

Contents lists available at ScienceDirect

Current Research in Food Science



journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Genomic characterization of molecular markers associated with antimicrobial resistance and virulence of the prevalent *Campylobacter coli* isolated from retail chicken meat in the United Arab Emirates



Ihab Habib^{a,b,*}, Mohamed-Yousif Ibrahim Mohamed^a, Akela Ghazawi^c, Glindya Bhagya Lakshmi^a, Mushtaq Khan^c, Dan Li^d, Shafi Sahibzada^{e,f}

^a Veterinary Public Health Research Laboratory, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al

^c Department of Medical Microbiology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates

^d Department of Food Science and Technology, National University of Singapore, Singapore

^e Antimicrobial Resistance and Infectious Diseases Laboratory, Murdoch University, Australia

^f CSIRO, Australian Centre for Disease Preparedness, Geelong, VIC, Australia

ARTICLE INFO

Handling Editor: Siyun Wang

Keywords: Campylobacter spp Poultry Antibiotic resistance Whole-genome sequencing UAE

ABSTRACT

Campylobacter is a major cause of gastroenteritis worldwide, with broiler meat accounting for most illnesses. Antimicrobial intervention is recommended in severe cases of campylobacteriosis. The emergence of antimicrobial resistance (AMR) in Campylobacter is a concerning food safety challenge, and monitoring the trends of AMR is vital for a better risk assessment. This study aimed to characterize the phenotypic profiles and molecular markers of AMR and virulence in the prevalent Campylobacter species contaminating chilled chicken carcasses sampled from supermarkets in the United Arab Emirates (UAE). Campylobacter was detected in 90 (28.6%) out of 315 tested samples, and up to five isolates from each were confirmed using multiplex PCR. The species C. coli was detected in 83% (75/90) of the positive samples. Whole-genome sequencing was used to characterize the determinants of AMR and potential virulence genes in 45 non-redundant C. coli isolates. We identified nine resistance genes, including four associated with resistance to aminoglycoside (aph(3')-III, ant(6)-Ia, aph(2")-Ib, and aac(6')-Im), and three associated with Beta-lactam resistance (blaOXA-61, blaOXA-193, and blaOXA-489), and two linked to tetracycline resistance (tet(O/32/O), and tet(O)), as well as point mutations in gyrA (fluoroquinolones resistance), 23S rRNA (macrolides resistance), and rpsL (streptomycin resistance) genes. A mutation in gyrA 2 p.T86I, conferring resistance to fluoroquinolones, was detected in 93% (42/45) of the isolates and showed a perfect match with the phenotype results. The simultaneous presence of blaOXA-61 and blaOXA-193 genes was identified in 86.6% (39/45) of the isolates. In silico analysis identified 7 to 11 virulence factors per each C. coli isolate. Some of these factors were prevalent in all examined strains and were associated with adherence (cadF, and jlpA), colonization and immune evasion (capsule biosynthesis and transport, lipooligosaccharide), and invasion (ciaB). This study provides the first published evidence from the UAE characterizing Campylobacter virulence, antimicrobial resistance genotype, and phenotype analysis from retail chicken. The prevalent C. coli in the UAE retail chicken carries multiple virulence genes and antimicrobial resistance markers and exhibits frequent phenotype resistance to macrolides, quinolones, and tetracyclines. The present investigation adds to the current knowledge on molecular epidemiology and AMR development in non-jejuni Campylobacter species in the Middle East and globally.

https://doi.org/10.1016/j.crfs.2023.100434

Received 26 September 2022; Received in revised form 24 November 2022; Accepted 2 January 2023 Available online 3 January 2023 2665-9271/© 2023 The Authors, Published by Elsevier B.V. This is an open access article under the CC BY-NC

Ain, United Arab Emirates

^b Department of Environmental Health, High Institute of Public Health, Alexandria University, Alexandria, Egypt

^{*} Corresponding author. Veterinary Public Health Research Laboratory, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain, United Arab Emirates.

E-mail address: i.habib@uaeu.ac.ae (I. Habib).

^{2665-9271/© 2023} The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Campylobacter is among the key foodborne bacterial pathogens causing gastroenteritis worldwide, with about one hundred million cases of foodborne illness per year (Majowicz et al., 2020). The species *Campylobacter jejuni* and *Campylobacter coli* are commonly associated with human illnesses, with an estimated infectious dose of just a few hundred bacterial cells (Asuming-Bediako et al., 2019). *Campylobacter* colonizes the gut of broiler chicken and can be transferred to the carcass during slaughter processing and spread further to retail chicken meat. Cross-contamination arising from poor hygienic handling of raw chicken is considered the leading risk factor impacting human exposure to *Campylobacter* (Henry et al., 2011; Asuming-Bediako et al., 2019).

Campylobacter harbors a sophisticated set of virulence and fitness determinants that facilitate host invasion and avoidance (adhesion, survival inside the epithelial cells of the human intestine, and invasion) (Bolton, 2015). Other factors include flagella, glycosylation of flagellins, capsular polysaccharide, lipooligosaccharide, and genotypic diversity owing to several phase-variable loci (Nauta et al., 2009). In severe cases of campylobacteriosis, ciprofloxacin (fluoroquinolone) and erythromycin (macrolide) are recommended as the first choice-antimicrobial treatment (Asuming-Bediako et al., 2019). However, there is a growing trend in many regions worldwide in Campylobacter resistance to antimicrobials, notably against fluoroquinolones in C. jejuni and erythromycin in C. coli (Asakura et al., 2019; Asuming-Bediako et al., 2022). The acquisition of various determinants mediates antimicrobial resistance in Campylobacter, and genetic mechanisms is related to a mixture of inherent and acquired mechanisms, such as resistance genes, point mutations, and efflux pumps (Asakura et al., 2019).

In the Gulf Cooperation Council (GCC) countries, 2-28% of human diarrheal cases are attributed to infection with Campylobacter (Kaakoush et al., 2015). Nevertheless, there is currently minimal surveillance data for Campylobacter at the human-food interface across the GCC nations. Worldwide, the United Arab Emirates (UAE) is a significant consumer of local and imported chicken meat, with ~60 kg/capita a year (USDA, 2021). The broiler industry has been expanding in recent years in the UAE and is foreseen as a growing sector to aid with the food security gap in the country (USDA, 2021). However, currently, there is limited research in the UAE on the status of microbial contamination in chicken meat (Habib et al., 2021). No baseline studies in the UAE have investigated the extent of antimicrobial resistance of Campylobacter in the broiler meat chain (Habib et al., 2021). Such baseline data are critical for establishing a national program to control the foodborne transmission of Campylobacter and are fundamental for surveillance AMR from a One Health perspective.

Because of the public health and food safety importance of *Campylobacter*, we aimed in this study to: (i) determine the prevalence of *Campylobacter* spp. (referring to the thermo-tolerant species *C. jejuni* and *C. coli*) in retail chilled whole chicken carcasses in the UAE; (ii) analyze the phenotypic resistance to clinically important antibiotics, and to characterize using whole-genome sequencing the determinants conferring AMR and virulence markers in a selection of the prevalent *Campylobacter* species presented in the UAE retail chicken meat.

2. Materials and methods

2.1. Sampling and survey

Between March to December 2021, a total of 315 whole chicken carcasses were purchased from the chilled display of various (n = 26) supermarkets in the UAE. The samples were obtained on two monthly occasions (15 per each). We targeted (after deliberation with national food control bodies) samples from seven different brands (denoted anonymously as A to G). These brands included major national processors (all from broiler flocks raised in the UAE), supplying more than 75% of the chilled chicken to the UAE retail. None of the seven

processors produce organic chicken (all are from conventional production systems). All samples were collected from chilled supermarkets display, where all the carcasses were packed and labeled by brand. Samples were shipped for testing on the same day in chilled containers, and all microbiological testing was achieved within 6 h of collection.

2.2. Campylobacter isolation and species confirmation

Standard Campylobacter enumeration was carried out using a modification of the ISO 10272:2006 method (Habib et al., 2011). This study adopted the enumeration method to generate quantitative data to support future risk assessment for Campylobacter contamination in chicken meat in the UAE. For the tested whole carcasses, the sample was excised from the neck skin. Neck skin is one of the most positive carcass sites for detecting Campylobacter (Baré et al., 2013). A 10 g of neck skin was mixed with 90 ml (nine volumes) of 0.1% peptone water (Oxoid, Basingstoke, England) and homogenized for 1 min in a bag-mixer blender. From this sample homogenate (10^{-1}) , a volume of 1 ml was spread plated (0.3, 0.3, 0.3, and 0.1 ml) over four modified charcoal cefoperazone deoxycholate agar plates (mCCDA) (Oxoid, Basingstoke, England). The enumeration procedures of Campylobacter using 1 ml of the initial homogenate over 3 or 4 plates has been utilized by several studies in order to improve the chance of Campylobacter enumeration, notably when the samples carry low loads (Habib et al., 2019). A further (10^{-2}) serial dilution was done in peptone water, of which 0.1 ml was spread over the surface of mCCDA. Plates were incubated micro-aerobically by introducing sachets of CampyGen (Oxoid) in a rectangular jar (2.5 L capacity). All plates were incubated at 41.5 °C and counted after 48 h.

Up to five presumptive colonies per sample were selected according to their colony morphology and stored at -80 °C for further confirmation. DNA extraction was achieved from an 18–24 h culture of the suspected colonies using a commercial kit (Wizard®, Promega, USA). A multiplex PCR assay amplifying 16S rRNA, *mapA*, and *ceuE* genes was used to confirm the genus as *Campylobacter* and distinguish between the thermo-tolerant species *C. jejuni* and *C. coli*. The PCR conditions and oligonucleotides were described previously by Denis et al. (1999).

2.3. Whole-genome sequencing and bioinformatic analysis

A subset of 45 non-redundant C. coli isolates (one isolate per positive sample from unrelated batches) was characterized further at the genome level. WGS was carried out using short reads technology (Illumina, NovaSeq) through a commercial service (Novogene, the United Kingdom). De Novo assembly was carried out using SPADES (https://cge.cbs.dtu.dk/services/SPAdes/) and assembled into contigs. The assembled contigs were uploaded to PathogenWatch (https://patho gen.watch) (accessed May 20, 2022) to confirm species identification. The genomes that passed the quality tests were used to predict resistance genes via NCBI Antimicrobial Resistance Gene Finder (AMRFinder; https://github.com/ncbi/amr), and virulence genes were detected via ABRicate (https://github.com/tseemann/abricate), and by using the REsfinder (https://cge.food.dtu.dk/services/ResFinder/) and Virulence finder (https://cge.food.dtu.dk/services/VirulenceFinder/) databases. Point mutations in specific genes conferring antimicrobial resistance were examined using SSI wrapper for calling and parsing the output of KMA (Kmer Aligner) for finding point mutations (https://github.com/ ssi-dk/punktreskma). Hits were considered only for gene identities \geq 60% and lengths \geq 90% (Dahl et al., 2021).

All read data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive, and the whole-genome-sequenced *Campylobacter coli* can be accessed under BioProject number PRJNA897955 (accession numbers are in continuous serial between SAMN31596899 and SAMN31596941). Novel sequence types (STs) were registered and assigned identification numbers through the designated multilocus

sequence types (MLST) database of *Campylobacter* (PubMLST; https://pubmlst.org/organisms/campylobacter-jejunicoli).

2.4. Antimicrobial resistance

For isolates characterized by WGS, antimicrobial resistance was evaluated using minimum inhibitory concentration (MIC) (EUCAMP2 plates; Thermo Scientific, USA). The following antibiotics (and dilution ranges) were evaluated in the panel: gentamicin (GEN; 0.12–16 mg/L), ciprofloxacin (CIP; 0.12–16 mg/L), nalidixic acid (NAL; 1–64 mg/L), tetracycline (TET; 0.5–64 mg/L), streptomycin (STR; 0.25–16 mg/L), and erythromycin (ERY; 1–128 mg/L). MIC classification was interpreted as wild-type (susceptible) or non-wild-type (resistant) using the epidemiological cutoff (ECOFF) defined by European Committee on Antimicrobial Susceptibility (EUCAST) (EFSA et al., 2022). The relatedness between AMR genotypes and phenotypes was calculated by dividing the count of resistant isolates based on their genotype by the count of isolates that showed phenotypic resistance (Dahl et al., 2021).

3. Analysis of data

Campylobacter detection frequencies across the different retail brands were compared using descriptive analysis and logistic regression procedures. Differences with *P* values less than 0.05 were considered significant. All analyses were done using the STATA software, version 16.0 (STATA Corporation. 2020).

4. Results and discussion

The study provides the first insight into the genomic characterization of antimicrobial resistance and virulence markers in *Campylobacter* isolated from retail chilled chicken carcasses from supermarkets in the UAE, a country among the biggest markets for per capita chicken meat consumption (USDA, 2021).

4.1. Overall campylobacter detection in chilled chicken carcasses

Campylobacter isolates were recovered from 90 of 315 chilled whole chicken carcasses. Results in Fig. 1 shows evident variation across the seven brands regarding the recovery of *Campylobacter* among their samples. Of note, all (n = 30) carcasses from Company F were *Campylobacter*-negative, while samples from company G (n = 30) showed a significantly higher rate (logistic-regression odds ratio [OR] = 11.4, *p-value* < 0.0001) of *Campylobacter* detection.

Lower recovery (28.6%) of Campylobacter in chicken carcasses was



Fig. 1. Prevalence of *Campylobacter* in retail chilled carcasses representing seven brands (denoted A to G) for sale in the United Arab Emirates supermarkets.

observed in our study compared to other Gulf Cooperation Council countries in the region, such as Qatar (36.5%) and Saudi Arabia (52.2%) (Mohammed et al., 2015; Alarjani et al., 2021). The recovery of *Campylobacter* from supermarket broiler carcasses based on the direct plating methods has been hypothesized to be lower as the bacterial load fall below the limit of detection of the plate count method (below one log₁₀ CFU/g) of such a method (Oyarzabal et al., 2007). To avoid potential underestimation in the prevalence estimate, a selective enrichment method for *Campylobacter* and direct plating for quantification should be considered fully determine baseline studies of *Campylobacter* in the UAE.

Except for samples from one producer [F], our results indicate that all other retail brands were positive for *Campylobacter* (Fig. 1). These results reflect variation in prevalence, may be due to variations in flock prevalence and processing approaches to control carcass contamination. Several authors have reported that the load of bacteria contaminating the surface of broiler carcasses might be affected by practices occurring across the processing line, as well as by chicken rearing and preharvest management (Sampers et al., 2008; Stella et al., 2017). In subsequent work, it will be essential to investigate if and how certain processing practices could influence the *Campylobacter* contamination risk profile across the different producers in the UAE.

4.2. Campylobacter coli is dominant in retail chicken for sale in UAE supermarkets

Using multiplex PCR (Fig. 2. A), C. coli was confirmed as the dominant single species detected in 75 of 90 Campylobacter-positive samples (Fig. 2. B). In this study, the high representation of C. coli in broiler carcasses essentially contradicts other studies in various regions across the world, where typically C. jejuni is more common than C. coli. Nevertheless, in some survey studies, in Australia, Argentina, and China, C. coli was reported to be the prevalent species isolated from chicken meat and offal (Ma et al., 2014; Walker et al., 2019; Schreyer et al., 2022). Additionally, in reporting antimicrobial resistance in the European Union, some countries in central and south Europe have also reported C. coli as more prevalent than C. jejuni in broiler meat (EFSA et al., 2022). Previous research noted that the colonization of C. coli in the broiler gut seems to depend on the studies' geographical setting, the broiler's age at slaughter, and potential selection due to antibiotic usage (Henry et al., 2011; Wang et al., 2016). The high occurrence of C. coli in the UAE retail chicken carcasses and its impact on public health warrants further investigation.

4.3. Whole-genome-based characterization of a subset of C. coli

4.3.1. Clonality and population structure

All *Campylobacter coli* isolates were assembled to a near-complete form with an average genome size of 1,921,359 bp. The genomes were assembled at high continuity with an average N50 of 142,172 bp, the largest N50 achieved for a single isolate is around 248,261 bp. The complete list of the basic statistics of the assemblies of all isolates is given in Supplementary Table 1.

The 45 genomes identified previously described five multilocus sequence types (ST464, ST830, ST1628, ST2718, and ST12096), all belonging to one clonal complex (CC-828). As presented in Table 1, eight novel STs were identified for the first time among 21 of the sequenced *C. coli* isolated from retail chicken in the UAE. Five of the eight newly assigned STs belong to CC-828, and were detected across several brands (Table 1).

The predominance of the CC-828 was expected since several studies demonstrated its global spread in both human and chicken *C. coli* populations (Hull et al., 2021; Tedersoo et al., 2022). The prevalent clonal complex we have found in the UAE retail chicken is concordant with the worldwide dynamics of the *C. coli* population (Kaakoush et al., 2015). Keeping track of molecular epidemiology in the local setting adds to the



Fig. 2. [A] Multiplex PCR analysis of *Campylobacter coli* (lanes 1–3) and *Campylobacter jejuni* (lanes 4,5). In lane 6, *C. coli* and *C. jejuni* were concurrently detected in the same sample. A 100 bp DNA marker is shown on the gel's first (left-hand) lane. [B] Venn diagram showing *Campylobacter* species distribution in 90 *Campylobacter*-positive samples out of 315 tested childed chicken carcasses sampled from United Arab Emirates supermarkets.

Table 1	
New multilocus sequence types (STs) identified in this study and their assigned clonal complexes (CC)	
	-

Sequence Type (ST)	Clonal Complex (CC)	aspA	glnA	gltA	glyA	pgm	tkt	uncA	Number of C. coli isolates	Source/brand
12243	CC-828	33	39	103	140	104	206	17	1	D
12244	CC-828	33	66	30	79	104	35	17	1	С
12245	CC-828	33	39	30	140	483	47	17	2	B, G
12246	NA ^a	33	38	30	79	189	206	17	1	G
12247	CC-828	33	39	30	79	483	47	17	6	C (4), D (1), E (1)
12248	NA	33	38	30	79	189	47	17	1	В
12249	NA	33	66	30	79	189	206	17	2	G
12250	CC-828	33	38	30	140	104	206	17	7	G (3), C (1), D (1), E (1)

^a NA, not assigned to a known clonal complex.

universal picture of the movement of dominant *Campylobacter* strains and subtypes.

4.3.2. Genotypic determinants of antimicrobial resistance

Fig. 3 provides an overview of the detected AMR genes and resistance-associated point mutations. The most frequently encountered point mutation was the *gyrA* 2 *p*.*T861* (nucleotide change, ACT –> ATT) conferring resistance to quinolones, which was detected in 42/45 (93.3%) of the sequenced *C. coli* isolates in this study (Fig. 3). The *T861* mutation in the *gyrA* gene has been reported as the most prevalent AMR mechanism in *Campylobacter* from animal and human sources (Zhang et al., 2003). In addition to the *gyrA* mutation, an RNA mutation *23S r.2075* conferring macrolide-resistance (nucleotide change, A - > G) was present among 30/45 (66.6%) of the characterized *C. coli* (Fig. 3). This point mutation is the most prevalent genetic determinant conferring high levels of erythromycin resistance in *Campylobacter* (Vinueza-Burgos et al., 2017).

Three types of β -lactam (*blaOXA*) genes were detected based on WGS analysis; *blaOXA-61* and *blaOXA-193* were concurrently present in 39/45 (86.6%). β -lactams are not typically prescribed for treating

campylobacteriosis, and most of the panels used for routine AMR monitoring for *Campylobacter* spp. currently do not include this class of antimicrobials (Ocejo et al., 2021). Four genes associated with amino-glycoside resistance (aph(3')-III, ant(6)-Ia, aph(2'')-Ib, and aac(6')-Im), and two linked to tetracycline resistance (tet(O/32/O) in 20 *C. coli*, and tet(O)) in eight isolates were also identified (Fig. 3). This study elaborates further the added value of WGS in the hazard characterization of foodborne pathogens, such as *C. coli*, providing genomic insight into determinants of AMR.

4.3.3. Concordance between phenotypic and genotypic antimicrobial resistance

As shown in Table 2, for the six antimicrobials included in the MIC test panel, there was an agreement ranging from 94 to 100% between MICs above the ECOFF breakpoint and the predicted resistance genes or mutations. Our results indicate a 100% concordance between genotype and phenotype for (fluoro)quinolones (nalidixic acid and ciprofloxacin) and erythromycin. Previous studies reported a similar perfect correlation level for *Campylobacter* in different populations, including human, poultry, and ruminants sourced isolates (Dahl et al., 2021; Ocejo et al.,



Fig. 3. Frequency of genetic determinants associated with resistance genes and point mutations on genomes of 45 Campylobacter coli isolated from chilled chicken carcasses sampled from United Arab Emirates supermarkets.

Table 2

Concordance between resistance genotype and phenotype for 45 Campylobacter coli isolated from chilled chicken carcasses sampled from United Arab Emirates supermarkets.

Classes of antimicrobials	Antimicrobials ^a	ECOFF ^b	No. of phenotypically resistant isolates $^{\rm c}$	AMR genes and mutations	Concordance between genotype and phenotype
(Fluoro)quinolone	NAL (but not CIP.)	>16 µg/ ml	1	gyrA 2 p.T861 (n = 1)	
	CIP (but not NAL.)	>0.5 µg∕ ml	1	$gyrA \ 2 \ p.T86I + gyrA \ p.T86V \ (n = 1)$	100% (42/42) ^c
	CIP + NAL		40	gyrA 2 p.T86I (n = 33); gyrA 2 p.T86I + gyrA p.T86I (n = 7)	
Macrolide	ERY	>8 µg/ ml	30	23S r.2075A > G (n = 30)	100% (30/30)
Aminoglycoside	GEN	>2 µg/ ml	17	<i>aph</i> (2")- <i>Ib</i> + <i>aph</i> (3')- <i>III</i> , + <i>aac</i> (6')- <i>Im</i> (n = 9); <i>aph</i> (3')- <i>III</i> (n = 7)	94.1% (16/17)
Aminoglycoside	STR	>4 µg/ ml	18	ant(6)-Ia (n = 5); ant(6)-Ia + $rpsL p.K43R$ (n = 2); ant(6)-Ia + $aph(3')$ -III (n = 3); $aph(2'')$ -Ib + $aph(3')$ -III + $aac(6')$ -Im + ant (6)-Ia (n = 7)	94.4% (17/18)
Tetracycline	TET	$>2~\mu\text{g/}$ ml	29	tet(O) (n = 8) tet(O/32/O) (n = 20)	96.5% (28/29)

^a CIP, Ciprofloxacin; ERY, erythromycin; GEN, Gentamicin; NAL, Nalidixic; STR, Streptomycin; TET, Tetracycline.

^b EUCAST epidemiological cut-off values (ECOFF) for *Campylobacter* coli; resistant phenotype = non-wild-type.

^c Overall concordance for the (fluoro)quinolone group.

2021). Hence, adopting outcomes based on WGS to identify the genetic markers conferring the phenotypic resistance against clinically significant antimicrobials should be considered much more broadly in national and regional antimicrobial monitoring programs for *Campylobacter*.

Although most campylobacteriosis cases are self-limiting, ciprofloxacin and erythromycin are recommended as empirical therapy to treat severe cases (Bolton, 2015). Our results from the UAE retail chicken align with other reports from different countries, raising the alarm about the emergence of AMR in thermophilic *Campylobacter*, mainly against fluoroquinolones in *C. jejuni* and notably against erythromycin in *C. coli* (Kaakoush et al., 2015; Asuming-Bediako et al., 2019). The high resistance rates for fluoroquinolones and erythromycin in the present study might be attributed to the common use of these antibiotics in UAE poultry farms. Stewardship programs to optimize the use of antibiotics in poultry production are essential for the future of animal and public health (Pinto Ferreira et al., 2022).

4.3.4. Virulence markers in C. coli from retail chicken

The prevalence of virulence genes detected among genomes of the 45 *C. coli* are presented in Fig. 4. *In silico* analyses revealed a range of seven to 11 virulence factors per each *C. coli* isolate. Some of these factors were prevalent in all (n = 45) of the isolates, and these were associated with adherence (*cadF* and *jlpA*), colonization and immune evasion (*capsule* biosynthesis and transport and lipooligosaccharides (LOS)), and invasion (*ciaB*). The results observed for the former genes are aligned with findings reported by several studies that such genes were detected in most of the strains associated with clinical cases (Zhang et al., 2003; Tedersoo et al., 2022).

The cytolethal-distending toxins (CDT), encoded by the *cdtABC* operon, was present in 88.8% (40/45) of the isolates. Previous studies have indicated a similarly high prevalence of CDT in *Campylobacter* recovered from patients with life-threatening diarrhea (Tegtmeyer et al., 2021). Additionally, about half (53.3% (24/45)) of the characterized



Fig. 4. Frequency of the *in silico* predicted virulence factors on genomes of 45 Campylobacter coli isolated from chilled chicken carcasses sampled from United Arab Emirates supermarkets.

C. coli isolates carried type 4 secretion system (T4SS) genes: *virB4*, *virB8*, *virB9*, *virB10*, and *virD4*. T4SS is encoded by genes present on the *pVir* plasmid, known for its potential role in intestinal epithelial adhesion and invasion. Plasmid *pVir* has also been hypothesized to facilitate the events of horizontal gene transferability that could add to increased virulence and fitness of some campylobacters (Mihaljevic et al., 2007). It is important to note that yet the role of many of the genes linked to the virulence potential of *Campylobacter* are not fully understood in the development of gastroenteritis (Bolton, 2015).

5. Conclusion

Campylobacter coli was found to be the most common species detected in retail broiler chicken carcasses in the UAE. Genomic data provided further insight on virulence and resistance profiles in a representative subset of *C. coli* for the first time in the UAE, a country among the leading markets for the per capita consumption of broiler meat. Our study reveals a disturbing pattern of AMR to quinolones and macrolides among *C. coli*, and we report a high resistance rate to tetracycline and aminoglycosides. The insight obtained from the present study provided a piece of evidence that help with filling the gap in the epidemiology of AMR in *Campylobacter* in the UAE. The use of WGS adds value to the surveillance of *Campylobacter* and can be considered a tool to support future One Health AMR surveillance from farm to fork.

CRediT authorship contribution statement

Ihab Habib: Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. Mohamed-Yousif Ibrahim Mohamed: Formal analysis, Investigation, Project administration. Akela Ghazawi: Formal analysis, Software, Visualization. Glindya Bhagya Lakshmi: Investigation, Project administration. Mushtaq Khan: Funding acquisition, Project administration, Resources, Writing – review & editing. Dan Li: Funding acquisition, Project administration, Writing – review & editing. Shafi Sahibzada: Formal analysis, Investigation, Software, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The work was funded by the United Arab Emirates University (UAEU)-Asian University Alliance (AUA) grant number 12R009. The grant was facilitated through the UAEU-Zayed Center for Health Sciences. The funders had no role in the study design, data analysis, publishing decisions, or manuscript preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100434.

References

- Alarjani, K.M., Elkhadragy, M.F., Al-Masoud, A.H., Yehia, H.M., 2021. Detection of Campylobacter jejuni and Salmonella typhimurium in chicken using PCR for virulence factor hipO and invA genes (Saudi Arabia). Biosci. Rep. 41.
- Asakura, H., Sakata, J., Nakamura, H., Yamamoto, S., Murakami, S., 2019. Phylogenetic diversity and antimicrobial resistance of *Campylobacter coli* from humans and animals in Japan. Microb. Environ. 34, 146–154.
- Asuming-Bediako, N., Kunadu, A.P., Jordan, D., Abraham, S., Habib, I., 2022. Prevalence and antimicrobial susceptibility pattern of *Campylobacter jejuni* in raw retail chicken meat in Metropolitan Accra, Ghana. Int. J. Food Microbiol. 376, 109760.
- Asuming-Bediako, N., Parry-Hanson Kunadu, A., Abraham, S., Habib, I., 2019. *Campylobacter* at the human-food interface: the African perspective. Pathogens 8.
- Baré, J., Uyttendaele, M., Habib, I., Depraetere, O., Houf, K., De Zutter, L., 2013. Variation in *Campylobacter* distribution on different sites of broiler carcasses. Food Control 32, 279–282.
- Bolton, D.J., 2015. Campylobacter virulence and survival factors. Food Microbiol. 48, 99–108.

I. Habib et al.

Current Research in Food Science 6 (2023) 100434

- Dahl, L.G., Joensen, K.G., Osterlund, M.T., Kiil, K., Nielsen, E.M., 2021. Prediction of antimicrobial resistance in clinical *Campylobacter jejuni* isolates from whole-genome sequencing data. Eur. J. Clin. Microbiol. Infect. Dis. 40, 673–682.
- Denis, M., Soumet, C., Rivoal, K., Ermel, G., Blivet, D., Salvat, G., Colin, P., 1999. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Lett. Appl. Microbiol. 29, 406–410.
- EFSA (European Food Safety), A., European Centre for Disease, P., Control, 2022. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019-2020. EFSA J. 20, e07209.
- Habib, I., Mohamed, M.I., Khan, M., 2021. Current state of Salmonella, Campylobacter and Listeria in the food chain across the Arab countries: a descriptive review. Foods 10.
- Habib, I., Coles, J., Fallows, M., Goodchild, S., 2019. A baseline quantitative survey of *Campylobacter* spp. on retail chicken portions and carcasses in metropolitan Perth, Western Australia. Foodb. Pathog. Dis. 16, 180–186.
- Habib, I., Uyttendaele, M., De Zutter, L., 2011. Evaluation of ISO 10272:2006 standard versus alternative enrichment and plating combinations for enumeration and detection of *Campylobacter* in chicken meat. Food Microbiol. 28, 1117–1123.
- Henry, I., Reichardt, J., Denis, M., Cardinale, E., 2011. Prevalence and risk factors for *Campylobacter* spp. in chicken broiler flocks in Reunion Island (Indian Ocean). Prev. Vet. Med. 100, 64–70.
- Hull, D.M., Harrell, E., van Vliet, A.H.M., Correa, M., Thakur, S., 2021. Antimicrobial resistance and interspecies gene transfer in *Campylobacter coli* and *Campylobacter jejuni* isolated from food animals, poultry processing, and retail meat in North Carolina, 2018-2019. PLoS One 16, e0246571.
- Kaakoush, N.O., Castano-Rodriguez, N., Mitchell, H.M., Man, S.M., 2015. Global epidemiology of *Campylobacter* infection. Clin. Microbiol. Rev. 28, 687–720. Ma, L., Wang, Y., Shen, J., Zhang, O., Wu, C., 2014. Tracking *Campylobacter*

Ma, L., Walg, F., Shen, J., Zhang, Q., Wu, C., 2014. If a King Campyiouditer contamination along a broiler chicken production chain from the farm level to retail in China. Int. J. Food Microbiol. 181, 77–84.

- Majowicz, S.E., Panagiotoglou, D., Taylor, M., Gohari, M.R., Kaplan, G.G., Chaurasia, A., Leatherdale, S.T., Cook, R.J., Patrick, D.M., Ethelberg, S., Galanis, E., 2020. Determining the long-term health burden and risk of sequelae for 14 foodborne infections in British Columbia, Canada: protocol for a retrospective population-based cohort study. BMJ Open 10, e036560.
- Mihaljevic, R.R., Sikic, M., Klancnik, A., Brumini, G., Mozina, S.S., Abram, M., 2007. Environmental stress factors affecting survival and virulence of *Campylobacter jejuni*. Microb. Pathog. 43, 120–125.
- Mohammed, H.O., Stipetic, K., Salem, A., McDonough, P., Chang, Y.F., Sultan, A., 2015. Risk of *Escherichia coli 0157:H7, non-0157* shiga toxin-producing *Escherichia coli,* and *Campylobacter* spp. in food animals and their products in Qatar. J. Food Protect. 78, 1812–1818.
- Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B., Havelaar, A., 2009. A comparison of risk assessments on *Campylobacter* in broiler meat. Int. J. Food Microbiol. 129, 107–123.

- Ocejo, M., Oporto, B., Lavin, 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, 8998.
- Oyarzabal, O.A., Backert, S., Nagaraj, M., Miller, R.S., Hussain, S.K., Oyarzabal, E.A., 2007. Efficacy of supplemented buffered peptone water for the isolation of *Campylobacter jejuni* and *C. coli* from broiler retail products. J. Microbiol. Methods 69, 129–136.
- Pinto Ferreira, J., Battaglia, D., Dorado Garcia, A., Tempelman, K., Bullon, C., Motriuc, N., Caudell, M., Cahill, S., Song, J., LeJeune, J., 2022. Achieving antimicrobial stewardship on the global scale: challenges and opportunities. Microorganisms 10.
- Sampers, I., Habib, I., Berkvens, D., Dumoulin, A., Zutter, L.D., Uyttendaele, M., 2008. Processing practices contributing to *Campylobacter* contamination in Belgian chicken meat preparations. Int. J. Food Microbiol. 128, 297–303.
- Schreyer, M.E., Olivero, C.R., Rossler, E., Soto, L.P., Frizzo, L.S., Zimmermann, J.A., Signorini, M.L., Virginia, Z.M., 2022. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *C. coli* identified in a slaughterhouse in Argentina. Curr Res Food Sci 5, 590–597.
- Stella, S., Soncini, G., Ziino, G., Panebianco, A., Pedonese, F., Nuvoloni, R., Di Giannatale, E., Colavita, G., Alberghini, L., Giaccone, V., 2017. Prevalence and quantification of thermophilic *Campylobacter* spp. in Italian retail poultry meat: analysis of influencing factors. Food Microbiol. 62, 232–238.
- Tedersoo, T., Roasto, M., Maesaar, M., Kisand, V., Ivanova, M., Meremae, K., 2022. The prevalence, counts, and MLST genotypes of *Campylobacter* in poultry meat and genomic comparison with clinical isolates. Poultry Sci. 101, 101703.

Tegtmeyer, N., Sharafutdinov, I., Harrer, A., Soltan Esmaeili, D., Linz, B., Backert, S., 2021. Campylobacter virulence factors and molecular host-pathogen interactions. Curr. Top. Microbiol. Immunol. 431, 169–202.

- USDA (United States Department of Agriculture), 2021. Poultry and Products Annual. Available via. https://www.fas.usda.gov/data/united-arab-emirates-poultry-andproducts-annual-2. (Accessed 12 January 2022).
- Vinueza-Burgos, C., Wautier, M., Martiny, D., Cisneros, M., Van Damme, I., De Zutter, L., 2017. Prevalence, antimicrobial resistance and genetic diversity of *Campylobacter coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age. Poultry Sci. 96, 2366–2374.
- Walker, L.J., Wallace, R.L., Smith, J.J., Graham, T., Saputra, T., Symes, S., Stylianopoulos, A., Polkinghorne, B.G., Kirk, M.D., Glass, K., 2019. Prevalence of *Campylobacter coli* and *Campylobacter jejuni* in retail chicken, beef, lamb, and pork products in three Australian states. J. Food Protect. 82, 2126–2134.
- Wang, Y., Dong, Y., Deng, F., Liu, D., Yao, H., Zhang, Q., Shen, J., Liu, Z., Gao, Y., Wu, C., Shen, Z., 2016. Species shift and multidrug resistance of *Campylobacter* from chicken and swine, China, 2008-14. J. Antimicrob. Chemother. 71, 666–669.
- Zhang, Q., Lin, J., Pereira, S., 2003. Fluoroquinolone-resistant *Campylobacter* in animal reservoirs: dynamics of development, resistance mechanisms and ecological fitness. Anim. Health Res. Rev. 4, 63–71.