International Journal of Food Microbiology 427 (2025) 110940

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Whole-genome comparative analysis of the genetic, virulence and antimicrobial resistance diversity of *Campylobacter* spp. from Spain

Irene Ortega-Sanz, Jordi Rovira, Beatriz Melero *

Department of Biotechnology and Food Science, University of Burgos, Burgos, Spain

ARTICLE INFO

Keywords:
Whole-genome sequencing
Genomics
Phylogeny
Virulence genes
Phase variation
Antimicrobial genes

ABSTRACT

Whole-Genome Sequencing has the potential to be an effective method for surveillance of foodborne diseases. This study aims to determine the genetic relatedness and prevalence of virulence-associated genes and antimicrobial resistance determinants in 135 *Campylobacter jejuni*, seven *Campylobacter coli* and three *Campylobacter lari* isolates from the poultry supply chain and a hospital in Spain. The isolates showed a wide genetic diversity between and within species with Clonal Complex 21 the most frequent lineage found. Among species, *C. jejuni* showed the highest prevalence of virulence genes (287/333) in which a high occurring variability was observed in the capsule biosynthesis and transport, O-linked flagellar glycosylation and lipooligosaccharide biosynthesis loci, with a great impact of phase-variation that led to 72 different virulence gene patterns among all isolates. High prevalence (> 90 %) of bla_{OXA}-type β-lactamase genes and mutations in DNA gyrase gene associated with fluoroquinolones resistance were observed, and at a frequency similar to the *tet(O)* gene in *C. jejuni* (93 %) and *C. coli* (86 %), both of which also harboured resistance determinants to aminoglycosides with a higher occurrence rate in *C. coli* (43 %), that was the only species in which mutations in the 23S ribosomal subunit conferring resistance to erythromycin were identified (43 %). The present study constitutes the largest genomic survey of *Campylobacter* isolates in Spain providing insight into the prevalence and diversity of the pathogen along the poultry supply chain in the country.

1. Introduction

Campylobacter spp. are the most common cause of foodborne gastrointestinal infection worldwide with >127,000 human reported cases in the EU in 2021 (EFSA and ECDC, 2022a). *Campylobacter jejuni* causes approximately 90 % of them, followed by *Campylobacter coli* (about 10 %) and less frequent species like *C. lari* (0.1 %). Poultry is the most important source of *Campylobacter* transmission, although direct contact with infected animals or environmental reservoirs (e.g., water sources) are potential risk factors associated with *Campylobacter* outbreaks (McLure et al., 2023). Moreover, certain lineages are frequently associated with specific hosts or niche, like the *C. jejuni* Clonal Complex (CC)257 defined as chicken-specialist lineage (Sheppard et al., 2014), while others dominant genotypes, such as CC21, CC45 or CC848, are host-generalist (Mehat et al., 2020).

The pathogenesis of *Campylobacter* is unclear due to the great diversity of bacteria, and the relevance of putative virulence factors is commonly poorly understood (García-Sánchez et al., 2018a). However, some virulence factors involved in flagella (FlaA and FlaB) and proteins associated with adhesion (CadF), invasion (CiaB, CiaC, CiaD and CiaI) and colonization (CapA) have been described as responsible for *Campylobacter* pathogenicity (Bolton, 2015). Moreover, the structure of the capsular polysaccharide (CPS) and lipooligosaccharide (LOS), together with the O-linked glycosylation of flagellin and genetic variations owing to phase variation, are also important determinants of the pathogenicity of the bacteria (Burnham and Hendrixson, 2018).

Campylobacteriosis is usually a self-limiting infection and antibiotics are only recommended for severe cases. However, their indiscriminate use in humans and in food-producing animals has led to the emergence of multidrug resistant strains that threaten global public health (Qin et al., 2023). Nevertheless, specific *Campylobacter* genotypes are more likely to resist antibiotics than others and a potential link was found between specific lineages and source of isolation (Wirz et al., 2010; Shin et al., 2013). Similarly, some *C. jejuni* genotypes have a better ability to survive and persist at certain stages of the food supply chain (Melero et al., 2012; García-Sánchez et al., 2017).

Currently, Whole-Genome Sequencing (WGS) is applied for epidemiology studies including surveillance of foodborne pathogens of public

* Corresponding author.
E-mail address: bmelero@ubu.es (B. Melero).

https://doi.org/10.1016/j.ijfoodmicro.2024.110940
Received 8 January 2024; Received in revised form 4 October 2024; Accepted 14 October 2024
Available online 18 October 2024

health concern, allowing outbreak detection and prevention of foodborne diseases (Joensen et al., 2021; Dessouky et al., 2022). However, in Spain, only a few studies have analysed the genetic diversity and genomic traits of *Campylobacter* strains and used a limited number of isolates from 16 to 70 (Cantero et al., 2018; García-Sánchez et al., 2019; Ocejo et al., 2021). The aim of this study was to investigate the diversity of *Campylobacter* spp. isolated from different steps of the poultry supply chain and from the hospital of Burgos (Spain), and compare their antimicrobial resistance and virulence profiles.

2. Material and methods

2.1. Campylobacter isolates population

A total of 135*C. jejuni*, seven *C. coli* and three *C. lari* was selected from our previous cross-sectional surveys carried out between June 2014 and July 2015 in different steps of the poultry supply chain of northern and central Spain ($n = 86$), such as farms ($n = 11$), a slaughterhouse ($n = 32$), a poultry processing plant ($n = 14$) and poultry products form different regions of Spain at retail shops ($n = 29$), and from human clinical samples of the region of Burgos (Spain) ($n = 59$) (García-Sánchez et al., 2017; García-Sánchez et al., 2018a; García-Sánchez et al., 2020). During that period, the slaughterhouse was exclusively supplied by the seven farms that were sampled, but the retail shops received meat from different poultry processing plants (including that of this study). Moreover, despite the slaughterhouse and poultry processing plant comprised different areas of the same location, they were studied independently due to the statistically significant association ($p < 0.05$) between the frequency of *C. jejuni* isolates and these areas (García-Sánchez et al., 2017). Isolate metadata is described in Supplementary Table S1.

2.2. Whole-Genome Sequencing (WGS) and genome analysis

Genomic DNA extraction and sequencing was performed as described by Ortega-Sanz et al. (2023a). Briefly, Bacterial DNA was extracted using the commercial QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. DNA concentrations were measured using the Qubit Fluorometer, while DNA purities were determined using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Library preparation and sequencing was performed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) and the MiSeq platform (Illumina) providing 2 × 300 bp paired-end reads.

Reads were filtered, assembled and annotated using CamPype (Ortega-Sanz et al., 2023b). Briefly, read quality for each sample was analysed using FastQC v0.11.9 (S. Andrews, http://www.bioinformatics.babraham.ac.uk/projects/fastqc) to optimize the read quality step. Trimmomatic v0.39 (Bolger et al., 2014) was used to remove adapters from reads (ILLUMINACLIP:adapters_and_sequences.fa:1:30:11), followed by read quality filtering using PRINSEQ (Schmieder and Edwards, 2011) with the following parameters: -min_len 50 -min_qual_mean 30 -trim_qual_right 25 -trim_qual_window 20. Overlapping reads were extended with FLASH v1.2.11 (Magoč and Salzberg, 2011) with default settings, followed by de novo assembly through SPAdes v3.14.0 (Bankevich et al., 2012) using the isolate mode. Contigs shorter than 500 bp were removed, and the quality and completeness of genomes were analysed through QUAST v5.0.2 (Gurevich et al., 2013) and BUSCO v.5.2.2 using the campylobacterales_odb10 dataset (Simão et al., 2015), respectively. Draft genomes were reordered against the reference genomes of *C. jejuni* NCTC 11168 (AL1111681.1), *C. coli* RM2228 (CP035927.1) or *C. lari* RM2100 (CP000932.1) using progressiveMAUVE v2.4.0 (Darling et al., 2010) and annotated using Prokka v1.14.6 (Seemann, 2014) with the corresponding reference genome annotation for each species mentioned above. CRISPR arrays and *cas* loci were also annotated using minced v0.2.0 (Bland et al., 2007), which was available in Prokka.

2.2.1. Genetic diversity, Multilocus Sequence Type (MLST) identification and core genome MLST (cgMLST) analysis

Average nucleotide identity (ANI) was calculated based on the BLAST algorithm using pyANI v0.2.11 (Pritchard et al., 2016) to assess the similarity within species and to each reference genome. Draft genomes were taxonomically typed based on the auto-detected MLST scheme by mlst v2.17.6 (T. Seemann, https://github.com/tseemann/mlst) and assigned to Clonal Complexes (CCs) by the PubMLST database (Jolley et al., 2018). For *C. jejuni* and *C. coli* isolates, the MLST scheme was composed of the following 7 housekeeping genes: *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt* and *uncA*, while for *C. lari* the gene targets were *adk*, *atpA*, *glnA*, *glyA*, *pgi*, *pgm* and *tkt*. Phylogenetic comparison of the genomes was performed using a Neighbour-Joining (NJ) tree based on the distance matrix calculated from the allelic differences in the core genome MLST (cgMLST).The genomes were analysed by chewBBACA v2.8.5 (Silva et al., 2018) with default configuration following the external pubMLST *C. jejuni/coli* cgMLST scheme of 1343 loci (Cody et al., 2017) (last updated on September 12, 2022) and the Prodigal training file provided for genome *C. jejuni* NCTC 11168-BN148 (https://www.ncbi.nlm.nih.gov/nuccore/HE978252). For allele assignment, a BLAST Score Ratio (BSR) equal to 0.6 was used. The allele profile data was subsequently used to obtain the cgMLST allele distance matrix by cgmlst-dists v0.4.0 (https://github.com/tseemann/cgmlst-dists). Finally, MEGA X v10.2.5 (Kumar et al., 2018) was used to calculate the NJ tree using the bootstrap method with 1000 replicates, and the "p-distance" model. Annotations and visualizations were performed using the web-based tool iTOL v6.5.8 (Letunic and Bork, 2021).

2.2.2. Identification of virulence genes

An in-house database of 333 *Campylobacter* virulence-associated genes (Supplementary Table S2) was blasted twice against the draft and annotated genomes through BLAST 2.10.1+ (Zhang et al., 2000). Results were manually corrected for overlapping blast hits (i.e., different genes found at the same location in the genome) based on analysing Bidirectional Best Hits (BBHs) using the orthologr R package (Drost et al., 2015) and gene synteny. BBHs confirmed the presence of a single gene at a specific location when overlapping hits were observed at the same genomic coordinates. When no BBH was possible, genes were assigned based on contiguous sequences and sequential order of locus tags in both the reference genome and the annotated genomes of study (gene synteny). Furthermore, truncations were considered as gene presence if the hit occurred at contigs boundaries (i.e., at the end of two contigs) (Ortega-Sanz et al., 2024) and confirmed through mapping the reads to the reference genome using BWA-maximal exact match (MEM) 0.7.17-r1188 (Li, 2013), followed by visualization of the alignment with IGV (Robinson et al., 2011). For those virulence-associated genes affected by phase-variation (PV) (Supplementary Table S2), the ON/OFF status was analysed using PhasomeIt (Aidley et al., 2018) with the cutoffs 7 6 10 5 5 and the filter W9 as recommended for *Campylobacter* analysis.

2.2.3. Identification of antimicrobial resistance genes

The detection of antimicrobial resistance (AMR) genes was performed using ABRicate 1.0.1 (T. Seemann, https://github.com/tseemann/abricate) against the databases ARG-ANNOT (Gupta et al., 2014), CARD (Comprehensive Antibiotic Resistance Database) (McArthur et al., 2013), ResFinder (Zankari et al., 2012), Megares (Lakin et al., 2017) and NCBI Bacterial Antimicrobial Resistance Reference Gene Database (NDARO) (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047). A minimum of 80 % identity and 60 % coverage was required for gene presence. In addition, point mutations conferring resistance were identified through PointFinder v3.1.1 (Zankari et al., 2017) in the genes existing in the database of the *Campylobacter* species and using BLASTN. No blast threshold for identity

or minimum coverage were specified for PointFinder and default configuration was used (90 % identity and 60 % coverage). The in silico data was finally compared with the antimicrobial in vitro susceptibility tests that were previously performed in the research group (García-Sánchez et al., 2018b; García-Sánchez et al., 2019; García-Sánchez et al., 2020).

2.3. Statistical analysis

Differences between means were examined using one-way analysis of variance (ANOVA), and Fisher's LSD (Least Significant Difference) test was applied whenever multiple comparisons were carried out. Besides, the significance of between-group differences in the frequency of positive isolates was verified with Fisher's exact test. The statistical significance level was set at $p \leq 0.05$.

3. Results

3.1. Genome annotation

The 145 draft genomes were assembled into 10–86 contigs with an average coverage of 162 ± 104× (Supplementary Table S3). The total genome length ranged from 1.61 to 1.87 Mbp in *C. jejuni*, 1.66 to 1.77 Mbp in *C. coli*, and 1.45 Mbp in *C. lari*. While the average size of the *C. jejuni* and *C. coli* genomes was statistically equal ($p < 0.001$), *C. lari* genomes were significantly shorter with ~240 kbp less, and so was the number of coding DNA sequences (CDS) found. The genome annotation revealed 1648 to 1967 CDS and 3–8 rRNAs in *C. jejuni*, 1674 to 1795 CDS and 3–6 rRNAs in *C. coli*, and 1447 to 1451 CDS and 3 rRNAs in *C. lari*. Moreover, 108 genomes (106*C. jejuni* and two *C. coli*) harboured a CRISPR-Cas (Clustered Regularly Interspaced Palindromic Repeats) system and genes encoding for Cas1, Cas2 and Cas9, but the remaining 29*C. jejuni* genomes possessed the *cas* locus with no companion CRISPR array, while no isolated *cas* locus (or CRISPR arrays) were found in the remaining eight genomes (five *C. coli* and three *C. lari*). The average GC content was 30.36 ± 0.10 % in *C. jejuni*, 31.33 ± 0.09 % in *C. coli* and 29.68 ± 0.01 % in *C. lari*. Genome completeness was ≥99 % for all genomes.

3.2. Genetic diversity and phylogenetic analysis of Campylobacter spp.

The ANI values calculated between isolates of the same species were above 96 % for *C. jejuni* (mean = 98.76 %, range = 96.98 %-100 %), *C. coli* (mean = 98.79 %, range = 98.45 %-99.73 %) and *C. lari* (mean = 99.04 %, range = 98.08 %-100 %). When the isolates were compared to the reference genome within each species, highly similar ANI values were obtained for *C. jejuni* (mean = 98.93 %, range = 97.26 %-99.97 %) and *C. coli* (mean = 98.82 %, range = 98.55 %-99.01 %), but lower values for *C. lari* (mean = 98.09 %, range = 98.08 %-98.10 %).

The *Campylobacter* strains showed a great variety of Clonal Complexes (CCs) with 126 isolates (87 %) being grouped in 14 previously described CCs, while the remaining 19 isolates belonged to yet undefined CC, including the three *C. lari* isolates (Supplementary Table S3). This diversity was predominant in *C. jejuni*, for which the most frequent Clonal Complex was CC21 with 69 isolates in total (51 %), followed by CC607 and CC464 with 10 isolates each (7 %). CC21 was the only lineage that was isolated from all sampling locations (farm, slaughterhouse, poultry processing plant, retail shop and hospital) with a predominance in all of them (Fig. 1A). The distribution of CCs along the food supply chain revealed that the slaughterhouse and poultry processing plant were the stages with the least diversity of lineages (four CCs each), while a high diversity of lineages was found in retail (nine CCs) and, in particular, in hospital (12 CCs). Moreover, a potential association was found between specific CCs and sampling locations ($p < 0.001$). Thus, isolates from slaughterhouse were more likely to belong to CC607 than isolates from other sites (25 % versus 2 %, $p < 0.001$). Similarly, CC257 was only found in human campylobacteriosis cases, while CC460 in surfaces in slaughterhouse and poultry processing plant, and CC658 in retail chicken. In contrast, CC464 was the only CC found in all sampling locations except in slaughterhouse. In the *C. coli* species, all isolates were grouped in CC828.

Phylogenetic analysis of the strains showed three main clades, one for each *Campylobacter* species (Fig. 1B). Of these, the highest diversity of Sequence Types (STs) was observed in the *C. jejuni* clade, and, specifically, in isolates from clinical origin (Fig. 1B). They were divided into 25 STs and most of them ($n = 130$) belonged to 13 distinct CCs. However, five *C. jejuni* strains did not have a ST reported in the pubMLST database (accessed on January 4, 2023), and belonged to an unknown not yet reported CC. The most frequent STs in *C. jejuni* were ST-3769 ($n = 36$), ST-50 ($n = 12$), ST-464 ($n = 10$) and ST-21 ($n = 8$). Despite such diversity of the *C. jejuni* strains, sampling locations were also significantly linked to specific STs ($p < 0.001$). Of these, ST-3769 and ST-904 were significantly predominant of environmental samples (83 % versus 9 %, $p < 0.001$) and in slaughterhouse and poultry processing plant over other STs (89 % versus 6 %, $p < 0.001$). Both STs included strains with little allelic variations among them and were highly clonal. Moreover, ST-3769 was the most persistent ST along the poultry supply chain (isolated from farm, slaughterhouse and processing plant) and in hospital with increasing prevalence in the slaughterhouse and processing plant (72 %). The *C. coli* strains were represented by four STs (ST-827, ST-854, ST-860 and ST-1595), of which ST-860 (n = 3) was the most frequent, and together with ST-854 persisted from retail to hospital. On the other hand, all *C. lari* strains were represented by ST-21 (*C. non-jejuni/coli* MLST scheme).

3.3. Virulence genes

A total of 191 virulence-associated genes were identified in the 145 *Campylobacter* spp. isolates, whereas differences were observed for 134 genes, and only the *ggt* gene (colonization) and seven genes of the Type IV Secretion System (T4SS) were neither found in any isolate nor the reference strains (Fig. 2). The distribution of genes indicated 72 different virulence profiles among the isolates. Prevalence of genes varied among the different categories of virulence factors, although the genes representing the flagellar Type III Secretion System (T3SS) and those related to invasion (IamA/B and Cia proteins) and secretion system (TatABC complex) were found in all isolates. Conversely, genes encoding enterobactin receptors (*cfrB*), autotransporters (*capB*), capsule components (*cj1426c*, *cj1432c*, *1433c*, *cj1435c-cj1438c* and *cj1440c*) and secretion systems (Type VI secretion system, T6SS) were less frequently detected (below 20 %).

Among species, a higher number of virulence genes was found for *C. jejuni* (mean = 287, range = 255–313), followed by *C. coli* (mean = 255, range = 249–258) and *C. lari* (mean = 222, range = 222–222), that was statistically significant between each pair ($p < 0.001$). A similar pattern was observed by virulence factor category with the exception of genes related to lipooligosaccharide (LOS), that were less prevalent in *C. coli* with on average three genes less compared to the 12 genes found in *C. lari*, although the difference was not significant between the two ($p > 0.05$). Compared to each selected reference genome per species, *C. lari* harboured on average 12 genes less and the difference was significant ($p < 0.001$), while no statistical difference was observed in the *C. jejuni* isolates despite harbouring 22 genes less neither in *C. coli* ($p > 0.05$). Hierarchical clustering analysis based on the 134 variable genes showed clear clustering patterns for each species and CC (Fig. 2). Within CC21, ST-21 and ST-883 formed a separate cluster due to large differences in the capsule biosynthesis and transport locus. This locus together with the O-linked flagellar glycosylation and LOS biosynthesis loci showed high intra- and interspecies variability due to phase-variable (PV) genes, especially in *C. jejuni* and *C. coli*. From the 24 virulence-related PV genes whose ON/OFF state was assessed, 19 were located in one of these three loci (Fig. 2). Differences in the phase state were found for 23 PV genes,

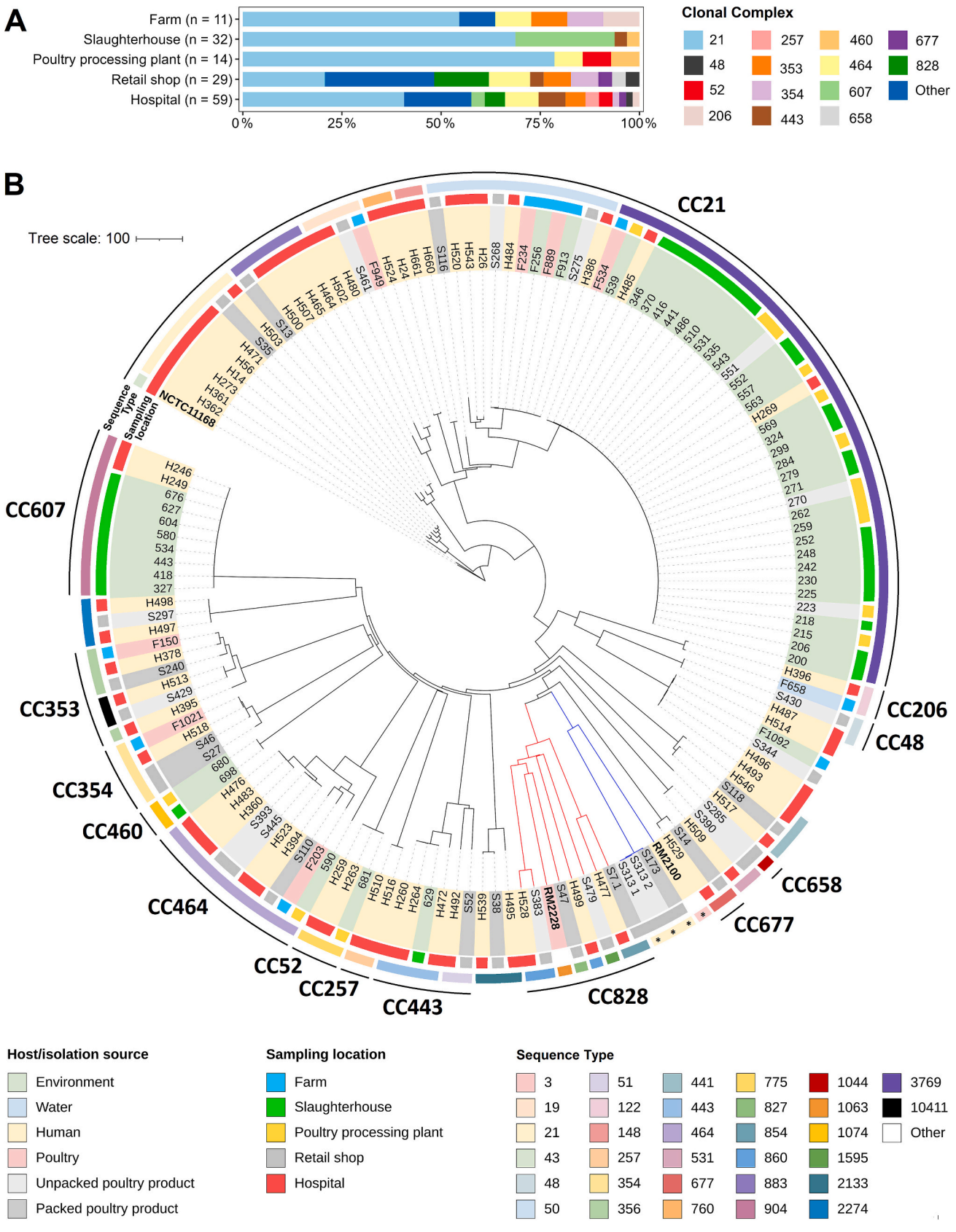

Fig. 1. Phylogenetic analysis of the *Campylobacter* species isolated from Spain in this study. Clonal Complex (CC) frequency distribution of *Campylobacter* spp. isolates grouped per sampling location are detailed (A). Phylogenetic tree is based on the pubMLST *Campylobacter jejuni/coli* cgMLST scheme (B). Tree branches are coloured based on species with *C. jejuni* in black, *C. coli* in red and *C. lari* in blue. Host or isolation source is shown in coloured boxes for each strain with reference genomes in bold. Sampling location and Sequence Types (STs) are highlighted in the outer rings for each strain. *, *C.* non-*jejuni/coli* MLST scheme.

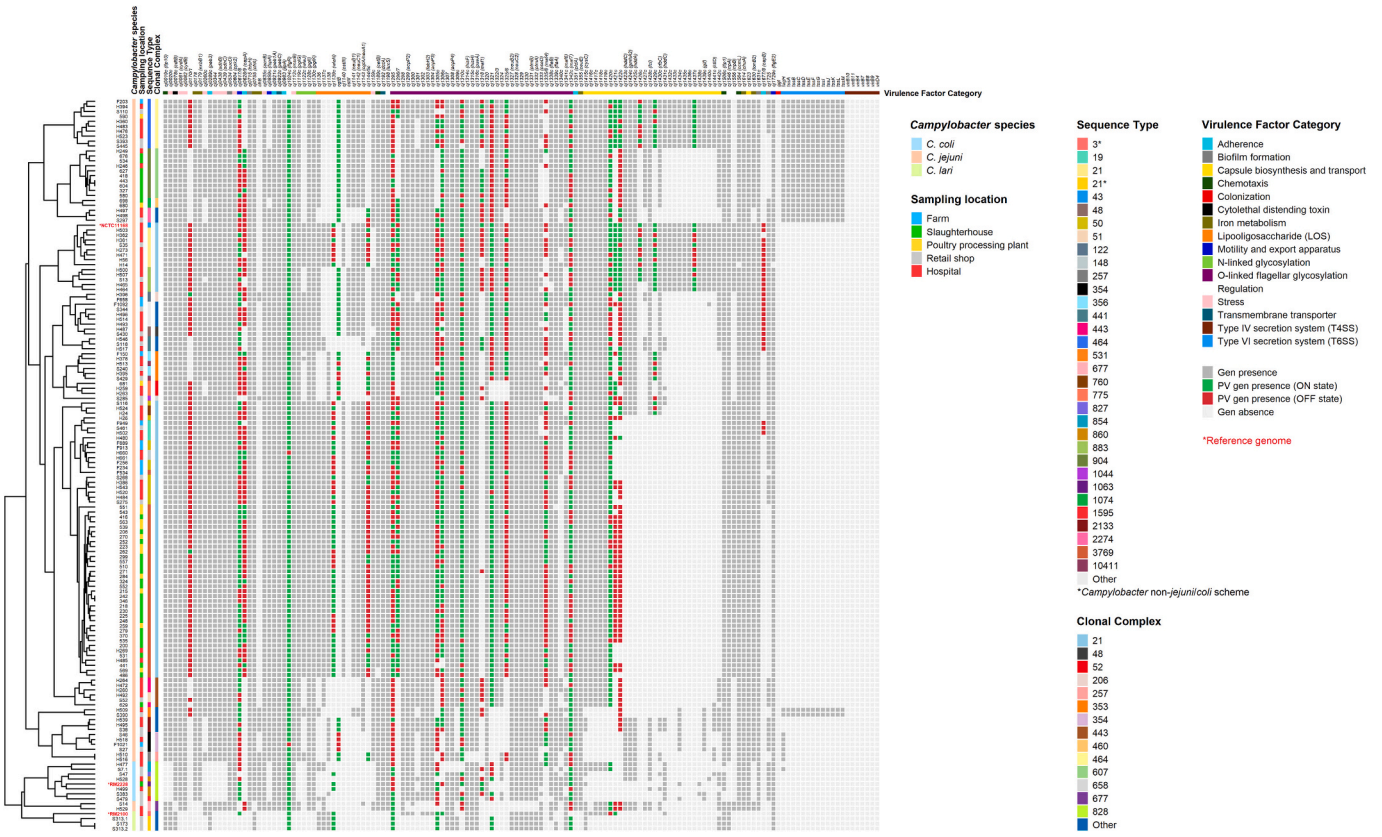

Fig. 2. Distribution of *Campylobacter* spp. virulence-associated genes. The heatmap shows the presence (dark grey, green and red) and absence (light grey) of variable virulence genes, phase-variable (PV) genes and absent genes among the 145 *Campylobacter* isolates and reference genomes (*C. jejuni* NCTC 11168, *C. coli* RM2228 and *C. lari* RM2100). PV genes are represented in green or red based on their ON/OFF status. The dendrogram in the left represents the similarity among isolates based on their virulence pattern and phase state of PV genes and was generated according to the result of the hierarchical clustering using the complete linkage method on the Euclidean distance matrix. Strains are colour-coded according to *Campylobacter* species, Clonal Complex and sampling location from where they were isolated. Virulence genes are highlighted by coloured boxes according to virulence factor categories. The heatmap was created using the ComplexHeatmap v.2.10.0 package (Gu et al., 2016) of the R software v.4.1.1.

while *capB* (autotransporter protein) was in the OFF state in all isolates that harboured it (19 %). Furthermore, certain genes like *cj1024c* (*flgR*) and *cgtB* were more likely to be in the ON state at rates higher than 86 %, while others, such as *cj0170/1* and *cj0628/9* (*capA*), were mainly in the OFF state and at frequencies >81 %. However, a predominance of the ON state was found among the variable PV genes among isolates along the poultry supply chain (52 %), although no significant difference was found between each stage ($p > 0.05$).

Furthermore, a total of 45 virulence-associated genes were only found in *C. jejuni*, including *capA* and *capB* (adherence), *cj1421c*, *cj1426c*, *cj1427c*, *cj1429c*, *cj1432c*, *cj1433c* and *cj1435c-cj1437c* (capsule biosynthesis and transport), *tonB2* (iron metabolism), *cgtB*, *cj1140-cj1144c/5c* and *cst-II* (lipooligosaccharide, LOS), *fspA* (motility and export apparatus), *pglG* and *wlaJ* (N-linked glycosylation), *cj1296/7*, *cj1306c*, *cj1322/3* and *cj1325/6* (O-linked flagellar glycosylation), *cj0170/1* and *rrpB* (regulation), *cj0260c*, *cj0344*, *sdhB*, *sdhC* and *cj1159c* (stress), and *tssA-tssM* (Type VI secretion system, T6SS). Although, the prevalence of virulence factors was widely distributed among the isolates per site of isolation, *cfrB* (ferric enterobactin receptor) was predominant in isolates from retail and hospital (18/19 isolates, $p < 0.001$), but was not found in any isolate from the slaughterhouse and poultry processing plant. Moreover, isolates from retail significantly possessed a reduced number of virulence-associated genes (mean = 275, range = 222–311) and PV genes (mean = 13, range = 6–23) on average compared to isolates from any other sampling location ($p < 0.05$), which showed increased number of virulence genes (mean = 287, range = 249–313) and PV genes (mean = 16, range = 7–23).

Among CCs, the greatest number of virulence genes was noted in isolates harbouring T6SS, which was significantly associated with CC464 (mean = 310, range = 308–313), CC607 (mean = 296, range = 296–297), CC460 (mean = 296, range = 296–296) ($p < 0.001$), followed by CC21 (mean = 289, range = 282–308) and CC206 (mean = 289, range = 288–289). Thus, the prevalence of virulence genes was significantly linked to bacterial lineages ($p < 0.001$). The sialyltransferase encoded by *cst-II* that determines LOS locus class A or B was significantly associated with CC48, CC206, CC460, CC464 and CC607 ($p < 0.001$), and the sialyltransferase encoded by *cst-III* that determines LOS locus class C was significantly associated with CC21 ($p < 0.001$). Similarly, the *Campylobacter* isolates that carried either *wlaN* or *cgtB* could elicit Guillain-Barré syndrome (GBS), and the presence of these genes was significantly associated with *C. jejuni* lineages, particularly CC21, CC48, CC52, CC206, CC257, CC353, CC354, CC460, CC464, CC607 and CC658 ($p < 0.001$).

3.4. Antimicrobial resistance genes

The analysis of the antimicrobial resistome revealed the presence of resistance determinants to five groups of antibiotics, including β-lactams (bla_{OXA}), tetracycline (*tetO*), (fluoro)quinolones (point mutation T86I/T86V in *gyrA*), erythromycin (point mutation A2075G in *23S rRNA*) and aminoglycosides (*aad9*, *aadE*, *aadE-Cc*, *aph(2″)-If*, *aph(3′)-IIIa*, *aph7*, *sat4* and point mutation K43R in *rpsL*), and the presence of two Cme efflux pumps (*cmeABC* and *cmeDEF*) with the *cmeR* gene (Fig. 3). Altogether 11 antimicrobial resistance (AMR) patterns were distinguished among

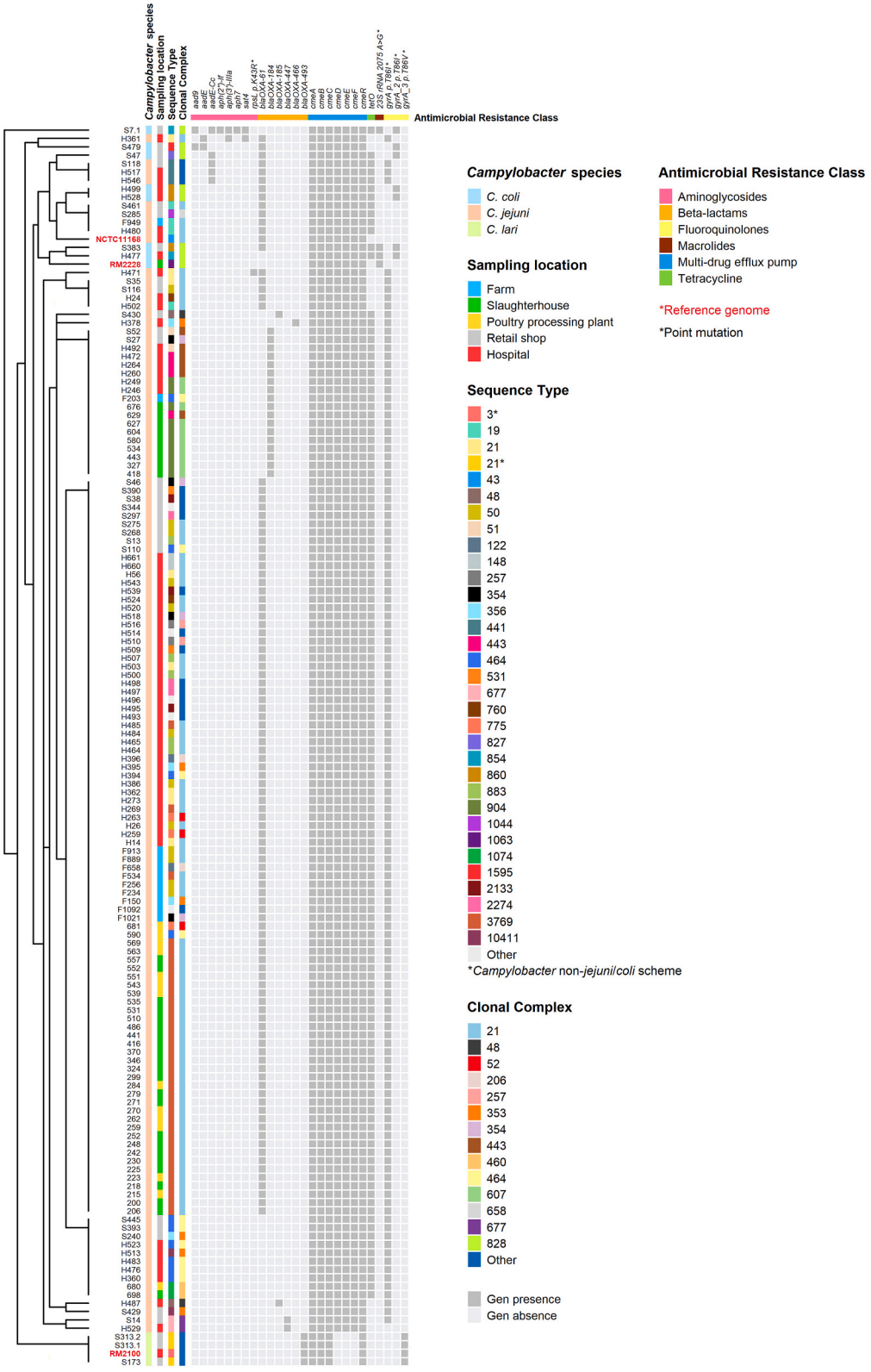

Fig. 3. Distribution of antimicrobial resistance (ARM) genes. The binary heatmap shows the presence (dark grey) and absence (light grey) of AMR genes among the 145 *Campylobacter* isolates and the reference genomes (*C. jejuni* NCTC 11168, *C. coli* RM2228 and *C. lari* RM2100). The dendrogram in the left represents the similarity among isolates based on their AMR pattern and was generated according to the result of the hierarchical clustering using the complete linkage method on the Euclidean distance matrix. Strains are colour-coded according to *Campylobacter* species, Clonal Complex and sampling location from where they were isolated. AMR genes are highlighted by coloured boxes according to AMR classes. The heatmap was created using the ComplexHeatmap v.2.10.0 package (Gu et al., 2016) of the R software v.4.1.1.

Campylobacter spp., and differences were observed between species (Table 1). *C. coli* harboured more resistance determinants against antimicrobials, especially for aminoglycosides, and showed an increased AMR diversity in proportion to *C. jejuni*, while *C. lari* possessed the lowest number of AMR markers with only the $bla_{OXA-493}$ gen, the CmeABC multidrug efflux pump with its transcriptional regulator CmeR, and the point mutation T86V in GyrA. Despite such diversity of patterns, β-LAC + TET + (F)QUI was not only predominant in *C. jejuni* (80 %) and *C. coli* (29 %), but also the most prevalent pattern in each stage of the poultry supply chain, particularly in farm, slaughterhouse and poultry processing plant accounting for >90 % of isolates each, and in hospital (75 %) (Table 1). Moreover, the diversity of AMR determinants was greater in isolates from hospital, followed by retail, than in the initial stages of the poultry supply chain (Fig. 4). Resistance to aminoglycosides was only found in isolates from retail and hospital, locations where the *tet(O)* gene was absent in certain strains.

In total, β-lactamase (bla_{OXA}) genes were present in 124*C. jejuni* isolates (92 %), five *C. coli* isolates (71 %) and *C. lari* (100 %) (Table 2). The bla_{OXA-61} gene was widely distributed among *C. jejuni* (75 %) and *C. coli* (71 %), except in CC443 and ST-904/CC607 that were linked to $bla_{OXA-184}$ (100 %), ST-48/CC48 with $bla_{OXA-185}$ (100 %), ST-677/CC677 with $bla_{OXA-447}$ (100 %), and *C. lari* with $bla_{OXA-493}$ (100 %) (Fig. 3). The tetracycline resistance (*tetO*) gene was found in *C. jejuni* (93 %) and *C. coli* (86 %). Higher prevalence of point mutations in the *gyrA* gene associated with resistance to (fluoro)quinolones were detected. While the T86I mutation in GyrA was found in *C. jejuni* (96 %) and in *C. coli* (100 %), the T86V substitution in GyrA was detected in *C. lari* (100 %). Neither bla_{OXA} gene was found in ST-1074/CC460 nor *tet(O)* in ST-677/CC677, nor *gyrA* mutations in ST-1044/CC658. Presence of erythromycin resistance determinant (*23S rRNA* A2075G) was exclusive to *C. coli* (43 %) and was combined with mutation in the DNA gyrase gene (T86I) associated with (fluoro)quinolones resistance. Moreover, nearly all isolates seemed to be sensitive to aminoglycosides as only eight isolates (five *C. jejuni* —ST-441 and ST-21/CC21— and three *C. coli* —ST-827/CC828, ST-854/CC828 and ST-1595/CC828—) acquired sporadically one or more aminoglycoside resistance determinants, except one of the two *C. coli* isolates representing ST-854 that had resistance genes to spectinomycin, streptomycin, gentamicin, kanamycin and streptothricin. Streptomycin resistance gene *aadE-Cc* was associated with *C. jejuni* ST-441 (100 %), although the eight *Campylobacter* isolates harboured some streptomycin resistance determinant (*aadE*, *aadE-Cc* or point mutation K43R in *rpsL*). While probable resistance to kanamycin and streptothricin were detected in a *C. jejuni* isolate (<1 %) and another *C. coli* (14 %), putative resistance to spectinomycin was only observed in *C. coli* (29 %). Multidrug resistance to four or more antibiotics was found in three *C. jejuni* (2 %) and three *C. coli* isolates (43 %). Furthermore, the multidrug efflux CmeABC pump, associated with an increased level of resistance towards fluoroquinolones, macrolides and tetracycline, complemented by the CmeDEF secondary efflux pump were highly predominant in the *Campylobacter* spp. isolates. Both efflux pumps were present in *C. jejuni* (100 %) and *C. coli* isolates (100 %), while *C. lari* only harboured the CmeABC efflux pump. However, *cmeR*, the transcriptional regulator of multidrug efflux CmeABC pump, was present in all isolates (100 %).

Table 1
Antimicrobial resistance (AMR) profiles for the *Campylobacter* species isolated from Spain in this study. For each AMR pattern, the number of isolates by species and sampling location is indicated when found, together with the Clonal Complexes (CCs) associated.

	AMR pattern	AMR determinant	***C. jejuni*** (***n*** = 135)						***C. coli*** (***n*** = 7)			***C. lari*** (***n*** = 3)		Clonal Complex (CC)*
			F	S	P	R	H	Total	R	H	Total	R	Total	
1	β-LAC + MEP	bla_{OXA} + cmeABC, cmeDEF, cmeR					1	1						677
2	β-LAC + MEP + TET	bla_{OXA} + cmeABC, cmeDEF, cmeR + tetO	1			2	1	4						21 (3), 658
3	β-LAC + MEP + TET + (F)QUI	bla_{OXA} + cmeABC, cmeDEF, cmeR + tetO + gyrA p.T86I	10	31	13	12	42	108		2	2			21 (60), 48, 52 (3), 206 (2), 257 (2), 353 (3), 354 (4), 443 (6), 464 (4), 607 (10), 828 (2), other (13)
4	β-LAC + MEP + (F)QUI	bla_{OXA} + cmeABC, cmeR + gyrA p.T86V										3	3	Other (3)
		bla_{OXA} + cmeABC, cmeDEF, cmeR + gyrA p.T86I				3	3	6						21 (4), 48, 677
5	AMI + β-LAC + MEP + (F)QUI	*rpsL* p.K43R + bla_{OXA} + *cmeABC*, *cmeDEF*, *cmeR* + *gyrA* p.T86I					1	1						21
		aadE, aph(3′)-IIIa, sat4 + bla_{OXA} + cmeABC, cmeDEF, cmeR + gyrA p.T86I					1	1						21
		aad9, aadE + bla_{OXA} + cmeABC, cmeDEF, cmeR + gyrA p.T86I							1		1			828
6	β-LAC + MEP + TET + ERY + (F)QUI	bla_{OXA} + *cmeABC*, *cmeDEF*, *cmeR* + *tetO* + 23S rRNA 2075 A > G + *gyrA* p.T86I							1		1			828
7	AMI + β-LAC + MEP + TET + (F)QUI	aadE-Cc + bla_{OXA} + cmeABC, cmeDEF, cmeR + tetO + gyrA p.T86I				1	2	3	1		1			828, other (3)
8	AMI + MEP + TET + ERY + (F)QUI	aad9, aadE-Cc, aph(2″)-If, aph(3′)-IIIa, aph7, sat4 + cmeABC, cmeDEF, cmeR + tetO + 23S rRNA 2075 A > G + gyrA p.T86I							1		1			828
9	MEP + (F)QUI	cmeABC, cmeDEF, cmeR + gyrA p.T86I				1		1						353
10	MEP + TET + (F)QUI	cmeABC, cmeDEF, cmeR + tetO + gyrA p.T86I		1	1	3	5	10						353 (2), 460 (2), 464 (6)
11	MEP + TET + ERY + (F)QUI	*cmeABC*, *cmeDEF*, *cmeR* + *tetO* + 23S rRNA 2075 A > G + *gyrA* p.T86I								1	1			828

AMR, antimicrobial resistance. F, farm. S, slaughterhouse. P, poultry processing plant. R, retail. H, hospital. β-LAC, beta-lactams. MEP, multidrug efflux pump. TET, tetracycline. (F)QUI, (fluoro)quinolones. AMI, aminoglycosides. ERY, erythromycin (macrolide). *In brackets: number of strains if more than one isolate was observed.

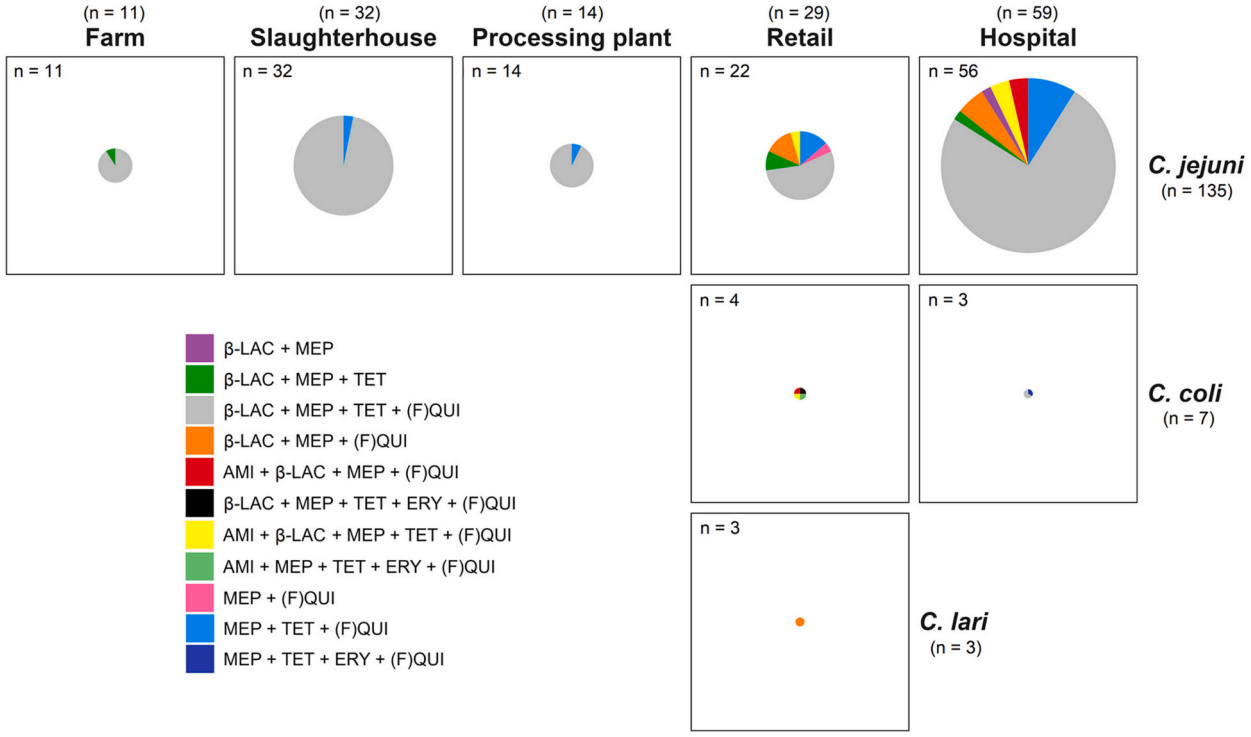

Fig. 4. Proportion of isolates displaying specific AMR patterns in each stage of the poultry supply chain within the *Campylobacter* species isolated from Spain in this study. The size of the charts is proportional to the number of isolates representing each stage for each species.

Table 2
Occurrence of isolates (%) harbouring resistance determinants conferring specific antibiotic resistance within the *Campylobacter* species isolated from Spain in this study.

Antibiotic class	*C. jejuni* (n = 135)	*C. coli* (n = 7)	*C. lari* (n = 3)
Aminoglycosides	4	43	0
β-lactams	92	71	100
Erythromycin	0	43	0
(Fluoro)quinolones	96	100	100
Tetracycline	93	86	0

4. Discussion

The study highlighted the wide diversity of Spanish *Campylobacter* strains between and within species, as previously reported Nafarrate et al. (2021). However, despite the isolates did not group into specific clusters based on the isolation source, isolates from slaughterhouse and processing plant were closely related and shared similar profiles of virulence-associated genes and antimicrobial resistance determinants. The ANI values indicated that the isolates of study were as closely related to each other as to the reference genomes, especially the *C. jejuni* and *C. coli* genomes, in a similar way to what Laconi et al. (2021) observed. Nonetheless, important differences in the gene content were demonstrated through the analysis of virulence genes and antimicrobial resistome.

Most *Campylobacter* lineages found in poultry, environment, water sources and poultry products, were also isolated from clinical isolates, except CC460 and CC658. This suggests that sources of human infection could be located anywhere leading to the existence of diverse patterns of transmission of the pathogen to humans. In agreement with many other analyses of different countries and regions, CC21 was the most common *Campylobacter* genotype, representing 18 % of all strains logged to the PubMLST database (consulted on January 4, 2023) (Rokney et al., 2018; Zhang et al., 2020). Nonetheless, although CC21 is widely distributed worldwide, in Spain <10 % of *Campylobacter* spp. accounted for this CC according to the PubMLST database, while approximately 30 % of reported isolates were grouped in CC828, which indicates that the actual number of isolates from Spain could be unregistered there. Predominant distribution of *C. coli* within CC828 has also been reported, which means that *C. coli* strains are genetically more conserved than *C. jejuni* (Duarte et al., 2014; Piccirillo et al., 2014; Ngulukun et al., 2016). Other less frequent CCs found in this study (CC257, CC353, CC354, CC443, CC464 and CC607) have also been reported in Portugal, Italy and UK (Duarte et al., 2014; Piccirillo et al., 2014; Cody et al., 2015). Of these, CC257 regarded as chicken-specific was found in isolates from clinical origin in this study, just like Piccirillo et al. (2014) observed. Therefore, although certain lineages seem to be host specific, most of *Campylobacter* lineages are widespread in the environment, supporting the ubiquitous nature of these bacteria.

A comparative virulome analysis was performed to characterize the prevalence of virulence-associated genes in the campylobacters. The wide diversity of virulence profiles found among the isolates, in which more than half of the genes analysed were common to all strains comprising up to 17 different virulence functional categories, indicates that the virulence of bacteria is a complex multifunctional mechanism involving phase-variable (PV) genes that requires specific virulence-associated genes (Bolton, 2015). Among these, data highlighted that secretion and invasion could be the key categories for the virulence of the bacteria. The Type III Secretion System (T3SS) is essential for flagella biosynthesis and required for exporting of virulence proteins, such as the *Campylobacter* invasion antigens (Cia) necessary for effective host cells invasion, but also for intracellular survival (Eucker and Konkel, 2012). High prevalence of invasion markers (87–99 %) has also been reported (Gahamanyi et al., 2021). The different virulence patterns observed were mainly the result of variation in the polysaccharide capsule, flagellar glycosylation, and LOS biosynthesis loci, as previously noticed by Fiedoruk et al. (2019).

Apart from vital virulence determinants, the absence in the isolates of study of the *ggt* gene and the Type IV Secretion System (T4SS) indicates that particular virulence factors might be dispensable for the

virulence of *Campylobacter*. The *ggt* gene encodes a gamma-glutamyl transpeptidase that promotes persistent colonization of animals and, contrary to this study, it was previously found in 23 % to 31 % *C. jejuni* isolates from humans and poultry (Floch et al., 2014; González-Hein et al., 2014). The T4SS is encoded by genes found in the plasmid *pVir* and contributes to the adhesion and invasion of intestinal epithelial cells (Bacon et al., 2000), as well as in the bacterial conjugation-dependent horizontal gene transfer. However, the plasmid *pVir* or some of the *vir* genes encoded might be likely to be absent in *Campylobacter* spp., in agreement with previous studies that also found a low prevalence of these genes (below 5 %) (Redondo et al., 2019; Panzenhagen et al., 2021). Hence, T4SS may had an unsignificant impact on the plasticity of the *Campylobacter* genomes of study delaying the dissemination of virulence and antibiotic resistance genes between strains. Therefore, it could be that this secretion system plays no role in promoting fitness and virulence of *Campylobacter* isolates from Spain.

The study corroborated the link between some virulence determinants and *Campylobacter* lineages. To CC460, CC464 and CC607, T6SS was also found to be linked to CC353 by Rokney et al. (2018), in disagreement with this study, but at similar frequency rate (17 %). Moreover, these lineages harbouring the T6SS also possessed the highest number of virulence genes due to longer genomes, which could increase their virulence. This study also highlighted the high prevalence of multiple virulence factors in *C. jejuni* in agreement with many authors (Cantero et al., 2018; Rokney et al., 2018). This suggests that the high number of virulence genes in this species may contribute to the pathogenicity of bacteria that would ultimately result in higher prevalence rates in the environment, although the specific set of genes existing only in *C. jejuni* could also be relevant. Nonetheless, more studies examining the virulence factors in species different than *C. jejuni* need to be performed as most of known virulence factors comes from *C. jejuni* and it is possible that the presence/absence of some genes in other species might have been missed (Bravo et al., 2021). Furthermore, the difference in the distribution of CRISPR-Cas system between the three species indicate that *C. jejuni* is on advantage against bacteriophages, plasmids and integrative elements, which is in accordance with a previous study reporting a higher prevalence in this species (Pearson et al., 2015). Moreover, it was demonstrated that CRISPR-Cas system does not only play a role in bacterial immune systems but also Cas9 promotes virulence and antimicrobial resistance in *C. jejuni* (Shabbir et al., 2018a; Shabbir et al., 2018b). Therefore, all this may contribute to the higher prevalence of *C. jejuni* worldwide.

The analysis of the resistome in the *Campylobacter* isolates revealed a high diversity of resistance determinants in strains from retail (from different Spain regions) and hospital, which is a cause of important concern in the region of Burgos that could also be extended to other regions in Spain due to the wide distribution of these products. Nonetheless, the lower diversity of resistomes found in the initial stages of the poultry supply chain could be the result of the lower number of genotypes representing these locations compared to retail stores or hospital, which agrees with previous studies reporting the emergence of new PFGE profiles in *C. jejuni* isolates from poultry products at retail and hospital (Melero et al., 2012; Thépault et al., 2018). Despite the chicken gut and biofilms provide suitable conditions for HGT in slaughterhouses and poultry processing facilities contributing to the spread of AMR genes (Samarth and Kwon, 2020; Ma et al., 2021), the absence of T4SS in the isolates suggests that HGT is not likely to be the process involved in dissemination of antibiotic resistance genes in Burgos, but the presence of *Campylobacter* bacteriophages, as those carrying antibiotic resistance genes persist longer in the environment than their bacterial hosts (Marti et al., 2014; Colavecchio et al., 2017). Therefore, phage-mediated transference of AMR genes could have developed the AMR diversity of *Campylobacter* in retail poultry and humans in the region. Nonetheless, retailers in Burgos could have been supplied by providers located in other areas of the country that could contribute to the higher diversity of genotypes and AMR profiles found at retail stores compared to the initial stages of the poultry supply chain, as well as consumers could have been infected after travelling outside the region, apart from the possible existence of several cross-contamination events along the chain (Domingues et al., 2012; Melero et al., 2012).

The prevalence of antibiotic resistance determinants was associated with *Campylobacter* species and lineages, particularly for bla_{OXA} genes. A higher antimicrobial resistance spectrum in *C. coli* was found in this study, that indicates that this species could be less sensible to antibiotics, while *C. lari* might be highly susceptible to antimicrobials, which could explain its lower prevalence in the poultry supply chain, together with a smaller genome and reduced virulence gene content. Previous reports showed a traditionally higher resistance rate to most antimicrobials of *C. coli* from both humans and animals (García-Sánchez et al., 2020; EFSA and ECDC, 2022b). Moreover, the $bla_{OXA-493}$ gene (resistance to β-lactams) and the T86V mutation in *gyrA* (resistance to fluoroquinolones) were exclusive to *C. lari*, just like mutations in the *23S rRNA* gene conferring erythromycin resistance in *C. coli*, while genes associated with aminoglycosides and tetracycline resistance were only found in *C. jejuni* and *C. coli*. Similar results were reported by Rivera-Mendoza et al. (2020) that highlighted the specificity of *Campylobacter* species to some of the genetic determinants associated with antibiotic resistance. Moreover, the association between different Sequence Types (STs) and AMR genes supports the idea that AMR is spread clonally (Elhadidy et al., 2018; Mouftah et al., 2021). Hence, the genomic characterization of clinical *Campylobacter* isolates through WGS may be crucial for tailoring efficient medical treatment plans for patients suffering from campylobacteriosis.

The isolates analysed here represent a subset of isolates from farm, slaughterhouse and retail whose antimicrobial susceptibility was evaluated in vitro in previous studies (García-Sánchez et al., 2018b; García-Sánchez et al., 2019; García-Sánchez et al., 2020). Frequency of AMR genes was comparable to the phenotypic data of these studies in all species, except in *C. lari*, for which resistance to tetracycline and gentamycin was encountered as opposed to this study, where no AMR determinant for these antibiotics was found. Other studies reported as well the resistance of *C. lari* isolates to tetracycline and aminoglycosides, although none of them analysed the presence of AMR markers in their genomes supporting this antibiotic resistance (Leatherbarrow et al., 2007; Murawska et al., 2022). However, all isolates presented the genes coding for the CmeABC efflux pump, that was reported to play a crucial role in conferring high-level resistance to tetracycline in *C. jejuni* isolates even when the *tet(O)* gene was present (Gibreel et al., 2007). This suggests that in *C. lari* an active CmeABC efflux pump could restore tetracycline resistance when the specific AMR determinant is absent, highlighting the improved effectiveness of this efflux pump over the Tet (O) protein. Nonetheless, more research should be done to elucidate this gap in *C. lari*.

This study confirmed the extremely high incidence of *Campylobacter* isolates in Spain harbouring resistance genes to β-lactams (> 90 %), tetracycline (> 90 %) and fluoroquinolones (> 96 %). A cross-sectional survey carried out in the region of the Basque Country in Spain showed a similar occurrence of bla_{OXA} genes in isolates from ruminants of 85 % in *C. jejuni* and 77 % in *C. coli* (Ocejo et al., 2021). Since the resistance to many β-lactam agents has been widely reported among *Campylobacter* spp. isolated from humans and poultry (Aarestrup and Engberg, 2001; Giacomelli et al., 2014; Redondo et al., 2019), β-lactams are not recommended for treating campylobacteriosis and the monitoring of β-lactams is optional in the EU since 2007 (EFSA, 2007). Likewise, a Spanish national surveillance program on AMR over the 2002–2018 period in *Campylobacter* spp. from animals, including poultry, revealed a proportion of isolates resistant to tetracycline and fluoroquinolones >80 % in both *C. jejuni* and *C. coli* (Lopez-Chavarrias et al., 2021). In *C. lari* isolates, Leatherbarrow et al. (2007) also reported maximum fluoroquinolone resistance rate (100 %), but encountered reduced resistance to ampicillin (β-lactam) (70 %). Prevalence of resistance determinants to erythromycin was only detected in *C. coli* and at levels as

high as those found in human isolates in Portugal (37 %) and Finland (43 %) (EFSA and ECDC, 2022b). Nonetheless, other studies also described resistance to erythromycin in *C. jejuni*, albeit at very low levels (3 %) (Rivera-Mendoza et al., 2020). Combined presence of erythromycin and (fluoro)quinolones determinants, which is considered critically important for treatment of human campylobacteriosis, was only found in *C. coli* (43 %), but in the clinical isolates the rate was lower (14 %) than in those isolates found in humans from Portugal (37 %) and Finland (41 %), while similarly uncommon detection was shown in *C. jejuni* isolates from humans in the EU (<1 %) (EFSA and ECDC, 2022b). Interestingly, data showed that ciprofloxacin-resistant isolates rose at a higher rate than erythromycin-resistant isolates, which is consistent with the 10,000-fold higher frequency mutations of the DNA gyrase gene associated with fluoroquinolone resistance than mutation frequency in the 23S ribosomal subunit that confers erythromycin resistance (Yan et al., 2006; Lin et al., 2007). Besides, several resistance markers associated with resistance to aminoglycosides were found in *C. jejuni* and *C. coli* with an increased occurrence of streptomycin resistance genes in both species, but a wider aminoglycoside resistance diversity in *C. coli*, which translates into higher aminoglycoside resistance in this species as previously described Lopez-Chavarrias et al. (2021). In agreement with recent studies, co-resistance to four or more antimicrobials (MDR) was higher in *C. coli* than in *C. jejuni* (Béjaoui et al., 2022; EFSA and ECDC, 2022b). Therefore, the prevalence of AMR determinants in the isolates of this study was similar to previous studies in other countries. Overall, the results of the *Campylobacter* spp. resistome indicate that the identification of species after bacterial infection is a good clinical practice for proper drug therapy in serious cases of *Campylobacter* infections, but WGS in combination with an automated and specific workflow for *Campylobacter* like CamPype (Ortega-Sanz et al., 2023b) would accelerate and optimize drug delivery to patients as part of precision medicine. Macrolides, such as erythromycin, and aminoglycoside drugs, such as gentamicin, would be more effective to treat severe human campylobacteriosis cases in Spain than common drugs like β-lactams, tetracyclines or fluoroquinolones like ciprofloxacin, whose use would be increasingly ineffective leading to higher medical costs.

Comparing the prevalence of virulence and antibiotic resistance genes between isolates per location, clinical isolates seem to be as virulent as isolates from the initial stages of the poultry supply chain (from farm to poultry processing plant) regarding their virulence gene content (i.e., number of virulence genes), despite they were clustered close to isolates from retail that could be less virulent due to their reduced virulome but similar antibiotic resistance patterns. Furthermore, no significant AMR profile was found along the whole set of isolates or among isolates from each sampling location that could be linked to potentially more virulent strains, except a possible sensitivity of *Campylobacter* isolates to aminoglycosides when the T6SS is present. However, in other cases, positive correlations between specific *Campylobacter* virulence-associated genes (*racR*, *ceuE*, *virB11* and *pldA*) and antibiotic resistance phenotypes has been described (Gharbi et al., 2022). Therefore, the multiple combinations of virulence genes, which are increased by the different ON/OFF states of those PV genes, seem to have a greater impact on the pathogenicity of the bacteria in the environment than diverse AMR profiles.

In conclusion, the present study provides valuable insights into the genomic diversity of *Campylobacter* spp. in Spain. The wide diversity of lineages between and within species was corroborated through the several virulence patterns and antimicrobial resistance profiles found, specifically in *C. jejuni*. Moreover, the mechanism underlying *Campylobacter* pathogenesis is the result of a complex combination of multifunctional virulence factors, some of which are affected by phase-variation that might increase fitness of bacteria, and antimicrobial resistance genes, that seemed to be species-specific and associated with lineages. The high rates of AMR genes observed explain the increased resistance of bacteria to drugs therapy, particularly, β-lactams, tetracycline and fluoroquinolones. Therefore, the use of WGS can provide important information for the monitoring of *Campylobacter* circulating in the country towards improved public health surveillance and the application of more effective One Health approaches, while serving as a valuable framework for future investigations.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110940.

Funding

This work was supported by "La Caixa" Foundation and Caja Burgos Foundation [LCF/PR/PR18/51130007], and the Ministry of Education of the Government of Castile and León [BU064P20]. Ortega-Sanz I received a predoctoral grant from the Junta of Castile and León, cofinanced by the Ministry of Education of the Government of Castile and León and the European Social Fund.

CRediT authorship contribution statement

Irene Ortega-Sanz: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Jordi Rovira:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Beatriz Melero:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no conflict of interest to declare.

Data availability

The draft genome sequences for all *Campylobacter* spp. strains analysed in this study are available in the European Nucleotide Archive (ENA) at EMBL-EBI under the accession numbers listed in Supplementary Table S1.

References

Aarestrup, F.M., Engberg, J., 2001. Antimicrobial resistance of thermophilic *Campylobacter*. Vet. Res. 32 (3–4), 311–321. https://doi.org/10.1051/vetres:2001127.

Aidley, J., Wanford, J.J., Green, L.R., Sheppard, S.K., Bayliss, C.D., 2018. PhasomeIt: an 'omics' approach to cataloguing the potential breadth of phase variation in the genus *Campylobacter*. Microb. Genom. 4 (11), e000228. https://doi.org/10.1099/mgen.0.000228.

Bacon, D.J., Alm, R.A., Burr, D.H., Hu, L., Kopecko, D.J., Ewing, C.P., Trust, T.J., Guerry, P., 2000. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infect. Immun. 68 (8), 4384–4390. https://doi.org/10.1128/IAI.68.8.4384-4390.200.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455–477. https://doi.org/10.1089/cmb.2012.0021.

Béjaoui, A., Gharbi, M., Bitri, S., Nasraoui, D., Ben Aziza, W., Ghedira, K., Rfaik, M., Marzougui, L., Ghram, A., Maaroufi, A., 2022. Virulence profiling, multidrug resistance and molecular mechanisms of *Campylobacter* strains from chicken carcasses in Tunisia. Antibiotics (Basel) 11 (7), 830. https://doi.org/10.3390/antibiotics11070830.

Bland, C., Ramsey, T.L., Sabree, F., Lowe, M., Brown, K., Kyrpides, N.C., Hugenholtz, P., 2007. CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. BMC Bioinform. 8, 209. https://doi.org/10.1186/1471-2105-8-209.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bolton, D.J., 2015. *Campylobacter* virulence and survival factors. Food Microbiol. 48, 99–108. https://doi.org/10.1016/j.fm.2014.11.017.

Bravo, V., Katz, A., Porte, L., Weitzel, T., Varela, C., Gonzalez-Escalona, N., Blondel, C.J., 2021. Genomic analysis of the diversity, antimicrobial resistance and virulence potential of clinical *Campylobacter jejuni* and *Campylobacter coli* strains from Chile. PLoS Negl. Trop. Dis. 15 (2), e0009207. https://doi.org/10.1371/journal.pntd.0009207.

Burnham, P.M., Hendrixson, D.R., 2018. *Campylobacter jejuni*: collective components promoting a successful enteric lifestyle. Nat. Rev. Microbiol. 16 (9), 551–565. https://doi.org/10.1038/s41579-018-0037-9.

Cantero, G., Correa-Fiz, F., Ronco, T., Strube, M., Cerdà-Cuéllar, M., Pedersen, K., 2018. Characterization of *Campylobacter jejuni* and *Campylobacter coli* broiler isolates by whole-genome sequencing. Foodborne Pathog. Dis. 15 (3), 145–152. https://doi.org/10.1089/fpd.2017.2325.

Cody, A.J., McCarthy, N.D., Bray, J.E., Wimalarathna, H.M., Colles, F.M., Jansen van Rensburg, M.J., Dingle, K.E., Waldenström, J., Maiden, M.C., 2015. Wild bird-associated *Campylobacter jejuni* isolates are a consistent source of human disease, in Oxfordshire. United Kingdom. Environ. Microbiol. Rep. 7 (5), 782–788. https://doi.org/10.1111/1758-2229.12314.

Cody, A.J., Bray, J.E., Jolley, K.A., McCarthy, N.D., Maiden, M., 2017. Core genome multilocus sequence typing scheme for stable, comparative analyses of *Campylobacter jejuni* and *C. Coli* human disease isolates. J. Clin. Microbiol. 55 (7), 2086–2097. https://doi.org/10.1128/JCM.00080-17.

Colavecchio, A., Cadieux, B., Lo, A., Goodridge, L.D., 2017. Bacteriophages contribute to the spread of antibiotic resistance genes among foodborne pathogens of the *Enterobacteriaceae* family - a review. Front. Microbiol. 8, 1108. https://doi.org/10.3389/fmicb.2017.01108.

Darling, A.E., Mau, B., Perna, N.T., 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PloS One 5 (6), e11147. https://doi.org/10.1371/journal.pone.0011147.

Dessouky, Y.E., Elsayed, S.W., Abdelsalam, N.A., Saif, N.A., Álvarez-Ordóñez, A., Elhadidy, M., 2022. Genomic insights into zoonotic transmission and antimicrobial resistance in *Campylobacter jejuni* from farm to fork: a one health perspective. Gut Pathog. 14 (1), 44. https://doi.org/10.1186/s13099-022-00517-w.

Domingues, A.R., Pires, S.M., Halasa, T., Hald, T., 2012. Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. Epidemiol. Infect. 140 (6), 970–981. https://doi.org/10.1017/S0950268811002676.

Drost, H.G., Gabel, A., Grosse, I., Quint, M., 2015. Evidence for active maintenance of phylotranscriptomic hourglass patterns in animal and plant embryogenesis. Mol. Biol. Evol. 32 (5), 1221–1231. https://doi.org/10.1093/molbev/msv012.

Duarte, A., Santos, A., Manageiro, V., Martins, A., Fraqueza, M.J., Caniça, M., Domingues, F.C., Oleastro, M., 2014. Human, food and animal *Campylobacter* spp. isolated in Portugal: high genetic diversity and antibiotic resistance rates. Int. J. Antimicrob. Agents 44 (4), 306–313. https://doi.org/10.1016/j.ijantimicag.2014.06.012.

Elhadidy, M., Miller, W.G., Arguello, H., Álvarez-Ordóñez, A., Duarte, A., Dierick, K., Botteldoorn, N., 2018. Genetic basis and clonal population structure of antibiotic resistance in *Campylobacter jejuni* isolated from broiler carcasses in Belgium. Front. Microbiol. 9, 1014. https://doi.org/10.3389/fmicb.2018.01014.

Eucker, T.P., Konkel, M.E., 2012. The cooperative action of bacterial fibronectin-binding proteins and secreted proteins promote maximal *Campylobacter jejuni* invasion of host cells by stimulating membrane ruffling. Cell. Microbiol. 14 (2), 226–238. https://doi.org/10.1111/j.1462-5822.2011.01714.x.

European Food Safety Authority (EFSA), 2007. Report of the task force on zoonoses data collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (Gallus gallus), turkeys and pigs and *Campylobacter jejuni* and *C. coli* in broilers. EFSA J. 96, 1–4. https://doi.org/10.2903/j.efsa.2007.96r.

European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), 2022a. The European Union one health 2021 zoonoses report. EFSA J. 20 (12), e07666. https://doi.org/10.2903/j.efsa.2022.7666.

European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), 2022b. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA J. 20 (3), 7209. https://doi.org/10.2903/j.efsa.2022.7209.

Fiedoruk, K., Daniluk, T., Rozkiewicz, D., Oldak, E., Prasad, S., Swiecicka, I., 2019. Whole-genome comparative analysis of *Campylobacter jejuni* strains isolated from patients with diarrhea in northeastern Poland. Gut Pathog. 11, 32. https://doi.org/10.1186/s13099-019-0313-x.

Floch, P., Pey, V., Castroviejo, M., Dupuy, J.W., Bonneu, M., de la Guardia, A.H., Pitard, V., Mégraud, F., Lehours, P., 2014. Role of *Campylobacter jejuni* gamma-glutamyl transpeptidase on epithelial cell apoptosis and lymphocyte proliferation. Gut Pathog. 6, 20. https://doi.org/10.1186/1757-4749-6-20.

Gahamanyi, N., Song, D.G., Yoon, K.Y., Mboera, L.E.G., Matee, M.I., Mutangana, D., Amachawadi, R.G., Komba, E.V.G., Pan, C.H., 2021. Antimicrobial resistance profiles, virulence genes, and genetic diversity of thermophilic *Campylobacter* species isolated from a layer poultry farm in Korea. Front. Microbiol. 12, 622275. https://doi.org/10.3389/fmicb.2021.622275.

García-Sánchez, L., Melero, B., Jaime, I., Hänninen, M.L., Rossi, M., Rovira, J., 2017. *Campylobacter jejuni* survival in a poultry processing plant environment. Food Microbiol. 65, 185–192. https://doi.org/10.1016/j.fm.2017.02.009.

García-Sánchez, L., Melero, B., Rovira, J., 2018a. *Campylobacter* in the food chain. In: Rodríguez-Lázaro, D. (Ed.), Advances in Food and Nutrition Research, vol. 86. Academic Press, Cambridge, pp. 215–252.

García-Sánchez, L., Melero, B., Diez, A.M., Jaime, I., Rovira, J., 2018b. Characterization of *Campylobacter* species in Spanish retail from different fresh chicken products and their antimicrobial resistance. Food Microbiol. 76, 457–465. https://doi.org/10.1016/j.fm.2018.07.004.

García-Sánchez, L., Melero, B., Jaime, I., Rossi, M., Ortega, I., Rovira, J., 2019. Biofilm formation, virulence and antimicrobial resistance of different *Campylobacter jejuni* isolates from a poultry slaughterhouse. Food Microbiol. 83, 193–199. https://doi.org/10.1016/j.fm.2019.05.016.

García-Sánchez, L., Melero, B., Diez, A.M., Jaime, I., Canepa, A., Rovira, J., 2020. Genotyping, virulence genes and antimicrobial resistance of *Campylobacter* spp. isolated during two seasonal periods in Spanish poultry farms. Prev. Vet. Med. 176, 104935. https://doi.org/10.1016/j.prevetmed.2020.104935.

Gharbi, M., Kamoun, S., Hkimi, C., Ghedira, K., Béjaoui, A., Maaroufi, A., 2022. Relationships between virulence genes and antibiotic resistance phenotypes/genotypes in *Campylobacter* spp. isolated from layer hens and eggs in the north of Tunisia: statistical and computational insights. Foods 11 (22), 3554. https://doi.org/10.3390/foods11223554.

Giacomelli, M., Salata, C., Martini, M., Montesissa, C., Piccirillo, A., 2014. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from poultry in Italy. Microb. Drug Resist. 20 (2), 181–188. https://doi.org/10.1089/mdr.2013.0110.

Gibreel, A., Wetsch, N.M., Taylor, D.E., 2007. Contribution of the CmeABC efflux pump to macrolide and tetracycline resistance in *Campylobacter jejuni*. Antimicrob. Agents Chemother. 51 (9), 3212–3216. https://doi.org/10.1128/AAC.01592-06.

González-Hein, G., Huaracán, B., García, P., Figueroa, G., 2014. Prevalence of virulence genes in strains of *Campylobacter jejuni* isolated from human, bovine and broiler. Braz. J. Microbiol. 44 (4), 1223–1229. https://doi.org/10.1590/s1517-83822013000400028.

Gu, Z., Eils, R., Schlesner, M., 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32 (18), 2847–2849. https://doi.org/10.1093/bioinformatics/btw313.

Gupta, S.K., Padmanabhan, B.R., Diene, S.M., Lopez-Rojas, R., Kempf, M., Landraud, L., Rolain, J.M., 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob. Agents Chemother. 58 (1), 212–220. https://doi.org/10.1128/AAC.01310-13.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29 (8), 1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

Joensen, K.G., Schjørring, S., Gantzhorn, M.R., Vester, C.T., Nielsen, H.L., Engberg, J.H., Holt, H.M., Ethelberg, S., Müller, L., Sandø, G., Nielsen, E.M., 2021. Whole genome sequencing data used for surveillance of *Campylobacter* infections: detection of a large continuous outbreak, Denmark, 2019. Euro Surveill. 26 (22), 2001396. https://doi.org/10.2807/1560-7917.ES.2021.26.22.2001396.

Jolley, K.A., Bray, J.E., Maiden, M.C.J., 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 3, 124. https://doi.org/10.12688/wellcomeopenres.14826.1.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Laconi, A., Drigo, I., Palmieri, N., Carraro, L., Tonon, E., Franch, R., Bano, L., Piccirillo, A., 2021. Genomic analysis of extra-intestinal *Campylobacter jejuni* and *Campylobacter coli* isolated from commercial chickens. Vet. Microbiol. 259, 109161. https://doi.org/10.1016/j.vetmic.2021.109161.

Lakin, S.M., Dean, C., Noyes, N.R., Dettenwanger, A., Ross, A.S., Doster, E., Rovira, P., Abdo, Z., Jones, K.L., Ruiz, J., Belk, K.E., Morley, P.S., Boucher, C., 2017. MEGARes: an antimicrobial resistance database for high throughput sequencing. Nucleic Acids Res. 45 (D1), D574–D580. https://doi.org/10.1093/nar/gkw1009.

Leatherbarrow, A.J., Griffiths, R., Hart, C.A., Kemp, R., Williams, N.J., Diggle, P.J., Wright, E.J., Sutherst, J., Houghton, P., French, N.P., 2007. *Campylobacter lari*: genotype and antibiotic resistance of isolates from cattle, wildlife and water in an area of mixed dairy farmland in the United Kingdom. Environ. Microbiol. 9 (7), 1772–1779. https://doi.org/10.1111/j.1462-2920.2007.01295.x.

Letunic, I., Bork, P., 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49 (W1), W293–W296. https://doi.org/10.1093/nar/gkab301.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997. doi:10.48550/arXiv.1303.3997.

Lin, J., Yan, M., Sahin, O., Pereira, S., Chang, Y.J., Zhang, Q., 2007. Effect of macrolide usage on emergence of erythromycin-resistant *Campylobacter* isolates in chickens. Antimicrob. Agents Chemother. 51 (5), 1678–1686. https://doi.org/10.1128/AAC.01411-06.

Lopez-Chavarrias, V., Ugarte-Ruiz, M., Barcena, C., Olarra, A., Garcia, M., Saez, J.L., de Frutos, C., Serrano, T., Perez, I., Moreno, M.A., Dominguez, L., Alvarez, J., 2021. Monitoring of antimicrobial resistance to aminoglycosides and macrolides in *Campylobacter coli* and *Campylobacter jejuni* from healthy livestock in Spain (2002-2018). Front. Microbiol. 12, 689262. https://doi.org/10.3389/fmicb.2021.689262.

Ma, L., Konkel, M.E., Lu, X., 2021. Antimicrobial resistance gene transfer from *Campylobacter jejuni* in mono- and dual-species biofilms. Appl. Environ. Microbiol. 87 (15), e0065921. https://doi.org/10.1128/AEM.00659-21.

Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27 (21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.

Marti, E., Variatza, E., Balcázar, J.L., 2014. Bacteriophages as a reservoir of extended-spectrum β-lactamase and fluoroquinolone resistance genes in the environment. Clin. Microbiol. Infect. 20 (7), O456–O459. https://doi.org/10.1111/1469-0691.12446.

McArthur, A.G., Waglechner, N., Nizam, F., Yan, A., Azad, M.A., Baylay, A.J., Bhullar, K., Canova, M.J., De Pascale, G., Ejim, L., Kalan, L., King, A.M., Koteva, K., Morar, M., Mulvey, M.R., O'Brien, J.S., Pawlowski, A.C., Piddock, L.J., Spanogiannopoulos, P., Sutherland, A.D., Tang, I., Taylor, P.L., Thaker, M., Wang, W., Yan, M., Yu, T., Wright, G.D., 2013. The comprehensive antibiotic resistance database. Antimicrob. Agents Chemother. 57 (7), 3348–3357. https://doi.org/10.1128/AAC.00419-13.

McLure, A., Smith, J.J., Firestone, S.M., Kirk, M.D., French, N., Fearnley, E., Wallace, R., Valcanis, M., Bulach, D., Moffatt, C.R.M., Selvey, L.A., Jennison, A., Cribb, D.M., Glass, K., 2023. Source attribution of campylobacteriosis in Australia, 2017-2019. Risk Anal. 43, 2527–2548. https://doi.org/10.1111/risa.14138.

Mehat, J.W., La Ragione, R.M., van Vliet, A.H.M., 2020. *Campylobacter jejuni* and *Campylobacter coli* autotransporter genes exhibit lineage-associated distribution and decay. BMC Genomics 21 (1), 314. https://doi.org/10.1186/s12864-020-6704-z.

Melero, B., Juntunen, P., Hänninen, M.L., Jaime, I., Rovira, J., 2012. Tracing *Campylobacter jejuni* strains along the poultry meat production chain from farm to retail by pulsed-field gel electrophoresis, and the antimicrobial resistance of isolates. Food Microbiol. 32 (1), 124–128. https://doi.org/10.1016/j.fm.2012.04.020.

Mouftah, S.F., Cobo-Díaz, J.F., Álvarez-Ordóñez, A., Elserafy, M., Saif, N.A., Sadat, A., El-Shibiny, A., Elhadidy, M., 2021. High-throughput sequencing reveals genetic determinants associated with antibiotic resistance in *Campylobacter* spp. from farm-to-fork. PloS One 16 (6), e0253797. https://doi.org/10.1371/journal.pone.0253797.

Murawska, M., Sypecka, M., Bartosik, J., Kwiecień, E., Rzewuska, M., Sałamaszyńska-Guz, A., 2022. Should we consider them as a threat? Antimicrobial resistance, virulence potential and genetic diversity of *Campylobacter* spp. isolated from Varsovian dogs. Antibiotics (Basel) 11 (7), 964. https://doi.org/10.3390/antibiotics11070964.

Nafarrate, I., Lasagabaster, A., Sevillano, E., Mateo, E., 2021. Prevalence, molecular typing and antimicrobial susceptibility of *Campylobacter* spp. isolates in northern Spain. J. Appl. Microbiol. 130 (4), 1368–1379. https://doi.org/10.1111/jam.14842.

Ngulukun, S., Oboegbulem, S., Klein, G., 2016. Multilocus sequence typing of *Campylobacter jejuni* and *Campylobacter coli* isolates from poultry, cattle and humans in Nigeria. J. Appl. Microbiol. 121 (2), 561–568. https://doi.org/10.1111/jam.13185.

Ocejo, M., Oporto, B., Lavín, J.L., Hurtado, A., 2021. Whole genome-based characterisation of antimicrobial resistance and genetic diversity in *Campylobacter jejuni* and *Campylobacter coli* from ruminants. Sci. Rep. 11 (1), 8998. https://doi.org/10.1038/s41598-021-88318-0.

Ortega-Sanz, I., García, M., Bocigas, C., Megías, G., Melero, B., Rovira, J., 2023a. Genomic characterization of *Campylobacter jejuni* associated with perimyocarditis: a family case report. Foodborne Pathog. Dis. 20 (8), 368–373. https://doi.org/10.1089/fpd.2023.0010.

Ortega-Sanz, I., Barbero-Aparicio, J.A., Canepa-Oneto, A., Rovira, J., Melero, B., 2023b. CamPype: an open-source workflow for automated bacterial whole-genome sequencing analysis focused on *Campylobacter*. BMC Bioinform. 24 (1), 291. https://doi.org/10.1186/s12859-023-05414-w.

Ortega-Sanz, I., Bocigas, C., Melero, B., Rovira, J., 2024. Phase variation modulates the multi-phenotypes displayed by clinical *Campylobacter jejuni* strains. Food Microbiol. 117, 104397. https://doi.org/10.1016/j.fm.2023.104397.

Panzenhagen, P., Portes, A.B., Dos Santos, A.M.P., Duque, S.D.S., Conte Junior, C.A., 2021. The distribution of *Campylobacter jejuni* virulence genes in genomes worldwide derived from the NCBI pathogen detection database. Genes 12 (10), 1538. https://doi.org/10.3390/genes12101538.

Pearson, B.M., Louwen, R., van Baarlen, P., van Vliet, A.H., 2015. Differential distribution of type II CRISPR-Cas systems in agricultural and nonagricultural *Campylobacter coli* and *Campylobacter jejuni* isolates correlates with lack of shared environments. Genome Biol. Evol. 7 (9), 2663–2679. https://doi.org/10.1093/gbe/evv174.

Piccirillo, A., Giacomelli, M., Salata, C., Bettanello, S., De Canale, E., Palù, G., 2014. Multilocus sequence typing of *Campylobacter jejuni* and *Campylobacter coli* from humans and chickens in North-Eastern Italy. New Microbiol. 37 (4), 557–562.

Pritchard, L., Glover, R.H., Humphris, S., Elphinstone, J.G., Toth, I.K., 2016. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. Anal. Methods 8, 12–14. https://doi.org/10.1039/C5AY02550H.

Qin, X., Wang, X., Shen, Z., 2023. The rise of antibiotic resistance in *Campylobacter*. Curr. Opin. Gastroenterol. 39 (1), 9–15. https://doi.org/10.1097/MOG.0000000000000901.

Redondo, N., Carroll, A., McNamara, E., 2019. Molecular characterization of *Campylobacter* causing human clinical infection using whole-genome sequencing: virulence, antimicrobial resistance and phylogeny in Ireland. PloS One 14 (7), e0219088. https://doi.org/10.1371/journal.pone.0219088.

Rivera-Mendoza, D., Martínez-Flores, I., Santamaría, R.I., Lozano, L., Bustamante, V.H., Pérez-Morales, D., 2020. Genomic analysis reveals the genetic determinants associated with antibiotic resistance in the zoonotic pathogen *Campylobacter* spp. distributed globally. Front. Microbiol. 11, 513070. https://doi.org/10.3389/fmicb.2020.513070.

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., Mesirov, J.P., 2011. Integrative genomics viewer. Nat. Biotechnol. 29 (1), 24–26. https://doi.org/10.1038/nbt.1754.

Rokney, A., Valinsky, L., Moran-Gilad, J., Vranckx, K., Agmon, V., Weinberger, M., 2018. Genomic epidemiology of *Campylobacter jejuni* transmission in Israel. Front. Microbiol. 9, 2432. https://doi.org/10.3389/fmicb.2018.02432.

Samarth, D.P., Kwon, Y.M., 2020. Horizontal genetic exchange of chromosomally encoded markers between *Campylobacter jejuni* cells. PloS One 15 (10), e0241058. https://doi.org/10.1371/journal.pone.0241058.

Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27 (6), 863–864. https://doi.org/10.1093/bioinformatics/btr026.

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30 (14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

Shabbir, M.A., Wu, Q., Shabbir, M.Z., Sajid, A., Ahmed, S., Sattar, A., Tang, Y., Li, J., Maan, M.K., Hao, H., Yuan, Z., 2018a. The CRISPR-cas system promotes antimicrobial resistance in *Campylobacter jejuni*. Future Microbiol. 13, 1757–1774. https://doi.org/10.2217/fmb-2018-023.

Shabbir, M.A.B., Tang, Y., Xu, Z., Lin, M., Cheng, G., Dai, M., Wang, X., Liu, Z., Yuan, Z., Hao, H., 2018b. The involvement of the Cas9 gene in virulence of *Campylobacter jejuni*. Front. Cell. Infect. Microbiol. 8, 285. https://doi.org/10.3389/fcimb.2018.00285.

Sheppard, S.K., Cheng, L., Méric, G., de Haan, C.P., Llarena, A.K., Marttinen, P., Vidal, A., Ridley, A., Clifton-Hadley, F., Connor, T.R., Strachan, N.J., Forbes, K., Colles, F.M., Jolley, K.A., Bentley, S.D., Maiden, M.C., Hänninen, M.L., Parkhill, J., Hanage, W.P., Corander, J., 2014. Cryptic ecology among host generalist *Campylobacter jejuni* in domestic animals. Mol. Ecol. 23 (10), 2442–2451. https://doi.org/10.1111/mec.12742.

Shin, E., Oh, Y., Kim, M., Jung, J., Lee, Y., 2013. Antimicrobial resistance patterns and corresponding multilocus sequence types of the *Campylobacter jejuni* isolates from human diarrheal samples. Microb. Drug Resist. 19 (2), 110–116. https://doi.org/10.1089/mdr.2012.0099.

Silva, M., Machado, M.P., Silva, D.N., Rossi, M., Moran-Gilad, J., Santos, S., Ramirez, M., Carriço, J.A., 2018. chewBBACA: a complete suite for gene-by-gene schema creation and strain identification. Microb. Genom. 4 (3), e000166. https://doi.org/10.1099/mgen.0.000166.

Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., Zdobnov, E.M., 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31 (19), 3210–3212. https://doi.org/10.1093/bioinformatics/btv351.

Thépault, A., Poezevara, T., Quesne, S., Rose, V., Chemaly, M., Rivoal, K., 2018. Prevalence of thermophilic *Campylobacter* in cattle production at slaughterhouse level in France and link between *C. Jejuni* bovine strains and campylobacteriosis. Front. Microbiol. 9, 471. https://doi.org/10.3389/fmicb.2018.00471.

Wirz, S.E., Overesch, G., Kuhnert, P., Korczak, B.M., 2010. Genotype and antibiotic resistance analyses of *Campylobacter* isolates from ceca and carcasses of slaughtered broiler flocks. Appl. Environ. Microbiol. 76 (19), 6377–6386. https://doi.org/10.1128/AEM.00813-10.

Yan, M., Sahin, O., Lin, J., Zhang, Q., 2006. Role of the CmeABC efflux pump in the emergence of fluoroquinolone-resistant *Campylobacter* under selection pressure. J. Antimicrob. Chemother. 58 (6), 1154–1159. https://doi.org/10.1093/jac/dkl412.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67 (11), 2640–2644. https://doi.org/10.1093/jac/dks261.

Zankari, E., Allesøe, R., Joensen, K.G., Cavaco, L.M., Lund, O., Aarestrup, F.M., 2017. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. J. Antimicrob. Chemother. 72 (10), 2764–2768. https://doi.org/10.1093/jac/dkx217.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7 (1–2), 203–214. https://doi.org/10.1089/10665270050081478.

Zhang, P., Zhang, X., Liu, Y., Jiang, J., Shen, Z., Chen, Q., Ma, X. (2020). Multilocus sequence types and antimicrobial resistance of *Campylobacter jejuni* and *C. coli* isolates of human patients from Beijing, China, 2017-2018. Front. Microbiol., 11, 554784. doi:https://doi.org/10.3389/fmicb.2020.554784.