO, 2012).

While some efforts have been made to link subtyping and susceptibility analysis of *C. jejuni* (see Appendix 1) our understanding of the link between epidemiologically-relevant subtyping, virulence and AMR is still in its infancy. Recently the relationship between mechanisms of AMR and virulence has been scrutinized (Beceiro *et al*., 2013). The authors concluded that antimicrobial resistance can increase the virulence or fitness of certain species in some environments, often helping these species to colonize new niches. Therefore, although antibiotic resistance is not in itself a virulence factor, in certain situations it is a key factor in development of infection, and it may be considered a virulence-like factor in specific ecological niches which antibiotic-resistant bacteria are able to colonize. Ultimately the association between virulence and resistance in a specific pathogen will depend on the interactions between the multiple factors associated with bacteria and their environments.

5.2 ESTIMATION OF RISK FOR NEW ZEALAND

Data on resistance of human *Campylobacter* isolates is limited, as it is not routinely tested in diagnostic laboratories for community-based cases. However, based on the data that are

available for fluoroquinolone and erythromycin, prevalence of resistance to each of these two antibiotics has varied between zero and 3% in recent years. Similar levels of resistance to these two antibiotics were identified among the animal isolates of *C. jejuni*, with 2.3% ciprofloxacin resistance and no erythromycin resistance (Heffernan *et al*., 2011; ESR 2015).

Although no longitudinal studies have been conducted to date, the sequence of events indicates emergence of a poultry-host adapted strain with the capability of causing human infections severe enough to self-present to health services. Current available exposure data suggest a high degree of correlation between the observed recent events both in poultry and human cases. Although only a few studies included both MLST and susceptibility testing, the current evidence is pointing towards the existence of a recently emerged poultry-associated *Campylobacter* strain (i.e. ST 6964), which shows a high level of resistance to fluoroquinolones and likely also tetracycline, and does not appear to be geographically limited. Co-resistance with macrolides would be of high clinical significance, but to date an increase in erythromycin resistance has not been observed. Although all isolates in the 2015 MoH study remained susceptible to erythromycin, the relatively high prevalence of fluoroquinolone resistance (19% overall) means that fluoroquinolone antimicrobials can no longer be assumed to be an appropriate empirical treatment for campylobacteriosis in New Zealand (ESR, 2015).

Given the current evidence, no conclusions can be made as to what has driven the emergence of ST 6964 and its associated AMR in New Zealand and what risk factors are at play. Further, the public health impact of ST 6964 can at this point only be qualitatively described. However results from on-going studies and in particular findings from the whole genome sequencing supported evolutionary analysis may provide important insight into the emergence of ST 6964 in New Zealand.

As such, the recent emergence of AMR in *Campylobacter* as described in Section 3.3 (Exposure) of this risk profile is of concern. To date the country has seen a cluster of fluoroquinolone-resistant *C. jejuni* in human samples and an apparent increased prevalence of AMR in *Campylobacter*, substantiated by a focused survey in response to the Auckland case cluster. However, the future risk to human health is difficult to quantify, in particular as no clinical information is available on the human cases from which the resistant clones were isolated; the increased number of observed cases infected with resistant *C. jejuni* already indicates a potential health burden due to the changes in identified resistance patterns.

Increased resistance in New Zealand could be indicative of both improper use of antimicrobials on broiler farms and also of transmission of resistance genes or the propagation and spread of acquired clones as described by Habib *et al*. (2009). Another hypothesis would be importation of AMR into the national flock via imported eggs. Investigations are currently underway to establish the genetic relatedness of these human cases to an emergent fluoroquinolone/tetracycline co-resistant ST 6964 *C. jejuni* clone in poultry (ESR, 2015) and results from these genomic studies should further increase our understanding of ST 6964 and its associated AMR in New Zealand. Although antimicrobial usage in livestock has been a key driver behind fluoroquinolone and tetracycline emergence internationally, given the antimicrobial usage patterns of the New Zealand poultry industry, other factors have likely contributed to the observed events. Importation of resistant pathogens from areas where multi-resistant pathogens are endemic has been identified as a factor contributing to the emergence and spread of antimicrobial-resistant pathogens in New Zealand (Williamson *et al.*, 2015). Although resistance in human pathogens is often attributed to incomplete or inappropriate use of antimicrobials, in the case of ST 6964 this is likely an insufficient explanation as in New Zealand most *Campylobacter* infections are considered sporadic

(Wilson, 2005) and hence there would be little opportunity for resistance to proliferate in humans due to limited human-to-human transmission.

It is unknown how this ST will continue to evolve. Previous evidence indicated that certain strains might gain increased fitness when acquiring fluoroquinolone resistance mutations and this could lead indirectly to the emergence of more widely competitive AMR strains (Safe Food, 2010). Regardless of ST 6964s future pre-dominance, its emergence could serve as an important case study to better understand the drivers behind the emergence of AMR in New Zealand in general. In consequence, further analysis of events observed to date could improve future risk management of similar hazards.

5.3 DATA GAPS

Significant data gaps currently exist which, if filled, would provide greater certainty of the risks attributable to the emergence of ST 6964. A better understanding of the prevalence of ST 6964 and its associated AMR could assist in investigating the current and potential future risks posed to human health by ST 6964. There is a need to understand in greater detail the genetic mechanism and drivers behind the resistance patterns in *Campylobacter* recently observed in New Zealand. This should be supported by standardised methods across different surveys to ensure that inferences made are as generalisable as possible and allow for sufficient resolution to detect any existing epidemiological and genetic effects at play. This would ideally include harmonised sample collection, culture, and susceptibility testing.

Structured attribution studies would be desirable to further clarify the role food plays in the transmission of AMR in New Zealand, compared with direct transmission of AMR from human-to-human and animal-to-human. Such attribution studies could elucidate the fraction of food-animal associated AMR infections and assist with the identification and prioritisation of hazards (SafeFood, 2010).

The data gaps identified in this risk profile are:

- Representative and parallel sampling of isolates from human cases and poultry to gain an understanding of the prevalence of resistance and the pre-dominance of ST 6964.
- Epidemiological and genetic assessment of the link between ST 6964 and the observed antimicrobial resistance emergence in isolates of poultry and human origin. It is critical for this to be done on representative samples. Ideally this would involve evolutionary analysis.
- Assessment of transmission routes from poultry to humans (and vice-versa) including in particular the importance of foodborne transmission of ST 6964 and its associated AMR into the human population.
- Factors leading to increased selection, maintenance and dissemination of ST 6964.
- Investigation of the presence of ST 6964 in other animal species in New Zealand, including AMR testing of detected isolates.
- Improved understanding of the virulence and clinical manifestation of ST 6964 in humans, and in particular those that display AMR.

6 Availability of Control Measures

The risk profile by Lake *et al*. (2013) provides a detailed overview of legislation of relevance to *Campylobacter* risk management in New Zealand, including the Animal Products Act, Animal Products Regulations and the Australia New Zealand Food Standards Code, and outlines mandatory requirements and codes of practice for poultry. This risk profile focuses on control measures specific to ST 6964 and its associated AMR.

Agricultural and agri-food interventions to reduce the impact of AMR bacteria in pigs and chickens on human health have previously been investigated (NCCID, 2009). The authors concluded that more research is needed on how and where resistance elements enter flocks/herds, the transmission dynamics once they are present, and the factors that allow persistence. Batches or flocks identified with AMR frequencies exceeding a stated threshold could be moved into high-risk production streams, such as further processed and pre-cooked meats. Such an approach has been taken with *Salmonella* and *Campylobacter* control in Europe.

Generally, alternatives to antimicrobials in animals should be considered (e.g. vaccines) and best management practices such as a high levels of biosecurity, reduced stress, use of modified genetics and investment in disease prevention measures can all contribute to high livestock productivity with less antimicrobial use (NCCID, 2009; Rushton *et al*., 2014); however for *Campylobacter* there are currently no effective, reliable and practical measure available (Lin, 2009).

While ideally hazards are controlled pre-harvest, slaughter and processing are considered key points in the food value chain where foodborne bacterial risk can be mitigated (NCCID, 2009).

In 2001, New Zealand adopted a prudent-use approach in response to the potential risk of AMR developing through the use of antimicrobial products in animals (MAF, 2011). Regulatory control of antibiotic use in animals in New Zealand lies with MPI's Agricultural Compounds and Veterinary Medicines (ACVM) Group. The use of antibiotics and the potential promotion of antibiotic resistance in bacteria pathogenic to humans have been subject to several reviews (e.g. Reid, 2006; MPI, 2011) and a sales analysis is regularly published by MPI (MPI, 2015).

Industry controls for *Campylobacter* contamination in poultry are in place in New Zealand and are described by Lake *et al*. (2013). Any interventions that aim to control contamination will indirectly affect resistance elements on meat and impact on AMR dissemination. However, there are currently no simple controls available that directly address the risk of contamination of carcasses with AMR bacteria and scientific research will be needed in case direct control should become a goal (NCCID, 2009).

Surveillance is increasingly considered as a key defence against AMR and countries are exploring how existing processes and resources can be used to build surveillance programmes in food processing animals (DAFF, 2007). The WHO has recently recommended that surveillance in animal health and agriculture sectors be strengthened (WHO, 2015).

It is widely accepted that patterns of antimicrobial use and selection for resistance in one part of the world affect health in other parts of the world, through international travel and trade. For example, it has been observed that fluoroquinolone-resistant infections are often associated with travel to developed or developing countries (WHO, 2012). No control measures are available to control "importation" of AMR from one country into another country's ecosystem via people.

Consequently, good food safety and hygiene practices are important to ensure the reduction in transmission of both resistant and susceptible *Campylobacter* from contaminated meat to people. This includes appropriate wrapping and placement of chicken in refrigerators, careful washing of surfaces and utensils that touch raw chicken, using different surfaces for preparing raw meat and vegetables/fruit, and thorough cooking (Agunos *et al*., 2015).

In summary, control measures for AMR in *Campylobacter* in poultry are currently limited and need to rely on general measures such as surveillance, kitchen hygiene, occupational safety as well as a focus on lowering frequency and levels of contamination.

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8 Appendix 1

Table A1: Overview of results from published studies retrieved by the rapid evidence assessment (REA) combining MLST analysis and susceptibility analysis of *C. jejuni* for fluoroquinolones, macrolides and tetracyclines.

Year published	Country	Samples	Key findings	Association between AMR & MLST[24]	Reference
2007	US	· Poultry[25]	Multidrug resistance: 87% of strains (including tetracycline, macrolides and fluoroquinolones)	Association between *C. coli* ST and multidrug resistance.	D'lima *et al*., 2007
2009	Belgium	· Poultry	*n=145* Ciprofloxacin resistance: 53% Tetracycline resistance: 48%	Correlation between *C. jejuni* clonal complexes and resistance to tetracycline and ciprofloxacin.	Habib *et al*., 2009
2009	Switzerland	· Human · Animal	Quinolone resistance: 31%	-	Korczak *et al*., 2009
2010	China	· Poultry	*n=120* Ciprofloxacin resistance: 13% Doxycycline resistance: 16%	-	Wang *et al*., 2010[26]
2010	China	· Human · Poultry	*n=44* Nalidixic acid resistance: 100% Levofloxacin resistance: 100% Ciprofloxacin resistance: 100%	-	Zhang *et al*., 2010
2010	Switzerland	· Human	*n=136* Ampicillin resistance: 8% Ciprofloxacin resistance: 36% Erythromycin resistance: 1% Nalidixic acid resistance: 38% Tetracycline resistance: 33%	-	Kittl *et al*., 2010

[24] Only significant or clearly described associations were considered.
[25] *C. coli* from turkeys.
[26] Due to lack of access to the full paper only the abstract of this publication was assessed.

Year published	Country	Samples	Key findings	Association between AMR & MLST[24]	Reference
2010	Switzerland	· Chicken	*n=340* Quinolone resistance: 18.9% (of *C. jejuni* with point mutation)	Three STs with point mutation associated with quinolone resistance.	Wirz *et al*., 2010
2011	US	· Retail meats	*n=202* Ciprofloxacin resistance: 15% Doxycycline resistance: 21 %	Subtyping data did not correlate with antimicrobial resistance phenotypes.	Wang *et al*., 2011
2012	UK	· Human[27]	*n=3,682* Ciprofloxacin resistance: 30.2% Erythromycin resistance: 1.8%	Significant association between nine clonal complexes and ciprofloxacin sensitivity and seven clonal complexes with ciprofloxacin resistance respectively.	Cody *et al*., 2012
2013	Grenada	· Poultry[23]	Farm A: Tetracycline resistance: 0% (0/23) Ciprofloxacin resistance: 74% (17/23) Farm B: Tetracycline resistance: 36% (10/28) Ciprofloxacin resistance: 18% (5/28)	No association between ST and quinolone resistance.	Stone *et al*., 2013
2013	Japan	· Human	Nalidixic acid resistance: 100% Fluoroquinolone resistance: 100% Ciprofloxacin resistance: 100% All resistant isolates of dominant ST 4526.	Significance differences in antimicrobial resistance between different clonal complexes.	Asakura *et al*., 2013
2013	Korea	· Human	*n=112* Ciprofloxacin resistant: 24% Enrofloxacin resistant: 46% Erythromycin resistant: 1% Tetracycline resistant: 46%	-	Shin *et al*., 2013

[27] *C. jejuni* and *C. coli* combined.

Year published	Country	Samples	Key findings	Association between AMR & MLST[24]	Reference
2013	Switzerland	· Human · Chicken · Dogs · Pigs · Cattle	-	Association between ST and quinolone resistance.	Kittl *et al*., 2013a
2013	Switzerland	· Human · Chicken	Human isolates: Quinolone resistance: 39% Chicken isolates Quinolones resistance: 27-53%	-	Kittl *et al*., 2013b
2013	UK	· Poultry[28]	*n= 214* Tetracycline resistance: 38% Quinolone resistance: 22% Erythromycin resistance: 5% Chloramphenicol resistance: 3%	Non-random distribution of antimicrobial resistance in different *Campylobacter* lineage clusters.	Wimalarathna *et al*., 2013
2014	Japan	· Human	Tetracycline resistance: 95% (38/40) Nalidixic acid resistance: 100% (40/40) Ciprofloxacin resistance: 88% (35/40)	-	Cha *et al*., 2014

[28] *C. jejuni* and *C. coli* combined (75% *C. jejuni*; 25% *C. coli*).

Year published	Country	Samples	Key findings	Association between AMR & MLST[24]	Reference
2014	Portugal	· Human · Retail food · Food animals	Human *n=79* Tetracycline resistance: 66% Ciprofloxacin resistance: 91% Nalidixic acid resistance: 100% Poultry meat *n=5* Tetracycline resistance: 60% Ciprofloxacin resistance: 100% Nalidixic acid resistance: 100% Poultry animal *n=5* Tetracycline resistance: 80% Ciprofloxacin resistance: 100% Nalidixic acid resistance: 100% 86% of isolates resistant to three or more antimicrobial families.	No association between MLST profile and antibiotic resistance pattern found.	Duarte *et al*., 2014
2014	Slovenia	· Human · Animal · Water	*n=52* Ciprofloxacin resistance: 61% Nalidixic acid resistance: 58% Tetracycline resistance: 25%	Most prevalent clonal complex showed highest level of resistance (95%).	Kovac *et al*., 2014
2014	South Korea	· Ducks	*n=46* Tetracycline resistance: 85% Nalidixic acid resistance: 85% Ciprofloxacin resistance: 87%	-	Wei *et al*., 2014
2014	US/UK	· Sheep	*n=95* Tetracycline resistance: varying between 5% (UK) to 19% (early US cases) and 100% (late US cases).	Correlation between rise in pre-dominance of a specific ST and antimicrobial susceptibility.	Wu *et al*., 2014

Year published	Country	Samples	Key findings	Association between AMR & MLST[24]	Reference
2015	France	· Broiler meat	*n=97* Tetracycline resistance: 54% Nalidixic acid resistance: 32% Ciprofloxacin resistance: 33% *3*1/97 isolates resistant to both ciprofloxacin and nalidixic acid. 22/97 isolates resistant to nalidixic acid, ciprofloxacin and tetracycline.	Significant association between AMR and certain clonal complexes. 100% (10/10) of isolates of ST 45 complex were pan-susceptible, 100% (8/8) of isolates of ST 464 complex were tetracycline resistant. However no association detected in the predominating ST-21 complex.	Guyard-Nicodeme *et al*., 2015
2015	Finland	· Human · Broiler · Bovine · Water · Wild birds · Zoo animals	Human: *n=95* resistance: 11.6% Poultry: *n=459* resistance: 5% (mainly streptomycin)	Significant association between resistance to tetracyclines and streptomycin and ST/clonal complex in isolates from zoo animals.	Olkkola *et al*., 2015