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Quantification of *Campylobacter* contamination on chicken carcasses sold in retail markets in the United Arab Emirates

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Abstract

Background: *Campylobacter* is among the leading causes of foodborne zoonotic disease worldwide, with chicken meat accounting for the majority of human illnesses. This baseline study generates the first quantitative data for *Campylobacter* contamination in the United Arab Emirates chicken meat. Such data will help inform risk analysis and develop evidence-based food safety management.

Methods: For a year, chilled whole chicken carcasses ($n = 315$) belonging to seven different companies were collected from retail supermarkets. According to standard methods, *Campylobacter* enumeration was achieved by a direct plating in all chicken samples, and isolates were confirmed using multiplex PCR.

Results: *Campylobacter* spp. were recovered from 28.6% (90/315) of the samples. *Campylobacter* enumeration results indicated that 71.4% of the tested samples were contaminated with < 1 $\log_{10}$ CFU (colony-forming units)/g, and 7% were contaminated with ≥ 3 $\log_{10}$ CFU/g. The mean *Campylobacter* concentration was 2.70 $\log_{10}$ CFU/g, with a standard deviation of 0.41 $\log_{10}$ CFU/g. *Campylobacter* counts varied significantly in relation to the sourcing chicken processing companies. Six out of the seven surveyed companies provided *Campylobacter* positive samples. Moreover, significantly higher (p-value< 0.0001) counts were found to be associated with smaller size chicken carcasses (weighted 600–700 g; compared to the other categories, 800 g and 900–1000 g). Interestingly, *C. coli* was present in 83% of the positive samples, while *C. jejuni* was only detected in 6.4% of the samples. Compared with studies from other countries utilizing the same enumeration method, the UAE chicken appears to have a lower prevalence but a higher *Campylobacter* count per gram of carcasses. Higher *Campylobacter* counts were significantly associated with smaller carcasses, and *C. coli* was the dominant species detected in this study's samples.

Conclusion: These results add to our understanding of the local, regional and global epidemiology of *Campylobacter* in chicken meat. Outputs of the current study may aid in developing a risk assessment of *Campylobacter* in the UAE, a country among the biggest per capita consumption markets for chicken meat worldwide.

Keywords: Campylobacteriosis, Middle East, UAE, Risk assessment, Chicken meat

Introduction

Campylobacter is among the leading bacterial pathogens causing human diarrheal illnesses worldwide, with an estimated 96 million foodborne illness cases per year (Majowicz et al. 2020). *Campylobacter jejuni* and *Campylobacter coli* are the most commonly implicated species in human cases, with an infectious dose as small as a few hundreds of such bacteria (Lopes et al. 2021).

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Campylobacter inhabits the gut flora of domestic poultry and could be transferred to carcass during slaughter processing and further on to retail chicken meat. Improper handling and undercooking of chicken are factors known to increase the exposure of humans to *Campylobacter* (Lopes et al. 2021; Majowicz et al. 2020).

Quantitative baseline studies of *Campylobacter* contamination in foods presented in retail markets are important for informing risk assessment and management (Stella et al. 2017). *C. jejuni* and *C. coli* cannot grow at refrigerated or room temperature; hence, research on the counts of *Campylobacter* on chicken presented at the retail level will insight a baseline evidence on one of the critical venues for foodborne infection among the public (Lopes et al. 2021). Additionally, quantitative data could guide the chicken meat processors to verify their process hygiene performance. *Campylobacter* enumeration data are crucial to better quantify the human exposure risk and evaluate relative reduction options for such risk (Habib et al. 2019). Previous studies estimated that a 2-log decline in *Campylobacter* loads on chicken meat could reduce campylobacteriosis infection incidence in humans by around thirty-fold (Rosenquist et al. 2003).

In the Gulf Cooperation Council (GCC) nations, 2–28% of human diarrheal cases are attributed to infection with *Campylobacter* (Kaakoush et al. 2015). Nevertheless, there are minimal surveillance studies for *Campylobacter* at the human-animal-food interface across the GCC nations. Worldwide, the United Arab Emirates (UAE) is among the leading countries in consuming chicken meat (~60 kg/capita per year) (USDA (United States Department of Agriculture) 2021). In the UAE, most broiler businesses are based in the Emirate of Abu Dhabi (encompassing three jurisdictions; Abu Dhabi Region, Al Ain Region, and Al Dhafra Region), as it accounts for more than 70% of the agricultural production in the UAE. The broiler industry has been expanding in the recent years in the UAE, and is foreseen as a promising sector to improve the country's food security (USDA (United States Department of Agriculture) 2021).

Nevertheless, the prevalence and enumeration data of *Campylobacter* contamination status in chicken meat are unavailable in the UAE (Habib et al. 2021). Such data are in high demand to fill a gap in the risk assessment of *Campylobacter* in one of the biggest per capita consumption markets for chicken meat worldwide. In this study, we conducted the first baseline study of *Campylobacter* spp. contamination loads in chilled whole chickens retailed in the UAE during 2021. Then, we evaluated the potential role of both producer and weight of the carcass on the resulting loads of the *Campylobacter* on chicken. We also evaluated the frequency of *C. jejuni* and *C. coli*, the most important species of thermotolerant *Campylobacter* impacting public health and food safety, among the isolates generated from whole chicken carcasses. The present baseline data offer novel insight into the status of *Campylobacter* in the GCC countries and adds to the global understanding of the epidemiology of such an important foodborne pathogen.

Materials and methods

Sampling design

The number of samples required in this study was calculated assuming a *Campylobacter*-prevalence of 50%, with the desired confidence level of 90% and a 5% margin of error. In total, 315 samples were collected from March to December 2021 (spread over 10 months). The samples were obtained on two occasions per month, and around 15 whole chicken carcasses were purchased per each (~30 samples per each month) from various supermarkets ($n=26$). We adopted a targeted sampling approach (after consultation with producers and local food control authorities) that involved retail samples from seven different companies (brands), of which the three biggest local companies, supplying more than 75% of the UAE distribution chains of chilled carcasses, are included. Six of the seven companies (brands) are UAE-based producers operating through processing facilities across Dubai, Al Ain and Abu Dhabi (Table 1); chicken meat produced from these companies is presented throughout the UAE-wide retail supermarkets. One of the seven companies (company F) is supplied from neighboring Saudi Arabia. All seven brands are from conventional production systems (none were organic).

All samples were collected from chilled supermarkets display, where all the carcasses were packed and labeled by brand. The samples were presented as sealed aerobically packaged units, and the on-label carcass weight for 268 of the 315 tested samples was recorded only based on the weight indicated on the label. Samples were shipped for testing on the same day in chilled containers (with ice bricks (6–8 °C)) to the Veterinary Public Health Research Laboratory at the United Arab Emirates University. All laboratory testing was completed within six hours of sampling.

Isolation and enumeration of *Campylobacter*

Standard *Campylobacter* enumeration was done in concordance with the ISO 10272:2006 methods (Habib et al. 2011; Jacobs-Reitsma et al. 2019). 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). Enumeration of *Campylobacter* in neck skin samples has been previously recommended by several baseline studies (Baré et al. 2013; Habib et al. 2008).

Table 1 Detection and average counts of *Campylobacter* in 315 chilled whole chicken carcasses sampled from supermarkets in the United Arab Emirates

Company[a]	Production and processing site	Samples number	No. recovered by direct plating (%)[b]	Average $\log_{10}$ CFU/g ± SD
A	Abu Dhabi/Al Ain (UAE)	52	12 (23.1)	2.91 ± 0.98
B	Al Ain (UAE)	30	3 (10.0)	2.62 ± 0.34
C	Abu Dhabi (UAE)	50	13 (26.0)	2.70 ± 0.42
D	Abu Dhabi (UAE)	53	20 (37.7)	2.73 ± 0.48
E	Dubai (UAE)	50	14 (28.0)	2.42 ± 0.34
F	Imported (Saudi Arabia)	30	0 (—)	–
G	Al Ain (UAE)	50	28 (56.0)	2.82 ± 0.31
Total		315	90 (28.6)	2.70 ± 0.41

[a] Arbitrarily identification letters were assigned for each company

[b] Number of carcasses with ≥ the quantification limit (1 $\log_{10}$ CFU/g) for direct plating methods

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.

Confirmation and species identification

Based on colony morphology, up to five suspected colonies per sample were re-streaked on mCCDA and further incubated micro-aerobically for 24 h at 41.5 °C. DNA was extracted from overnight culture using a kit according to the manufacturer's instructions (Wizard® Genomic DNA Purification Kit (Promega, USA)). *Campylobacter* genus and the thermo-tolerant species of *C. jejuni* and *C. coli* were identified with multiplex PCR assay targeting 16S rRNA, *mapA*, and *ceuE* genes utilizing primers and conditions recommended by Denis et al. (1999). The amplification reactions were carried out using a polymerase chain reaction (PCR) thermocycler (QIAamplifier 96, Qiagen, Germany) with an initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, an annealing step at 59 °C for 1.5 min, and an extension step at 72 °C for 1 min, and the last extension step at 72 °C for 10 min. PCR products were run on 1.5% agarose gel electrophoresis at 110 V for 30 min and then visualized using the GelDoc-Go Imaging System (BioRad, USA). The product size of 800 bp and 589 bp was interpreted as *C. jejuni* positive, and the product size of 800 bp and 462 bp was interpreted as *C. coli* positive.

Analysis of data

Enumeration results of *Campylobacter* on whole chicken carcasses were reported as the number of CFU (colony-forming units) per gram (g). Enumeration data were transformed to a base-ten logarithmic scale to approximate the data to normality. The tested chicken carcasses were assumed to be clustered within the sourcing processors; hence for comparing variation across processing companies and across the different categories of carcasses weight (based on the weight indicated on the label), we used the random-effects Poisson regression model (xtpoisson) for handling counts data (CFU/g), and random-effects logistic regression model (xtlogit) procedures for presence/absence results. 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). Enumeration results presented a skewed distribution, and a negative binomial model was applied in the case of enumeration data with an evident extra-Poisson variation.

Results and discussion

Overall *Campylobacter* recovery and enumeration

Campylobacter isolates were recovered from 28.6% [90/315] (95% confidence interval: 23.6%;33.9%) of the tested retail whole chicken carcasses. The average overall *Campylobacter* count was 2.70 $\log_{10}$ CFU/g, with a standard deviation of 0.41 $\log_{10}$ CFU/g (Table 1). The count data presented with a left-skewed frequency distribution,

where 71.4% of the carcasses were contaminated with < 1 $\log_{10}$ CFU/g (below the quantification limit) (Fig. 1). On the other hand, 21.6% of the carcasses exhibited a count range of ≥1 $\log_{10}$ to < 3 $\log_{10}$ CFU/g and 7% of the tested carcasses were contaminated with *Campylobacter* loads of ≥3 $\log_{10}$ CFU/g (Fig. 1).

The findings from this baseline study provide the first published investigation on the status of *Campylobacter* in chilled chicken meat sold in supermarkets in the UAE. Lower recovery of *Campylobacter* in chicken carcasses was observed in our study (28.6%) compared to results from neighboring countries, such as Qatar (36.5%) and Saudi Arabia (52.2%) (Abu-Madi et al. 2016; Alarjani et al. 2021). In this study, 71.4% of the tested chicken carcasses were contaminated with *Campylobacter* at a level below 1 $\log_{10}$ CFU/g (the quantification limit for the designated direct plating method). Such finding is comparable with counts data of *Campylobacter* spp. in chicken carcasses in a comprehensive survey done in the U.S.A (74% ($n=2400$)) and considerably larger than what was concluded in a recent study on Halal chicken slaughtered in UK, where 49.6% of whole carcasses ($n=405$) had *Campylobacter* of < 1 $\log_{10}$ CFU/g (FSIS (Food Safety and Inspection Service) 2008; Royden et al. 2021). It is likely that the recovery of *Campylobacter* from retail chicken using direct plating to become lower as the bacterial loads fall below the quantification limit of such a method (Oyarzabal et al. 2007). Given that the *Campylobacter* contamination level revealed in this study is frequently below 10 CFU per g, the use of a selective enrichment method in parallel to direct plating could be justifiable in future studies in the UAE context.

Comparing the UAE *Campylobacter* situation with the international context

The enumeration data generated in this study indicate that the mean count of *Campylobacter* in chicken carcasses ($n=315$) from the UAE retails was 2.70 $\log_{10}$ CFU/g (~ 500 CFU/g) (Table 1), with 7% of the samples being contaminated with ≥3 $\log_{10}$ CFU/g (Fig. 1). Compared to our results, a study in the UK found that 13.8% of the neck skin of Halal chicken carcasses ($n=265$) sampled from retail had counts ≥3 $\log_{10}$ CFU/g (Royden et al. 2021). On the other hand, a Belgium-wide survey of *Campylobacter* counts in chicken meat ($n=656$) showed an average of ~ 50 CFU/g (1.69 $\log_{10}$ CFU/g), and 11.6% of the chicken meat carried ≥2 $\log_{10}$ CFU/g [3]. A multistate survey in the U.S. revealed that about 1.1% of post-chill chicken carcasses had > 2 $\log_{10}$ CFU/mL of carcass rinse (FSIS (Food Safety and Inspection Service) 2008). Given that exposure to a relatively low number (~ 800 cells) of ingested *Campylobacter* could lead to enteric illness in humans (Lopes et al. 2021), understanding the distribution range of *Campylobacter* counts residing in chicken carcasses should be a priority for developing evidence-based management plans for both producers and food safety bodies. The output from this study could contribute to the future optimization of a quantitative risk assessment of *Campylobacter* contamination at the chicken meat-human interface in the UAE.

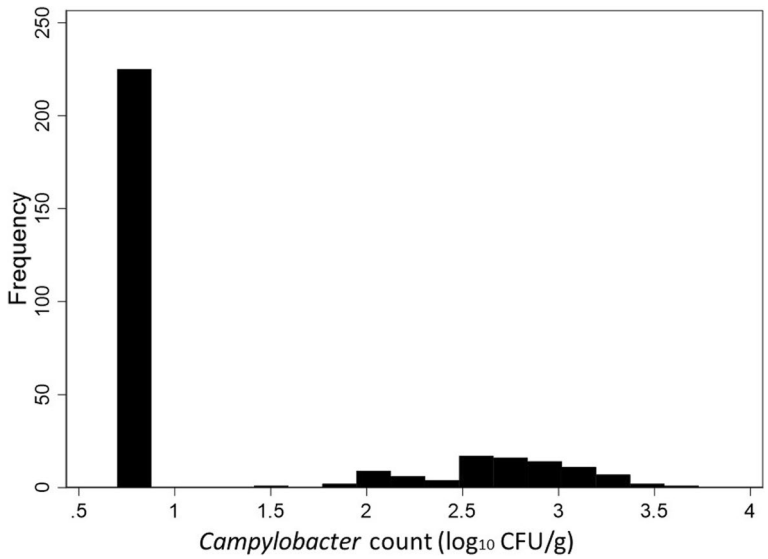

Fig. 1 Distribution frequency of the counts of *Campylobacter* recovered from 315 chicken carcasses sampled from supermarkets in the United Arab Emirates

Campylobacter contamination is associated with processing companies

Results in Table 1 demonstrate evident variability between the seven companies regarding the recovery of *Campylobacter* among their samples. All carcasses ($n=30$) originating from company F were below the limit of quantification (Table 1). Using a random-effects (negative binomial) regression analysis (with company A as a reference category) for counts data, significantly lower *Campylobacter* counts (incidence rate ratio [IRR] = 0.34, p-value = 0.009) were associated with chicken carcasses from company E (Table 1), with none of its samples ($n=50$) passed a contamination load of ≥ 3 $\log_{10}$ CFU/g (Fig. 2). Random-effects logistic regression analysis (with company A as a reference category) indicates that company G was the most significant (odds ratio [OR] = 11.4, p-value < 0.0001) in providing *Campylobacter*-contaminated samples (Table 1). Aligning with that, 20% of the samples belonging to company G had *Campylobacter* at a level of ≥ 3 $\log_{10}$ CFU/g, followed by 10% and 7.5% for companies C and D, respectively (Fig. 2).

Except for samples originating from one company [F], our results indicate that all other companies provided *Campylobacter* positive samples with a variable degree in recovery frequency and count distributions. These results possibly reflect some potential variability in the hygienic processing and quality management practices exhibited across the UAE suppliers of fresh chicken meat. Several studies have reported that bacterial contamination in chicken carcasses varies depending on processing practices and how the chickens were reared and processed (Sampers et al. 2008; Stadlmuller et al. 2017). In subsequent work, it will be investigated if and how certain processing practices could influence the *Campylobacter* contamination risk profile across producers in the UAE. A quantitative *Campylobacter* monitoring program could be of value in prioritizing a *Campylobacter* risk-based inspection and tracing sources of unacceptable contamination.

Carcasses weight is a potential contamination determinant

Campylobacter was recovered from 51.5% of carcasses weighted 600–700 g ($n=132$), as compared to 11% and 24% of carcasses weighed 800 g ($n=82$), and 900–1000 g ($n=54$), respectively. The variation in *Campylobacter* contamination levels across carcass weight categories is presented in Fig. 3. The descriptive data were tested using a random-effects (negative binomial) regression analysis; compared to carcasses weighed 600–700 g (the reference category in the model), very significantly lower (p-value < 0.0001) *Campylobacter* counts were associated with chicken carcasses weighed 800 g, and also lower counts (p-value < 0.012) was evident in carcasses weighted 900–1000 g.

Compared to many other countries, the demand for smaller chicken carcasses is unique in the UAE market.

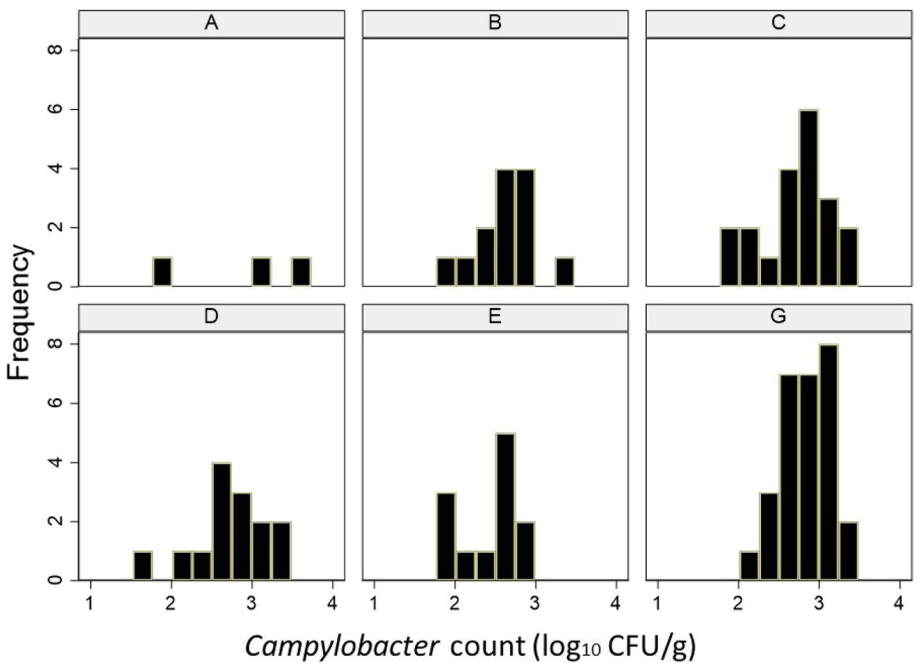

Fig. 2 Variability, across companies, in the distributions of *Campylobacter* counted data in chicken carcasses sampled from supermarkets in the United Arab Emirates

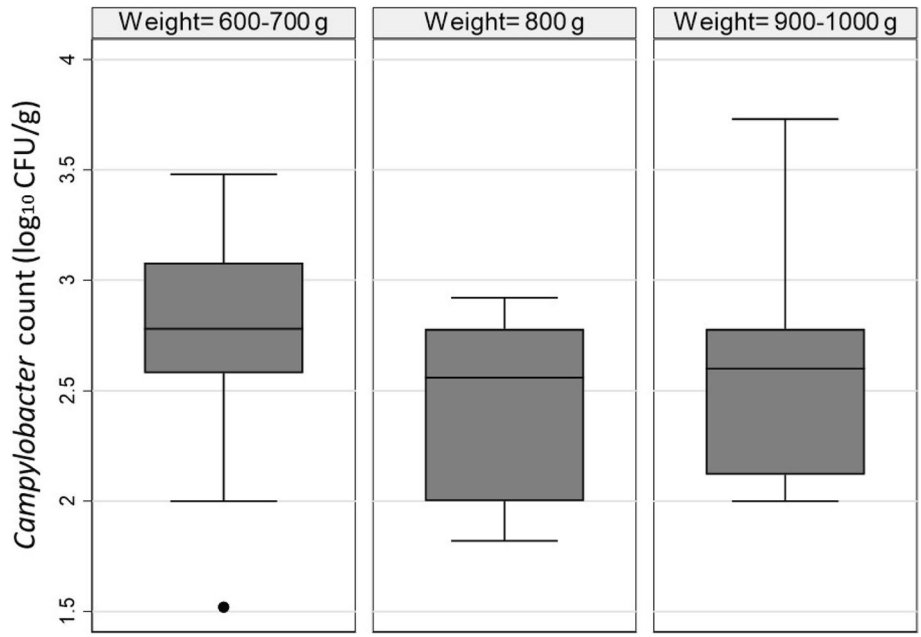

Fig. 3 Variability, in relation to carcasses weight (as indicated on samples' labels), in the distributions of *Campylobacter* counted data in chicken carcasses sampled from supermarkets in the United Arab Emirates. The upper and lower hinges represent the 75th and the 25th percentiles, while the line in each box denotes the median value

In the UAE, live chickens are generally slaughtered once reaching 35 days or less (USDA (United States Department of Agriculture) 2021). This practice is meant to satisfy the needs of the household consumers; generally, they prefer whole chilled carcasses that are small in size (between 0.8 to 1.3 kg) (USDA (United States Department of Agriculture) 2021). The prevalence of flock positivity is directly related to slaughter age, and reducing the slaughter age could be an effective action to reduce *Campylobacter* spp. prevalence in flocks. This conclusion stemmed from a baseline European study, suggesting that human campylobacteriosis would be reduced by 21% to 43% if the chicken slaughter age was reduced to 28 days (EFSA (European Food Safety BIOHAZ Panel) 2011). Additionally, van Wagenberg et al. (2016) estimated that if all flocks were slaughtered by 35 days or less, there would be a reduction in human campylobacteriosis of 10–18%. The reduced slaughter age (commonly practiced in the UAE poultry sector) might lower the overall *Campylobacter* recovery in retail chicken carcasses, as was indicated in this study.

In the present study, we noted that significantly higher (p-value< 0.0001) *Campylobacter* counts were associated with smaller size chicken carcasses (weighted 600–700 g; compared to the other categories, 800 g, and 900–1000 g) (Fig. 3). The reduced slaughter age might be associated with increasing the overall *Campylobacter* counts on small-sized carcasses. This might be attributed to the fact that smaller birds may lead to more carcass contamination due to gut rupture at slaughter if automated evisceration equipment is designed for larger birds (Koutsoumanis et al. 2020). It will be important in future work to investigate closely if and how certain processing practices such as carcass size and slaughter age could influence the *Campylobacter* contamination risk profile.

Dominance of *Campylobacter coli*

All isolates retained from samples with countable results (n = 90; up to 5 colonies per sample) were identified by multiplex PCR as either *Campylobacter jejuni* or *Campylobacter coli* (Fig. 4). Interestingly, *C. coli* was the most dominant species in whole chicken carcasses sampled from retail in the United Arab Emirates. *C. coli* was present in 93.6% of the positive samples (n = 90), while *C. jejuni* was only detected in 17.0% of the positive samples. Concurrently, *C. jejuni* and *C. coli* were detected (mixed) in isolates from 10.6% of the positive samples.

The dominance of *C. coli* in chicken carcasses from the retail stores detected in this work differs from many previous studies in other countries, where *C. jejuni* typically concluded as the more recovered species (Hlashwayo et al. 2021). Nevertheless, in some other survey studies, *C. coli* was reported to be the most prevalent species isolated from chicken meat and offals, as in some studies in

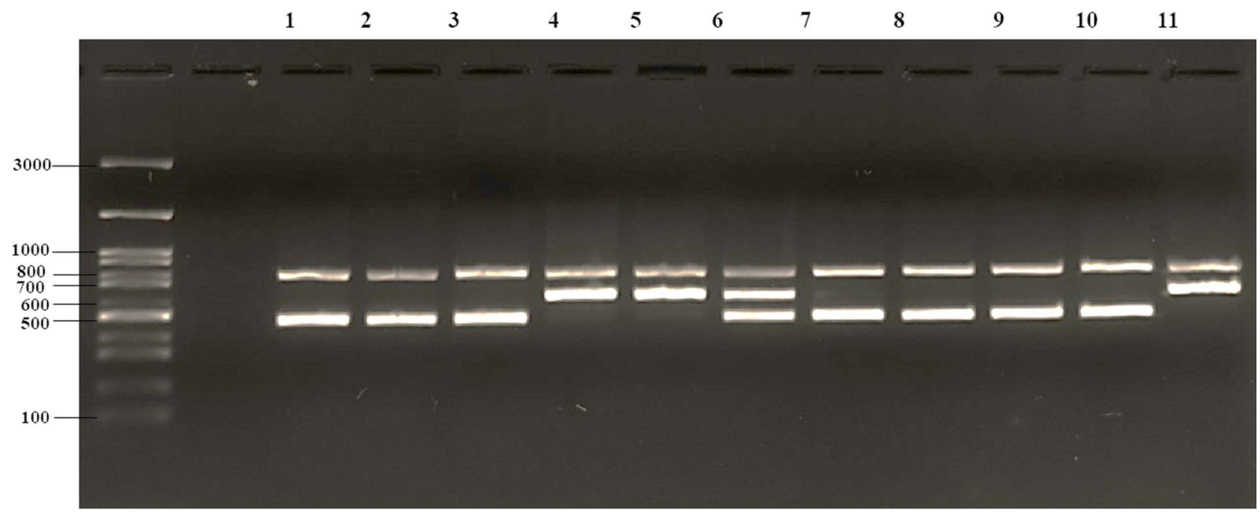

Fig. 4 Multiplex PCR analysis of *Campylobacter coli* and *Campylobacter jejuni*. *Campylobacter* genus-specific 16S rRNA amplicons were resolved at 800 bp. Lanes 1–3, and 7–10, *C. coli* only (462 bp); lanes 4,5,11, *C. jejuni* only (589 bp); lane 6, *C. coli* and *C. jejuni* were concurrently detected. A DNA ladder (in base pairs) is shown on the left-hand edge of the gel

Australia, Argentina, and China (Ma et al. 2014; Schreyer et al. 2022; Walker et al. 2019). In reporting antimicrobial resistance in the European Union, several countries (south and central Europe) have reported *C. coli* as the most prevalent species in chicken meat (EFSA (European Food Safety, European Centre for Disease, Control) 2022). Previous research indicated that *C. coli* in broiler intestinal tracts seems to depend on the geographical area, the age of the chicken, and antibiotic selection pressure (Henry et al. 2011; Wang et al. 2016). The dominance of *C. coli* in the UAE retail chicken carcasses and its impact on public health is worth investigating further in future work.

Conclusions

This study generates the first-of-its-kind data on contamination levels of *Campylobacter* in the UAE retailed chilled chicken carcasses. In comparison with studies from other countries utilizing the same enumeration method, our results conclude that the UAE chicken appears to have a lower prevalence but a higher *Campylobacter* count per gram of carcasses. Higher *Campylobacter* counts were significantly associated with smaller carcasses, and *C. coli* was surprisingly the dominant species detected in this study's samples. Future research is needed to ascertain the influence of certain processing practices such as carcass size and slaughter age on *Campylobacter* contamination risk profile. The output from this study may contribute to the future setting of a quantitative risk assessment modeling approach to understand better *Campylobacter* risk impact in the UAE, one of the biggest per capita consumption markets for chicken meat worldwide. Given the gap in knowledge on the status of *Campylobacter* contamination levels in the food chain across the Gulf Cooperation Council countries, the results generated in this study add to our understanding of the local, regional and global risk profiling of *Campylobacter* in chicken meat.

Abbreviations

CFU: Colony-forming units; GCC: Gulf Cooperation Council; log: Logarithm; IRR: Incidence rate ratio; mCCDA: Modified charcoal cefoperazone deoxycholate agar; n: Number; OR: Odds Ratio; PCR: Polymerase chain reaction; UAE: United Arab Emirates.

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Authors' contributions

Conceptualization, I.H; Formal analysis, M.I.M and G.L; Funding acquisition, I.H, M.K and D.L; Project administration, I.H, M.K and D. L; Supervision, M.I.M and G.L; Writing – original draft, I.H; Writing – review & editing, M.I.M and G.L, M.K and D. L. All authors have read and approved the final manuscript.

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Availability of data and materials

All data generated during this study are included in the manuscript.

Declarations

Competing interests

The authors declare no conflict of interest.

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