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Campylobacter jejuni ST50, a pathogen of global importance: A comparative genomic analysis of isolates from Australia, Europe and North America

Global comparison of *C. jejuni* ST50

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Australian whole-genome sequences and metadata were obtained from the CampySource study (2016-2020), led by Prof. Martyn Kirk, that aims to understand the sources of *Campylobacter* in Australia. The authors thank Dr Chris Whitehouse (U.S. Food and Drug Administration) for sharing additional metadata to compliment the sequence data obtained from NCBI for the USA *C. jejuni* isolates. The authors thank Dr Catherine Carrillo (Canadian Food Inspection Agency) and Dr Eduardo Taboada (Public Health Agency of Canada) for sharing draft genome sequences and respective metadata for the isolates collected in Canada.

Summary

Campylobacter jejuni is the leading cause of bacterial gastroenteritis globally and infections are often transmitted through consumption of raw or undercooked poultry. *Campylobacter jejuni* ST50 is among the top ten sequence types (STs) reported in the collected isolates listed at PubMLST records from poultry, food and clinical sources for Asia, Europe, North America, Oceania and South America. This study was designed to determine the most commonly reported *C. jejuni* STs globally using the PubMLST database and assess similarities between genomes of *C. jejuni* ST50 isolates from geographically distinct locations. To gain a better understanding of *C. jejuni* diversity, we compared draft genome sequences of 182 ST50 isolates recovered from retail or caecal poultry samples in Oceania,

Europe and North America that were collected over a period of nine years (2010 to 2018). Overall, phylogenetic analysis revealed that isolates from geographically distinct locations tended to cluster based on the continent where the sample was collected. Among ST50 isolates from Europe and North America, we identified resistance determinants associated with phenotypic resistance to beta-lactams (EU: 55%; GB: 43.1%), tetracyclines (CA: 77.3%; EU: 37.5%; GB: 9.8%; US: 43.5%) and fluoroquinolones (EU: 60.0%; GB: 15.7%); no resistance determinants were identified in isolates from Australia. In general, the majority of the virulence genes, with rare exceptions such as *wlaN*, *cj1138*, *hddA* and *rfbC,* were evenly distributed throughout the genomes of all ST50 isolates in this study. Genomic-based characterization of *C. jejuni* ST50 isolates from poultry on three continents highlighted that geographically distinct isolates have evolved independently but only represent a glimpse into the diversity of *C. jejuni*.

Keywords: antimicrobial resistance (AMR), *Campylobacter jejuni*, chicken, ST50, virulence, Whole genome sequencing (WGS)

Impacts

- *C. jejuni* ST50 is one of the most frequently reported *C. jejuni* STs globally and is increasingly being reported in both developed and developing countries.
- *C. jejuni* ST50 is a genetically diverse pathogen with geographically distinct isolates from poultry evolving independently.
- Virulence factors are generally conserved in *C. jejuni* ST50 with some exceptions such as *wlaN*, while the prevalence of resistance determinants may be influenced by differing practises in the use of antimicrobials in poultry production.

1. Introduction

Campylobacter is a leading cause of acute bacterial gastroenteritis in humans worldwide and are a major contributor to the global burden of foodborne disease (The World Health Organization, 2015). Rates of infection were observed to rise in the European Union (EU) between 2009 (58.2 cases per 100,000) and 2018 (64.1 cases per 100,000) (Kaakoush et al., 2015; The European Food Safety Authority, 2019a; European Centre for Disease Prevention and Control, 2021) and in Australia (AU) where the notification rate has risen from 110.0 cases per 100,000 in 2009 to 143.5 cases per 100,000 reported in 2019 (Australian Government Department of Health, 2020). Among other developed countries, *Campylobacter* infections are similarly the most commonly reported cause of bacterial gastroenteritis, with notification rates of 96.8 per 100,000 in the United Kingdom (GB) in 2017 (Public Health England, 2018), 30.2 per 100,000 in Canada (CA) in 2017 (Public Health Agency of Canada, 2018), and 19.5 per 100,000 in the United States (US) in 2018 (Tack et al., 2019). Variation in laboratory methods and surveillance and reporting requirements between countries makes direct comparison of these rates difficult.

Campylobacteriosis is typically self-limiting and is characterised by fever, abdominal cramping and diarrhoea (Altekruse et al., 1999), with the majority of cases making a full recovery. However, morbidity can be associated with campylobacteriosis, including inflammatory bowel disease, reactive arthritis, meningitis, Miller Fisher Syndrome and Guillain-Barré Syndrome (GBS) (Kaakoush et al., 2015). *Campylobacter* infections are generally sporadic and not associated with recognizable outbreaks (Kaakoush et al., 2015).

Campylobacteriosis is a zoonotic disease, transmitted to humans from contaminated food, water, or animals including wildlife, companion animals, poultry, and livestock (Horrocks et al., 2009). Healthy broiler chickens commonly carry *Campylobacter* as part of their gut microbiome (Horrocks et al., 2009) and one of the main sources of *Campylobacter* infection in humans is through consumption of poorly handled and/or poorly prepared poultry products including cross-contamination of other food during handling of raw poultry meat (Hermans et al., 2011).

Antibiotics are rarely used for *Campylobacter* infections, but may be prescribed to immunocompromised individuals, children or elderly patients. Also, a German study found that about one-third of hospitalised campylobacteriosis patients were given antibiotic treatment (Harvala et al., 2016; Kaakoush et al., 2015; Rosner et al., 2017; Wieczorek et al.,

2018). The treatment of non-self-limiting campylobacterosis is complicated by the emergence of antimicrobial resistance (AMR) among which fluoroquinolone and macrolide resistance are a significant problem globally (Wieczorek et al., 2018; Lapierre et al., 2016). Understanding the prevalence and persistence of resistance in a key, globally distributed lineage of *Campylobacter* may prove useful in the development of effective strategies to manage the AMR problem.

Understanding of the vertical and horizontal inheritance of other genes in such lineages may be useful too. A range of *Campylobacter* genes have been associated with pathogenesis, severe disease and post infection complications such as GBS. These nominal virulence genes are involved in motility, adhesion and cell invasion and are all potentially important in disease development. In relation to the development of GBS, there is an association with a particular *Campylobacter* lipooligosaccharide (LOS) that mimics a host ganglioside (Nguyen et al., 2016; Kim et al., 2016; Lapierre et al., 2016; Thakur et al., 2010; Wieczorek et al., 2018). Variation in *Campylobacter* strains causing human disease, unanswered questions in *Campylobacter* mechanisms of pathogenesis, and host factors influencing disease severity and complications leaves virulence factors as an open issue.

Multi-locus sequence typing (MLST), based on the internal sequences of seven housekeeping genes, is used to classify isolates and identify groups of related *Campylobacter* species (Dingle et al., 2001; Colles & Maiden, 2012; Skarp et al., 2015). The genetic diversity of these species is reflected in the more than 11,800 sequence types (STs) registered on the PubMLST database; notably more than 8,000 *C. jejuni* sequence types (PubMLST). *C. jejuni* ST21 clonal complex (CC) isolates are among the most common lineages causing human disease and are regarded as generalists as they are able to colonise a variety of different hosts (Dearlove et al., 2016; Revez et al., 2014). They are also frequently recovered from poultry products. Analysis of data in the PubMLST database showed that one quarter of the *C. jejuni* isolates in PubMLST are ST21 CC and ST50 is one of the most commonly reported STs in this CC (PubMLST).

C. jejuni ST50 isolates have been frequently reported globally from environmental, food and clinical sources (PubMLST). In this manuscript, we compare *C. jejuni* ST50 isolates previously recovered from retail chicken products in AU, as part of the CampySource study (Varrone et al., 2018; Wallace, Bulach, Jennison, et al., 2020), with poultry isolates from North America and Europe. The aims of this study were to i) assess genetic diversity, ii) examine the population structure and iii) compare the prevalence of genetic markers of resistance and virulence in *C. jejuni* ST50 isolates from poultry.

2. Materials and Methods

2.1. Bacterial isolates

The 182 *C. jejuni* ST50 poultry isolates included in this study were collected in 11 different countries: AU (n = 23), CA (n = 22), Germany (DE; n = 8), Denmark (DK; n = 16), Spain (ES; n = 4), GB (n = 51), Italy (IT; n = 3), the Netherlands (NL; n = 1), Poland (PL; n = 5), Romania (RO; n = 3) and the US (n = 46). AU isolates were obtained from chicken meat and offal (liver and giblet) products collected from retail outlets in the states of New South Wales, Queensland and Victoria between March 2017 and March 2019, as previously described (Walker et al., 2019); additional samples were collected in the Australian Capital Territory between May and September 2018. Danish *C. jejuni* isolates were collected as part of official surveillance programs by the Danish Veterinary and Food Administration (Joensen et al., 2020). Isolates from Scotland in GB were collected as part of the i-CaMPS-3 study commissioned by Food Standards Scotland (Food Standards Agency Scotland, 2015). Isolates from the US were collected as part of the National Antimicrobial Resistance Monitoring System (NARMS) by the US Food and Drug Administration (FDA) (The National Antimicrobial Resistance Monitoring System, 2016). Nineteen of the 40 isolates from the EU states were collected as part of the GENCAMP project (Leekitcharoenphon et al., 2018). The CA and DE isolates were from previously unpublished studies. Collection dates of the isolates ranged from 2010 to 2018. Read data for all isolates from AU, CA, US, EU and GB were retrieved as paired end reads via the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) or from laboratories in their respective countries. Metadata, data sources and study information for all isolates used in this study are listed in Table S1.

2.2. Assembly of Draft Genome Sequences and Genome Sequence Annotation

Isolate read data and metadata was predominantly obtained from previously published sources (Forbes, 2009; Joensen et al., 2020; Leekitcharoenphon et al., 2018; Wallace, Bulach, McLure, et al., 2020). Isolates that were included in this study had read data generated on the Illumina platform, at least 50x read depth coverage and no evidence of contaminating DNA (non-*C. jejuni* DNA). Read data processing was performed using the Nullarbor pipeline v2 (https://github.com/tseemann/nullarbor). Kraken (Wood & Salzberg, 2014) was used to confirm isolate classification and check for contaminating DNA. Where necessary, reads

were processed using Trimmomatic v0.39 to remove adapters and low-quality sequences. Reads were *de novo* assembled using SPAdes v3.14.0 (Prjibelski et al., 2020) and annotated using Prokka v1.14.5 (Seemann, 2014).

2.3. Typing of draft genome sequences

The ST was determined from assembled contigs for each isolate using *mlst* (https://github.com/tseemann/mlst), and the PubMLST "*Campylobacter jejuni/coli"* allele database sited at the University of Oxford (Jolley et al., 2018).

Draft genome sequences were screened for known AMR genes and virulence genes using NCBI's AMRFinderPlus (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/) and the Virulence Factor Database (VFDB) (http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Campylobacter) respectively, in conjunction with Abricate (https://github.com/tseemann/abricate). Hits were filtered using a cut-off set at 95% nucleic acid sequence identity and 90% sequence coverage.

Chromosomal mutations in the quinolone resistance determining region of *gyrA*, the promoter region of bla_{OXA-61} and the 23S rRNA gene, associated with AMR, were investigated as described previously (Wallace, Bulach, Jennison, et al., 2020). Resistance to quinolones (Hakanen et al., 2002) and macrolides/lincosamides/ketolides (Ladely et al., 2009) were determined by examining the amino acid at position 86 of GyrA (T86I confers resistance) and the nucleotides at positions 2074 and 2075 of the 23S rRNA gene, respectively. A point mutation at position 57 in the promoter region of bla_{OXA-61}, associated with inactivation of bla_{OXA-61} gene expression (Zeng et al., 2014), was also examined to enable accurate prediction of the ampicillin (AMP) phenotype. Table 1 summarizes the genes and mutations used to infer phenotypic resistance. This genotypic characterisation has been used to infer AMR phenotype based on good genotype-phenotype correlations reported in previous studies (Wallace, Bulach, McLure, et al., 2020; Whitehouse et al., 2018; Zhao et al., 2016).

2.4. Core Genome Sequence Comparison

C. jejuni RM1285 (ST50, Accession: CP012696) was used as the reference genome sequence for the mapping of isolate read sets using Snippy v4.4.6/BWA-MEM v0.7.17-r1188 as part of the core genome comparison of the 182 *C. jejuni* ST50 isolates. IQ Tree v1.6.12 (Nguyen et al., 2014) using the Jukes-Cantor model and the maximum likelihood method was

used to infer the relationship between isolates. The interactive tree of life (iTOL) v4 (Letunic & Bork, 2019) was used for tree and metadata visualization.

2.5. Virulome analysis

We evaluated the regional distribution of virulence genes by comparing the virulence gene content of isolates from each geographic region. Virulence genes present or absent in all 182 ST50 isolates were excluded from the clustering analysis. The distances between virulence profiles for each geographic region were calculated with the Euclidean distance measure. These analyses were performed using the heatmap and dist functions and the ggplot2 library in R (version 3.6.2.) (R Core Team, 2019; Wickham, 2016).

2.6. Accession numbers

Read data for the isolates used in this study were obtained from bioprojects PRJNA591966, PRJEB10936, PRJEB4848, PRJNA292664, PRJEB31119 or PRJEB23492, available at NCBI (https://www.ncbi.nlm.nih.gov/) or the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena). GenBank accession numbers are listed in Table S1.

3. Results

More than 77,000 *C. jejuni* isolates from around the world are listed in the PubMLST isolate database, with 47.0% of these being from clinical sources (Table 2 & Table S2). Among the *C. jejuni* submissions, ST50 was commonly reported from Asia (3.11%; 93/2,993), Europe (5.17%; 2,477/47,903), North America (3.99%; 828/20,722), Oceania (8.10%; 421/5,197) and South America (1.96%; 11/560). There were no ST50 isolates from Africa, although there are only limited data (<300 *C. jejuni* isolates). Figure 1 summarizes the number of *C. jejuni* ST50 isolates accumulated over time in the PubMLST database; in total, there are 3,870 ST50 isolates from more than 40 countries, however, 1,057 isolates have no year of isolation noted. The number of ST50 isolates, as well as the proportion of all isolates that are ST50, is generally increasing. The greatest number of ST50 isolates deposited into the database were collected in 2016 (n = 314) and 2015 (n = 251), representing 7.8% and 7.0% of all *C. jejuni* isolates added to the database in that year, respectively (Figure 1).

The tree in Figure 2, inferred using the maximum likelihood method, shows the relationship between the core genomes of the 182 ST50 isolates. The core genome covers about 80% of the *C. jejuni* RM1285 reference genome sequence with 11,717 variable sites.

Pairwise single nucleotide polymorphism (SNP) distances between isolates ranged from zero to 2,826 SNPs. Based on the tree, isolates are largely clustered by geographical region, with exceptions between GB and EU where trade barriers have been minimal. At least one genetic marker associated with inferred phenotypic antibiotic resistance was detected in isolates from each geographic location shown in Figures 2 & 3, with the exception of AU. It should be noted that 22 out of 23 AU isolates carried the inactive bla_{OXA-61} gene.

ST50 isolates from the EU were diverse in the context of the compared core genomes (0-2,633 SNPs; median = 1,094 SNPs) (Figure 4). The majority (n = 22; 95.7%) of isolates from AU were clustered exclusively (Figure 2). However, these isolates were highly diverse (0-1,128 SNPs). One isolate from a chicken meat sample in Queensland, AU was genetically distinct from all of the other AU isolates (1,421-1,706 SNPs). In contrast, isolates from CA (median = 163 SNPs) and the US (median = 118 SNPs) were less diverse (Figure 4). Identical isolates (0 SNPs) were present in all regions examined (Figure 2 & Figure 4). One group of isolates (n = 42), representing the majority (n = 40; 78.4 %) of isolates from GB, collected from retail chicken meat in Scotland, were nearly identical (0-13 SNPs) (Figure 2). Two very closely related isolates from DK (2-43 SNPs) were clustered with these 40 isolates from GB.

Genetic markers associated with inferred phenotypic AMP resistance were only observed in isolates from the EU (55.0%, CI_{95} 38.5-70.7%) and GB (43.1%, CI_{95}, 29.3-57.8%) (Figure 2 & Figure 3). Similarly, the mutation causing the T86I change in GyrA protein, associated with ciprofloxacin (CIP) resistance, was only observed in isolates from the EU (60.0%, CI_{95} 43.3-75.1%) and GB (15.7%, CI_{95} 7.0-28.6%). None of the isolates examined had evidence of resistance to erythromycin (ERY). Isolates from CA had the highest prevalence of genes associated with resistance to gentamicin (GEN; 36.3%) and tetracycline (TET; 77.3%). The *tet*(O) gene was detected in ST50 isolates from all geographic regions, except AU.

Hierarchical clustering of virulence factors shows that *C. jejuni* ST50 isolates from CA were genetically closer to isolates from the US (Euclidian distance = 0.81) than isolates from AU (Euclidian distance = 1.01), GB (Euclidian distance = 1.00) or the EU (Euclidian distance = 1.10) (Figure 5A). ST50 isolates from AU were genetically closer to isolates from the US (Euclidian distance = 0.92) than isolates from any other geographic region. Virulence genes *wlaN* and *cj1138* were present in 96.1% and 74.5% of isolates from GB, respectively, but were less frequently (21.7-54.5%) detected in isolates from other regions. Genes *hddA, hddC, gmhA2* and *rfbC* were more prevalent (93.5-95.7%) in isolates from CA, US and GB than isolates from AU and the EU (60.9-72.5%). On average, the number of virulence

markers identified ranged from 96 in isolates from AU to 102 in isolates from GB, with the least variability in isolates from CA (96-102 virulence genes) (Figure 5B).

4. Discussion

Nearly 5% of all *C. jejuni* isolates in the *Campylobacter jejuni/coli'* PubMLST isolate database are ST50; these ST50 isolates are from food and human sources from at least 40 countries. In AU, a recent study reported that 17.9% (51/285) of isolates from retail meat and 16.7% (95/569) of isolates from human fecal samples were ST50 (Wallace, Bulach, Jennison, et al., 2020). Several studies have similarly analyzed isolate genomes within a country or region (Cantero et al., 2018; Fiedoruk et al., 2019; Marotta et al., 2019; Rokney et al., 2018; Wallace, Bulach, McLure, et al., 2020), however no investigation of relationship between the genomes of ST50 isolates across continents has been undertaken. In the present study, we combined published genomic data for ST50 isolates to examine genetic diversity, population structure, and the prevalence of antibiotic resistance and virulence gene markers within this group. Analysis was restricted to poultry isolates to minimise diversity associated with different animal sources. Moreover, in the countries from which these isolates have been obtained, biosecurity measures generally preclude interaction between poultry and other potential source species.

4.1. Antimicrobial resistance

AMR is recognized as a serious current threat to public health globally (The World Health Organisation, 2017). Antibiotic use in food animal production, particularly in broiler flocks, is a driver of resistance and is now banned as a growth promoter in the EU (2006), AU (2007) and the US (2017), and for growth and preventive uses in CA (2014 for category I antibiotics (i.e. fluoroquinolones) and 2018 for category II antibiotics (i.e. aminoglycosides and macrolides)) (Agunos et al., 2017; Aidara-Kane et al., 2018; Australian Chicken Meat Federation, 2018; Chicken Farmers of Canada, 2020; Diarra & Malouin, 2014; Government of Canada, 2009; Prestinaci et al., 2015; Roth et al., 2019; The World Health Organization, 2017). Although the ban of antibiotic use in poultry farming as growth promoters largely occurred before the isolates in this study were collected, we still detected resistance determinants that are associated with phenotypic resistance to beta-lactams, fluoroquinolones and TET. These resistance determinants are likely to be neutral to *Campylobacter* due to their persistence despite the absence of selective pressure. While the persistence of resistance determinants is an issue, it highlights the importance of AMR stewardship in livestock

production. The monitoring of changes in resistance profiles in widely distributed pathogens, like *Campylobacter*, potentially provides a sensitive means of monitoring compliance in livestock production. Among the 40 EU isolates included in this study, more than 50% of isolates are resistant to AMP and more than 60% of isolates are resistant to fluoroquinolones. This seems to be a high level of persistent resistance in an animal production environment where antibiotic use as growth promoters has been banned, however both classes of antibiotics are still broadly used in the EU in food animal production (Roth et al., 2019). High levels of resistance impact the management of human campylobacteriosis. For example, fluoroquinolone resistance levels are so high in some countries (ES, 90% of isolates (Sáenz et al., 2000)) that this antibiotic is no longer recommended for treatment of campylobacteriosis (The European Food Safety Authority, 2019b).

In the present study, functional genetic determinants of resistance were absent from AU ST50 isolates. This contrasts findings from a recent report by the Australian Chicken Meat Federation where 37% of *C. jejuni* poultry isolates were phenotypically resistant to at least one antimicrobial among nine tested, which included: azithromycin, CIP, clindamycin, ERY, florfenicol, GEN, nalidixic acid, telithromycin and TET. Of note, 14.8% of *C. jejuni* were resistant to CIP, an antibiotic that is not approved for use in AU livestock (Australian Chicken Meat Federation, 2018). We did not observe genetic evidence for resistance to CIP in any of the 23 AU ST50 isolates, suggesting this mutation is associated with *C. jejuni* lineages other than ST50.

TET resistance was detected in isolates from all regions but was more common in isolates from CA (77.3%). Similarly, *aph(3')-Illa*, associated with GEN resistance, was primarily found in isolates from CA (36.4%), as well as a handful of isolates from the US (6.5%). These findings are not unexpected as aminoglycosides and tetracyclines, as well as lincosamides, macrolides, penicillins and sulfonamides were still used in broiler chickens as recently as 2017 in the US (Singer & Porter, 2019). An increase in GEN resistance in CA is likely a result of preferential use of this antibiotic for treating hatchery chicks, after ceftiofur was banned (Agunos et al., 2017; Rosengren L. B et al., 2009).

Fewer than 10% of isolates examined in this study could be classified as multidrug resistant; of the 15 isolates that had genetic evidence for resistance to three or more classes of antimicrobials, the majority of these (80%) were from the EU and were predicted to be AMP-CIP-TET resistant. Interestingly, all of these isolates that possessed the *aph(3')-Illa* gene also contained a bla_{OXA-61} gene with an active promoter (thus phenotypically AMP resistant). As

previously noted, the ongoing use of antimicrobial in food production in the EU has likely contributed to the resistance profiles observed.

4.2 Virulence

C. jejuni possess unique host factors and pathways that promote diarrhoeal disease in humans and commensalism in animals that are not typical in other enteric pathogens (Burnham & Hendrixson, 2018; Dasti et al., 2010). However, *C. jejuni* is unique in that it lacks many classical virulence and colonization factors (Burnham & Hendrixson, 2018; Fiedoruk et al., 2019). *C. jejuni* is a highly genetically variable pathogen with high rates of recombination and strains of the same ST may possess distinct virulence and resistance profiles. The key forces for variability in *C. jejuni* include horizontal gene transfer and recombination, causing distinct genetic boundaries between genotypes to be less apparent. Genes recognised as markers of human pathogenic *C. jejuni* strains have previously been discovered as significantly higher in ST50 (Fiedoruk et al., 2019). Similarly, a report of over 1,000 individuals with domestically acquired *C. jejuni* infection in Sweden showed that patients infected with ST50 were associated with higher numbers of hospitalisations compared to those infected with other STs, and cases that do seek medical attention tend to be in younger age groups (Harvala et al., 2016).

We found the number of virulence genes present in ST50 isolates was conserved across the three continents, with little variability between regions (a range of 96-102 virulence factors per region as detected using VFDB). Although the prevalence of some virulence factors varied with geographical location, many such as *cdtA*, *cdtB* and *cdtC* (production of cytolethal distending toxin) were present in all isolates examined in this study. Examples of genes not found in all isolates include *cj1136,* a LOS gene responsible for the production of glycosyltransferase, that was more abundant in isolates from the EU. The *flgE* gene, encoding the flagellar hook protein, was more common in isolates from AU and GB. The *wlaN* gene was detected in a high proportion of isolates from GB (96.1%), but less frequently in isolates from other regions (21.7-60.0%). Additionally, the *cj1138* gene was also found in a high proportion of GB isolates (74.5%) compared to other regions (26.1-47.5%). Genes responsible for heptose synthesis (*hddA, hddC, gmhA2*) appear to be more prevalent in isolates from AU, US and GB.

The significance of these differences in the prevalence of virulence factors on human infection globally remains to be elucidated, however the observation that ST50 isolates are associated with higher rates of hospitalisation suggests that the genes associated with this phenotype are part of the core genome. We observed a highly conserved core genome

comprising ~1,450 genes (~80% of the reference genome) and while this combination of genes may incidentally result in higher levels of hospitalisation, ST50 is a lineage that persists over time, is globally distributed in poultry and is by and large inherited vertically. By contrast, the accessory genome of *C. jejuni* ST50 contains features that are variably present, and presence is associated with geographic location to some extent. These genes are likely to be shared horizontally with compatible cohabitating *Campylobacter*; these geographically distinct accessory gene sets are likely important for the adaptation of the ST50 lineage to different environments.

4.3 Phylogenomics

The relationship between the core genomes of this collection of ST50 isolates revealed evidence of the evolution of a single exclusive lineage of ST50 isolates within AU, with the exception of isolate 17Q3056F1. This is consistent with the animal biosecurity measures applied in AU where no exotic livestock can be imported and the export of livestock is very limited. While our selection of isolates is conditional on the conservation of seven loci (MLST) and is providing a subset of highly related genomes, there is a strong selective pressure that preserves this core genome lineage despite being part of a heterogeneous *Campylobacter* population that is capable of recombination. Conservation of an ST50 lineage is indicated by the core genome analysis where around 80% of the reference genome is included in the core genome and the greatest pairwise difference between core genomes is 2,826 SNPs. The global distribution of ST50 and its conserved core genome is likely to be highly adapted to an optimal environment in a reservoir host that is likely to be chicken. In relation to the AU outlier isolate (17Q3056F1), it is not clear if this isolate is the result of an import of an exotic ST50 or the result of a recombination in the core region that has not altered the ST. The group of 42 ST50 isolates predominantly from GB (including 2 DK) were closely related and recently evolved from a common ancestor, perhaps originating from the same producer. These isolates all carry the bla_{OXA-61} gene, however only 18 out of 42 have an active promoter, while the remainder have lost the beta-lactam resistance genotype due to a single point mutation. The geographic origins of isolates in this group being from both GB and DK is consistent with the possibility that there has been a recent livestock exchange between these countries. Other more distant grouping relationships between US and GB, and CA and US isolates is consistent with past livestock exchange between these countries.

4.4 Limitations

Our study has some clear limitations with surveillance, reporting and characterisation of isolates by genome sequencing varying between countries and as a consequence our comparative genomics has been limited to isolates from a small number of developed countries. The process used to select ST50 as the dominant sequence type internationally restricted our analysis to isolates that were voluntarily submitted to the PubMLST isolate database. Samples from Africa were clearly underrepresented and thus our analysis focused on isolates from developed countries. Sampling bias is also possible with 40 of the GB isolates being near identical; these isolates arose from a study performed in Scotland (i-CaMPS-3) where isolates were obtained from retail chicken samples around Aberdeen.

4.4 Conclusions

ST50 represents a globally distributed stable lineage of *C. jejuni* that appears to be evolving independently in each of the regions covered as part of this study. Our analysis of this single lineage has enabled us to disentangle, to a small extent, the complex genetic exchange systems that exist within the species *C. jejuni* and the genus *Campylobacter* more generally. The link between production practices around the use of antimicrobials and genotypic AMR profiles highlights the potential for the use of genomic monitoring of *Campylobacter* isolates as a means of monitoring compliance.

Conflict of interest statement

The study team declares that there are no conflicts of interest.

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Tables

Table 1 Known antimicrobial resistance genes and mutations in *Campylobacter* spp. used to infer phenotypic resistance.

Drug class	Antimicrobial	Gene	Mutation	Reference
Aminoglycoside	Gentamicin	*aph(3')-Illa*	None	Ramirez & Tolmasky, 2010
Beta-lactam	Ampicillin	bla_{OXA-61},	G57T	Zeng et al., 2014
		$bla_{OXA-193}$	None	
Quinolone	Ciprofloxacin	*gyrA*	T86I (GyrA)	Hakanen et al., 2002
Macrolide	Erythromycin	*erm*(B),	None	Qin et al., 2014,
		23S rRNA	A2074G,	
			A2074C,	Ladely et al., 2009
			A2075G	
Tetracycline	Tetracycline	*tet*(O)	None	Whitehouse et al., 2018

Table 2

Ten most commonly reported *C. jejuni* sequence types (STs) recovered from clinical, animal and environmental samples in the PubMLST database† (*n* = 76,699‡) from each continent.

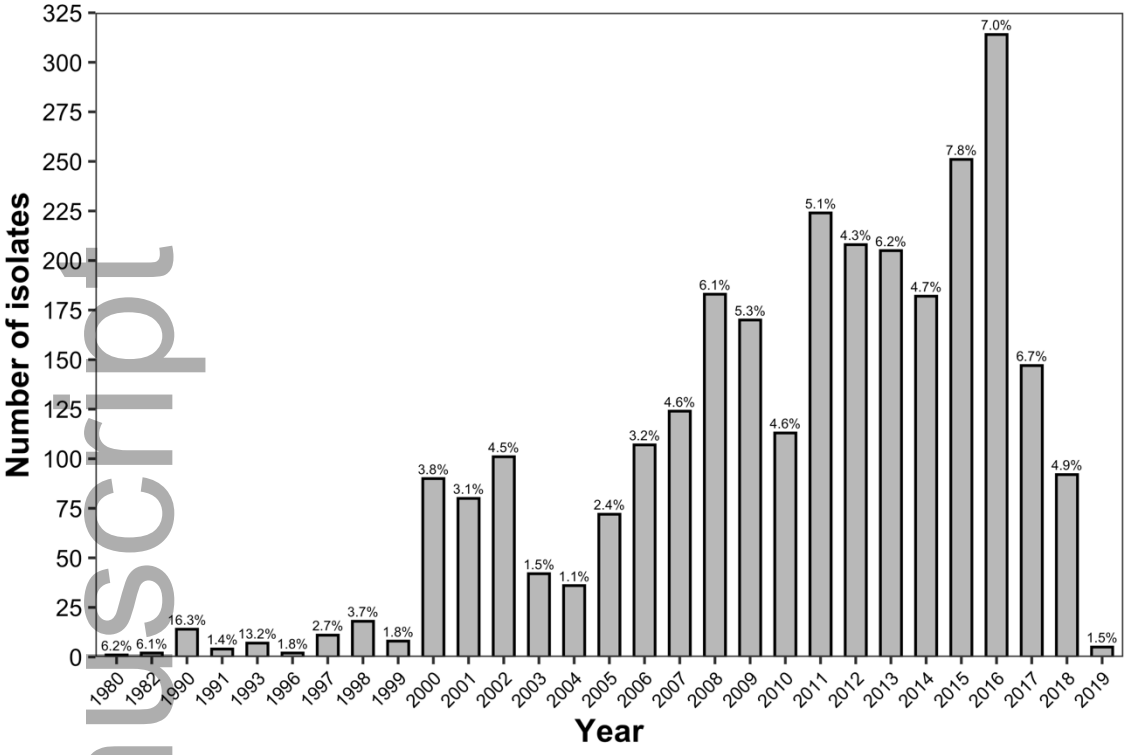
	Africa (*n* = 277)		Asia (*n* = 2,993)		Europe (*n* = 47,903)		North America (*n* = 20,722)		Oceania (*n* = 5,197)		South America (*n* = 560)	
Rank	ST	*n* (%)	ST	*n* (%)	ST	*n* (%)	ST	*n* (%)	ST	*n* (%)	ST	*n* (%)
1	1036	14 (5.05)	50	93 (3.11)	21	3263 (6.81)	45	1015 (4.90)	45	579 (11.14)	353	52 (9.29)
2	362	13 (4.69)	21	69 (2.31)	257	2539 (5.30)	982	962 (4.64)	474	422 (8.12)	8741	27 (4.82)
3	1035	8 (2.89)	354	67 (2.24)	50	2477 (5.17)	353	928 (4.48)	50	421 (8.10)	1919	23 (4.11)
4	19	7 (2.53)	45	47 (1.57)	45	2342 (4.89)	8	837 (4.04)	6964	310 (5.96)	403	23 (4.11)
5	1932	7 (2.53)	1919	46 (1.54)	48	2256 (4.71)	50	828 (4.00)	53	246 (4.73)	475	22 (3.93)
6	440	7 (2.53)	257	46 (1.54)	19	1293 (2.70)	48	800 (3.86)	42	222 (4.27)	607	17 (3.04)
7	52	7 (2.53)	22	39 (1.30)	53	1259 (2.63)	21	663 (3.20)	48	218 (4.19)	52	16 (2.86)
8	881	7 (2.53)	768	39 (1.30)	51	1226 (2.56)	459	656 (3.17)	583	194 (3.73)	137	12 (2.14)
9	658	6 (2.17)	572	37 (1.24)	61	1071 (2.24)	806	642 (3.10)	61	173 (3.33)	463	12 (2.14)
10	7784	6 (2.17)	51	32 (1.07)	354	999 (2.09)	922	562 (2.71)	190	145 (2.79)	50	11 (1.96)

† Data was extracted from https://pubmlst.org/campylobacter/ on 06 September 2020.

‡ Isolates were excluded if the ST was missing or if the continent was not provided. Antarctica was excluded due to only one isolate being submitted from this continent.

603 **Figure legends**

604 **Figure 1.** Summary of all *C. jejuni* ST50 isolates (*n* = 3,870) deposited into the PubMLST
605 database by year of collection. Numbers above the bars indicate the percentage of *C. jejuni*
606 isolates collected in the respective year that were ST50. Data were extracted from the
607 PubMLST database on 06 September 2020.
608
609 **Figure 2.** A Maximum likelihood phylogenetic tree showing the core genome relationship (0-
610 2,820 SNPs) between *C. jejuni* ST50 (*n* = 182) from poultry in Australia (AU), Canada (CA),
611 United States (US), European Union (DK, RO, DE, NL, IT, PL and ES) and the United
612 Kingdom (GB). The circle lanes from inner to outer indicate the country of isolation,
613 geographic region, number of genetic determinants of antimicrobial resistance, the presence
614 of genes and/or mutations associated with resistance to ampicillin (AMP), ciprofloxacin
615 (CIP), erythromycin (ERY), gentamicin (GEN), tetracycline (TET) and the year of isolation.
616
617 **Figure 3.** Prevalence of genes and mutations associated with resistance in *C. jejuni* ST50 (*n* =
618 182), from Australia (AU), Canada (CA), European Union (EU), United Kingdom (GB) and
619 the United States (US), associated with resistance to ampicillin (AMP), ciprofloxacin (CIP),
620 erythromycin (ERY), gentamicin (GEN) and tetracycline (TET). Error bars indicate 95%
621 confidence intervals.
622
623 **Figure 4.** Boxplots showing pairwise SNP differences between *C. jejuni* ST50 isolates (*n* =
624 182) from the same geographic region: Australia (AU), Canada (CA), European Union (EU),
625 United Kingdom (GB) and the United States (US).
626
627 **Figure 5. (A)** Hierarchal clustering of *C. jejuni* ST50 isolates (*n* = 182) from Australia (AU),
628 Canada (CA), European Union (EU), United Kingdom (GB) and the United States (US) and
629 the associated virulence genes (*n* = 59) based on Euclidian distance. Dark red indicates the
630 presence of the gene in all isolates from that region, while dark blue indicates the absence of
631 the gene in all isolates from that region. See Table S3 for heatmap data. **(B)** Boxplots
632 showing the abundance of virulence genes in each region.



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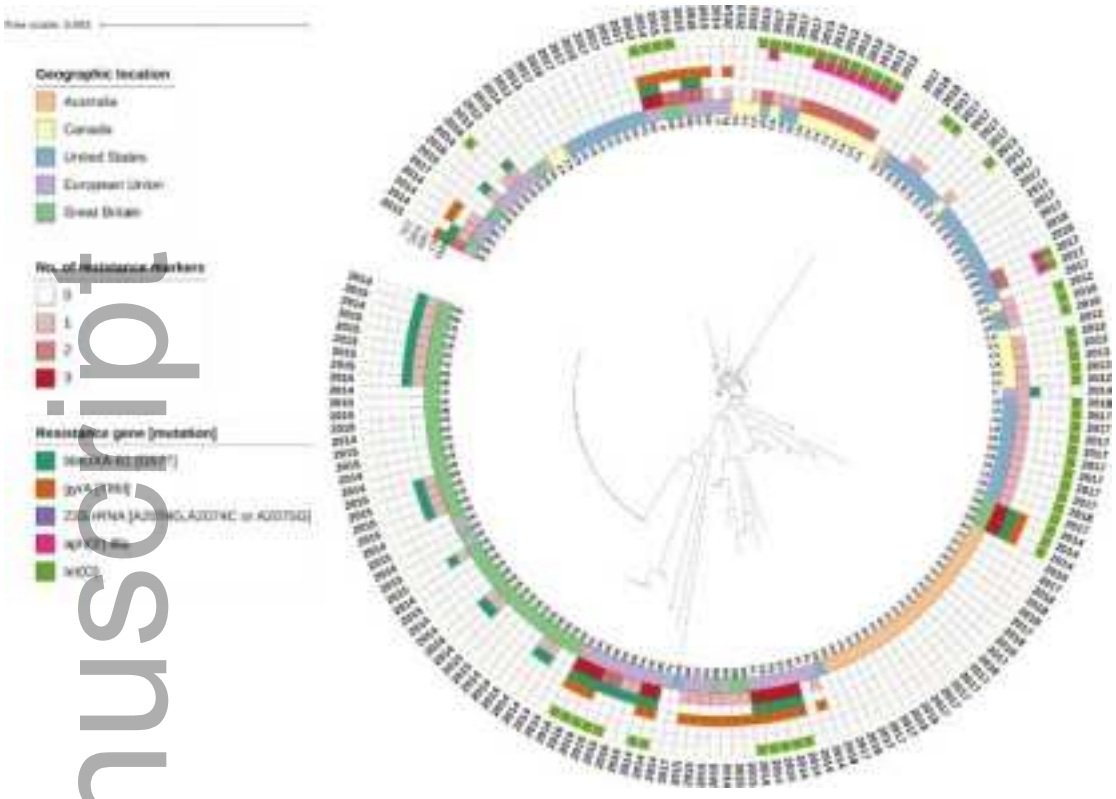

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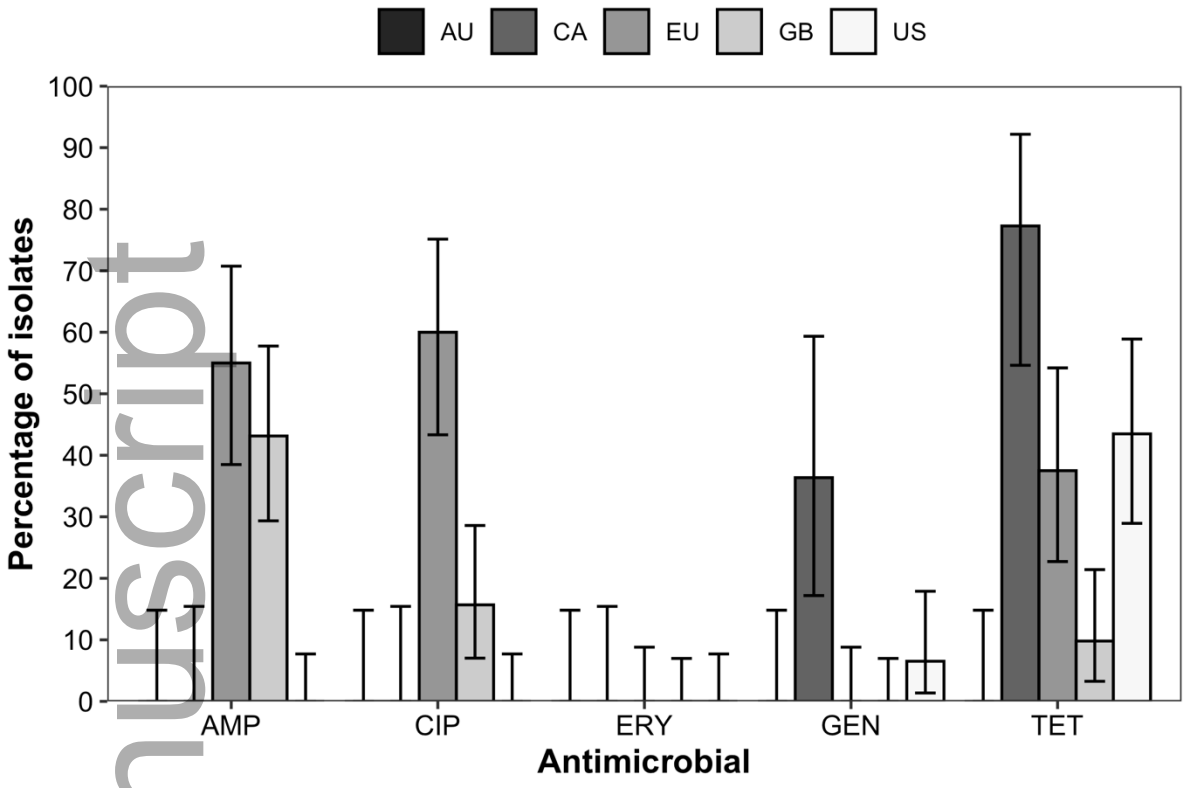

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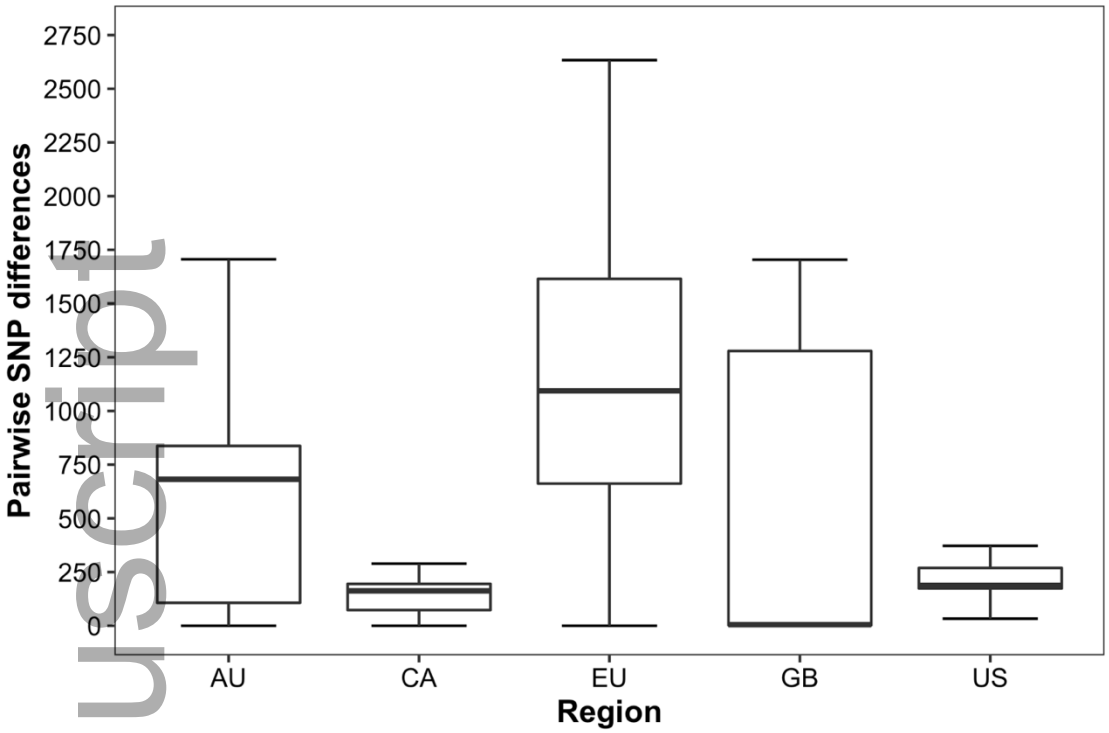

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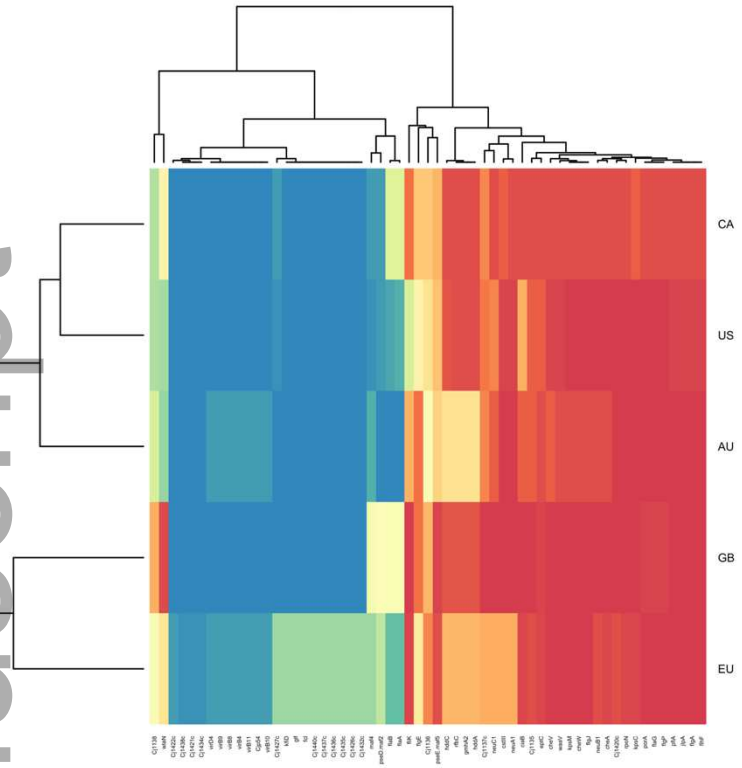

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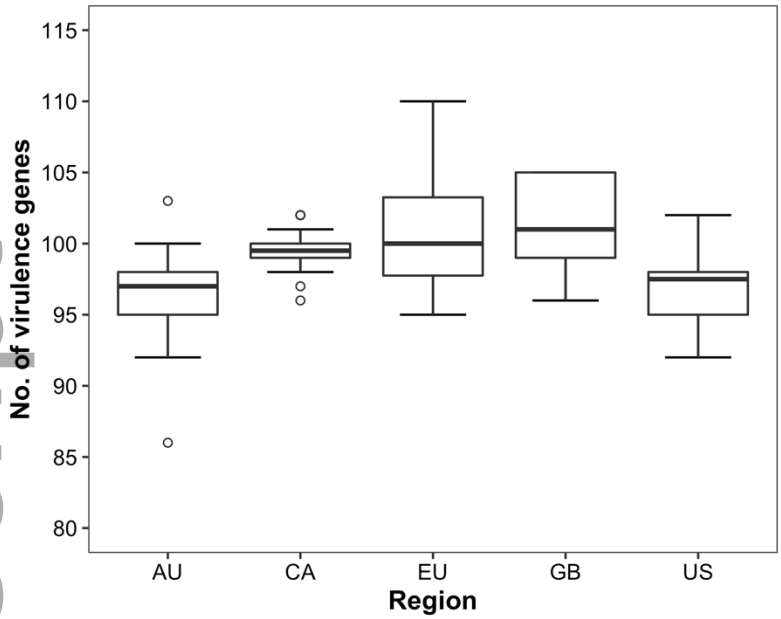

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