**Research Paper**

**Running Title: *Campylobacter* Contamination of UK-Produced Halal Chicken at Retail**

***Campylobacter* Contamination of UK-Produced Halal Chicken at Retail**

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**Abstract**

*Campylobacter* is the leading cause of human bacterial diarrhoeal disease worldwide, with poultry meat products accounting for the majority of human cases. Recent surveys by the Food Standards Agency estimate the *Campylobacter* prevalence in fresh UK retail chicken to be 41.2%. However, such surveys have not distinguished between broiler chickens produced for different consumer demographics, such as the Halal market. *Campylobacter* colonisation of broilers is difficult to prevent, especially during routine partial depopulation of flocks. Broilers produced for the Halal market may undergo multiple depopulation events, which may increase the risk of colonisation and subsequent *Campylobacter* contamination of chicken meat. This project aimed to determine the prevalence and levels of *Campylobacter* contamination of chicken meat produced for the UK Halal market. *Campylobacter* was identified and enumerated from the neck skin and outer packaging of 405 Halal chickens. Following culture, isolates were assigned to species via PCR and disc diffusion antimicrobial susceptibility tests determined. Logistic regression analysis assessed risk factors for *Campylobacter* isolation, the level of *Campylobacter* contamination among positive carcasses and antimicrobial resistance outcomes. *Campylobacter* spp. were confirmed in 65.4% of neck skin samples and 17.1% of packaging samples. 13.8% of neck skin samples had the highest level of contamination (>1000 cfu/g). Large birds had a significantly higher number of samples with >1000 cfu/g (p<0.001) and as chicken carcass weight increased, birds were more likely to be *Campylobacter-*positive (p<0.05). A high prevalence of resistance was seen to ciprofloxacin (42.0%) and 38.5% of samples contained at least one multi-drug resistant *Campylobacter* isolate. This study demonstrates that Halal chicken has a higher *Campylobacter* prevalence than non-Halal chicken. Interventions should be introduced to reduce this increased public health risk to consumers.

**Highlights**

* 405 intensively-reared Halal chickens were purchased from retailers in England.
* *Campylobacter* were isolated from 65.4% of neck skin and 17.1% of packaging samples.
* 13.8% of neck skin samples were contaminated with >1000 cfu/g (the highest level).
* 38.5% and 42% of samples contained (a) MDR or ciprofloxacin-resistant isolate(s).
* Halal chicken has a higher prevalence of *Campylobacter* than non-Halal.

*Campylobacter* is the leading cause of human bacterial diarrhoeal disease worldwide. In England and Wales in 2017, there were 56,729 reported cases *(40)*. However, as only one in ten cases are reported to national surveillance schemes, this figure underestimates the true impact of the disease, which is estimated to affect approximately 571,949 people every year in the UK *(45)*.The majority of cases (>90%) are caused by *C. jejuni*, with *C. coli* accounting for a further 5-10% of cases *(25)*. Poultry meat and products are estimated to account for >70% of human campylobacteriosis cases, due to the consumption of undercooked meat or cross-contamination of raw meat within the kitchen *(4)*.

In 2010, the Food Standards Agency (FSA) and the UK poultry industry set a joint target to reduce *Campylobacter* in UK-produced chicken; aiming to reduce the prevalence of the most contaminated chickens (>1000 cfu/g) at the end of the slaughter process to below 10% by the end of 2015 *(23)*. From 2014-2018, the FSA conducted UK-wide surveys of *Campylobacter* contamination on fresh chickens at retail *(14, 17, 18, 20)*. The results of the first survey in 2015 revealed that the joint FSA-industry target had not been met, as the prevalence of *Campylobacter* was 73.3% with 19.4% of the chickens found to be contaminated with >1000 cfu/g *(20)*. However, by 2018, overall prevalence had reduced to 41.2% and the percentage of most contaminated chickens to 3.5% *(15)*. These surveys did not distinguish between broiler chickens produced for different consumer demographics, largely sampling chickens produced for the conventional meat market.

Ritual or religious slaughter is the slaughter of an animal for meat for consumption by people of a particular religion, performed by someone of that particular faith who holds an appropriate licence *(2)*. In the UK, nearly 5% of the population (2.7M people) identify as Muslim *(39)*, with 90% of Muslims consuming Halal meat from animals which have been ritually slaughtered *(10)*. Of poultry produced in the UK, 21% conforms to Halal specifications *(21)*.

‘Thinning’ (or ‘partial depopulation’) is common practice throughout the UK broiler industry; allowing farmers to maximise productivity by utilising available space, whilst ensuring that the birds are kept at the correct stocking density to meet necessary welfare requirements. Thus, at the beginning of each flock cycle, sheds are over-stocked, and subsequently ‘thinned’, ensuring legal stocking densities are maintained. For the majority of chickens produced for the non-Halal market, flocks undergo thinning once before final depopulation. However, broiler chicken flocks produced for the Halal market may undergo multiple depopulation events *(3)*. Colonisation of broiler chickens with *Campylobacter* is difficult to prevent, especially during these thinning events *(29)*, and many studies have demonstrated that thinning is a risk factor for a broiler chicken flock to become colonised with *Campylobacter (1, 13, 24, 29, 32)*. These multiple thinning events take place to supply consumer demand for different sized birds within the Halal meat market *(10)*.

Furthermore, Halal chicken meat is more likely to be purchased from small independent retailers and butchers *(10)*, where the meat may not be sold in plastic packaging. Such conditions may decrease *Campylobacter* survival on chicken meat but increase cross-contamination between chickens. Thus, the increased number of thinning events and different retail practices may lead to differing levels of *Campylobacter*-contamination of Halal compared to non-Halal chicken.

Studies have shown that the incidence of campylobacteriosis is higher in certain ethnic groups *(7, 26, 37)*. The reason(s) for this increase in incidence are unknown but could be hypothesised to be due to these variations in farming and packaging methods within different markets, or differences in purchasing behaviour within different consumer demographics, such as Halal consumers.

This project aimed to: (i) determine the prevalence and levels of *Campylobacter* contamination of chicken meat and it’s outer packaging, produced for the Halal market in the UK; (ii) determine risk factors for *Campylobacter* contamination of UK Halal chicken meat and it’s outer packaging; (iii) determine the antimicrobial susceptibility of *Campylobacter* isolates collected at retail from chicken neck skin samples and packaging swabs of UK-produced Halal chicken meat to enable comparisons with reported data for chicken meat originating from other sectors of the industry; and (iv) investigate the genetic background of AMR phenotypes and relatedness of a selection of isolates by whole genome sequencing (WGS).

**Materials and Methods**

**Sampling.** Whole chickens were purchased from independent and supermarket retailers in England. The sampled chickens fulfilled the following criteria: (1) whole, raw, chilled chickens; (2) confirmed as reared intensively indoors (neither free-range nor organic), slaughtered and retailed in the UK by the slaughterhouse approval code displayed on the packaging or by the retailer; (3) where packaged, the packaging was unopened and undamaged; (4) clearly labelled as Halal on the packaging or, if not packaged, confirmed to be Halal by the retailer; and (5) not frozen, basted, herbed, stuffed, marinated, portioned or otherwise modified. Samples were transported to the laboratory and stored between 2-5°C before processing. All samples were processed within 48 hours of purchase and before their use-by-date, if provided.

During study design, the sampling technique and laboratory methodology was based on the FSA’s retail surveys to facilitate comparisons of the prevalence and levels of *Campylobacter* contamination between Halal and non-Halal chickens *(22)*. The study protocol was reviewed and approved by The University of Liverpool Veterinary Research Ethics Committee (Reference VREC478).

**Sample Size Calculation.** A sample size calculation was performed using the online EpiTools Epidemiological Calculator designed to estimate a proportion or apparent prevalence with specified precision *(44)* to calculate the required sample size needed to estimate the prevalence of *Campylobacter* in Halal chicken in the UK. The prevalence of *Campylobacter* in fresh Halal chicken in the UK was estimated to be similar to the most recent available survey completed by the FSA at the time of sampling (i.e. 73%) *(20)*. The calculation was based upon an infinite population size; thus, with an expected prevalence of 73%, precision of 5% and confidence level of 95%, 303 samples were required to accurately estimate the prevalence of *Campylobacter* in Halal chicken in the UK.

**Identification of Campylobacter on Packaging.** The chicken or batch of chickens was placed onto a disinfected surface and the outer packaging was swabbed with a Polywipe pre-moistened with peptone saline (MW726, Medical Wire & Equipment, Corsham, UK) using aseptic technique. If not formally packaged prior to retail, the box or bag the chicken was supplied in was swabbed. The entire packaging was swabbed twice using both sides of the Polywipe. The Polywipe was placed in a Stomacher 80 bag (Seward, Sussex, UK) with 10ml modified Exeter selective enrichment [1100ml nutrient broth (Lab M Ltd, Heywood, UK), 55ml lysed defibrinated horse blood (Southern Group Labs, Corby, UK), *Campylobacter* enrichment supplement SV59 (containing trimethoprim (10mg/l), rifampicin (5mg/l), polymyxin B (2500iu/l), cefoperazone (15mg/l) and amphotericin B (2mg/l); Mast Group Ltd, Bootle, UK) and *Campylobacter* growth supplement SV61 (containing sodium pyruvate (250mg/l), sodium (250mg/l), metabisulphite and ferrous sulphate (250mg/l); Mast Group Ltd)] and homogenised in the Seward Stomacher 80 Laboratory Blender for 30 seconds. Following homogenisation, the enrichment broth was poured into a sterile universal container (Starlab (UK) Ltd, Milton Keynes, UK) and incubated under microaerobic conditions (80% N2, 12% CO2, 5% O2 and 3% H2) at 41°C. After 24 hours incubation, 5μl of enrichment broth was streaked onto *Campylobacter*-selective blood-free agar supplemented with cefoperazone (32mg/l) and amphotericin B (10mg/l) [known as modified charcoal-cefoperazone-deoxycholate agar (mCCDA); Lab M Ltd]. Following 48-72 hours incubation under microaerobic conditions at 41°C, the plates were examined for typical *Campylobacter* colonies. Colonies were confirmed as *Campylobacter* spp. as described below.

**Identification and Enumeration of Campylobacter on Chicken Neck Skin.** The chicken was removed from its packaging, taking care not to allow contact between the chicken and its outer packaging. Using sterile instruments, 25g of skin was aseptically removed from the neck area. If less than 25g of neck skin was available, then breast skin was removed, and the amount required to make a 25g skin sample recorded. The subcutaneous fat was avoided for the sample. The 25g skin sample was placed into a Stomacher 400 bag (Seward) with 225ml buffered peptone water (Lab M Ltd) and homogenised in the Stomacher for 60 seconds. Following homogenisation, 100µl of the chicken skin homogenate was spread over the entire surface of two mCCDA plates. A 10-fold dilution of the homogenate was prepared by adding 0.5ml of the homogenate to 4.5ml Maximum Recovery Diluent (MRD; Lab M Ltd) and 100µl of this dilution was spread in duplicate onto another two mCCDA plates. These four mCCDA plates were incubated under microaerobic conditions at 41°C for 48 hours. Samples were enumerated by counting plates with <150 suspect-*Campylobacter* colonies.

In addition, 1ml of the broth was poured into a sterile universal container with 9ml modified Exeter selective enrichment broth (prepared as detailed above) and incubated under microaerobic conditions at 41°C. After 24 hours incubation, 5μl of enrichment broth was streaked onto mCCDA. Following 48-72 hours incubation under microaerobic conditions at 41°C, the plates were examined for typical *Campylobacter* colonies. Colonies were confirmed as *Campylobacter* spp. as described below.

**Confirmation of *Campylobacter* spp.** Up to four colonies per packaging or chicken neck skin sample were selected based on typical colony morphology for *Campylobacter*. Colonies were streaked in duplicate onto Columbia blood agar (Columbia Agar Base (Lab M Ltd) containing 5% (v/v) defibrinated horse blood (Southern Group Labs)). One plate was incubated for 48 hours under microaerobic conditions at 41°C and the other under aerobic conditions at 30°C, to distinguish morphologically similar *Campylobacter* and *Arcobacter* species. Genus- and species-specific polymerase chain reaction (PCR) assays were performed on suspect *Campylobacter* colonies from each sample, as described previously *(43)*.

**Antimicrobial Susceptibility Testing.** Antimicrobial susceptibility disc diffusion testing was performed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines v7.1 *(11)*. Briefly, following growth on Columbia blood agar for 48 hours under microaerobic conditions, a suspension of McFarland 0.5 standard was prepared. The inoculum was spread for confluent growth on Mueller Hinton agar (Lab M Ltd), supplemented with 5% (v/v) defibrinated horse blood and 20mg/L β-Nicotinamide adenine dinucleotide sodium salt (β-NAD, Sigma-Aldrich, Dorset, UK). Seven antimicrobial discs were applied to each plate: 30μg nalidixic acid, 5μg ciprofloxacin, 15μg erythromycin, 30μg tetracycline, 10μg gentamicin, 10μg ampicillin and 30μg clavulanate-amoxicillin (Mast Group Ltd). These antimicrobials were chosen to represent the main antimicrobial classes used in human and veterinary medicine and specifically to include those antimicrobials used within UK poultry production and those of critical importance to both human and veterinary medicine. Plates were incubated microaerobically at 41°C for 24-48 hours. The inhibition zones were measured and interpreted according to Table 1.

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**Minimum Inhibitory Concentration (MIC) to Ciprofloxacin.** A selection of 150 *C. jejuni* isolates from chicken skin samples resistant to ciprofloxacin on antimicrobial susceptibility disc diffusion testing were selected to determine the minimum inhibitory concentration (MIC) of these isolates to ciprofloxacin. Isolates were selected to ensure they were not duplicated and as many chicken samples and batches were represented as possible with a variety of antimicrobial susceptibility profiles. A pre-inoculated Mueller Hinton agar plate was prepared, supplemented as above, and a M.I.C.Evaluator (MICE) ciprofloxacin strip (Oxoid, Altrincham, UK) applied. Plates were incubated microaerobically at 41°C for 24-48 hours. The inhibition zones were measured, and the MIC interpreted according to EUCAST clinical breakpoint tables *(11)*.

**Statistical Analysis.** Calculation of the level of *Campylobacter* contamination was adapted from the Public Health England (PHE) *‘Detection and enumeration of Campylobacter species: Food, Water & Environmental Microbiology Standard Method FNES15 (F21) Version 2’* *(41)* for samples expected to have low numbers:

Where is the sum of the colonies counted on all the plates retained from two successive dilutions; is the number of plates counted at the first dilution; is the number of plates counted at the second dilution; is the dilution from which the first counts were obtained (e.g. 0.1 for 10-1 dilution) adjusted for variation in the number of combined grams of neck skin and breast skin used per sample (i.e. (Total Grams Skin/225)); and is the volume of the inoculum, in millilitres, applied to each plate.

Descriptive statistics were used to summarise key demographic characteristics of the Halal chicken neck skin samples. The main variables considered were weight, cost (£/chicken), cost (pence/kg (p/kg)), type of retailer, type of packaging, purchase date, slaughterhouse approval code and presence of visible liquid in the packaging. All statistical tests were performed using R (R 3.5.1 for Mac OS X, R Foundation for Statistical Computing, Vienna, Austria) *(42)*.

The Pearson chi-squared test of association or, if the expected frequency of any outcome <5, Fisher’s exact test was used to test the null hypothesis of no association between the explanatory variable of interest and *Campylobacter* contamination. McNemar’s chi-squared test was used for paired data. For continuous variables of interest, the distribution of data was assessed using QQ plots. If data was considered sufficiently normally distributed, parametric tests, including the Two-Sample T-test, were used to test the significance of associations. If data was not normally distributed, non-parametric tests, including Mann-Whitney U (Wilcoxon Rank Sum) and Kruskal-Wallis, were used. The threshold p-value of p<0.05 for stating statistical significance was used for all statistical tests.

**Multivariable Analysis.** Binary logistic regression analysis was used to assess risk factors for Halal chicken neck skin samples to (i) be *Campylobacter-*positive, (ii) have the highest level of *Campylobacter* contamination (>1000 cfu/g), or contain at least one *Campylobacter* isolate resistant to (iii) erythromycin, (iv) ciprofloxacin, (v) at least one test antimicrobial class (AMR) and (vi) at least three tested antimicrobial classes, whilst controlling for potential confounding. Outcome data were collapsed to the sample level. Therefore, a chicken neck skin sample with at least one resistant *Campylobacter* isolate was classed as resistant for analysis. The following independent, binomial, continuous and categorical predictor variables were created from data collected about each chicken neck skin sample: (i) Weight (g), (ii) Cost (£/chicken), (iii) Cost (p/kg), (iv) Month Purchased (March, April or May), (v) Presence of Visible Liquid in Packaging (Scored from 0-3), (vi) Presence of Visible Liquid in Packaging (Binomial Outcome: Yes/No), (vii) Approval Code, (viii) Type of Retailer (Supermarket or Specialist Halal Retailer), (ix) Type of Retailer (Supermarket, Halal Butcher or Online Halal Retailer), (x) Chicken Supplied Individually or Supplied in a Batch, (xi) Packaged Chicken or Chicken Retailed Loose, and (xii) Campylobacter Status of Packaging.

Univariable logistic regression was used to assess the association between the independent predictor variables and the six binary (yes/no) outcome variables. For the antimicrobial resistance outcomes, the following three predictor variables were explored: Weight, Presence of Visible Liquid in Packaging (Yes/No) and Packaged Chicken or Chicken Retailed Loose. For each predictor variable explored, a likelihood ratio chi-squared test (LRT) was used to assess fit compared to a null model. Where appropriate for categorical variables, the reference category was altered to aid model interpretation. Generalised additive models (GAM) were created and plotted to assess if continuous explanatory variables had a linear relationship with the outcome. If not, polynomial terms were fitted to the explanatory variables as appropriate. Independence between explanatory variables was assessed using the Pearson correlation coefficient. For highly correlated variables (-0.7 ≥ ρ ≥ 0.7), only variables with the lowest P-value were considered for inclusion in the GLMMs. To aid model convergence, continuous explanatory variables were rescaled using the scale() function in R, which subtracts the mean and divides by the standard deviation of the covariate. The odds ratios (ORs) and 95% CI for scaled continuous variables (Cost (£/chicken), Cost (p/kg) and Weight (g)) were adjusted by dividing the output by the standard deviation of the variable. Due to clustering of chicken neck skin samples in a nested data structure (i.e. chicken neck skin sample within package within shop within slaughterhouse), explanatory variables were tested in an initial mixed-effects logistic regression model if the LRT p-value <0.25. The package, retail outlet and slaughterhouse were included as random variables within the model. Final models were constructed by manual backwards stepwise procedures where variables with LRT p-value <0.05 were retained in order to produce a model fit with the lowest Akaike Information Criterion (AIC) possible. The predictor variables weight, cost (£/chicken) and cost (p/kg) were highly correlated; only weight was included in the initial mixed-effects logistic regression models because it was considered a better proxy for bird age than cost variables. As birds were purchased in multipacks, in boxes and as part of money-saving deals, weight was also the most accurately measured variable of these. The intra-class correlation coefficient (ICC) was estimated using the Binary Linear Model Method as described by Goldstein, Browne and Rasbash (2002). In this method, the outcome is assumed to be linear and the mixed-effects model is rerun as a linear mixed-effects model. The null and final models were re-run as linear mixed-effects models and the variation due to each random effect and the fixed effects overall estimated. All statistical modelling was performed using R (R 3.5.1 for Mac OS X, R Foundation for Statistical Computing, Vienna, Austria) (R Core Team, 2015).

**Whole Genome Sequencing.** Fifty *C. jejuni* isolates, including ten packaging isolates and 40 chicken neck skin isolates, from fifty different samples, were selected for further investigation by WGS. Selected isolates displayed a multidrug-resistant (MDR; resistance to ≥3 of the tested antimicrobial classes) resistance profile and were selected to represent as many shops and slaughterhouses, with known approval codes, as possible. Isolates were not selected if the slaughterhouse or cutting plant of origin was unknown.

Briefly, following growth of 20 selected isolates on Columbia blood agar for 48 hours under microaerobic conditions, DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Manchester, UK). DNA was then quantified and assessed for purity using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Cheshire, UK), a Qubit 3.0 fluorometer, dsDNA Broad Range (BR) assay (Invitrogen Life Technologies, Cheshire, UK), and by gel electrophoresis, before being forwarded to the Centre for Genomic Research (CGR) at the University of Liverpool for WGS by HiSeq Illumina next-generation sequencing (Illumina, San Diego, USA). At CGR, libraries were made by Dr Margaret Hughes and sequenced by Dr Anita Lucaci, using methods previously described *(30)*.

For an additional 30 isolates, a single colony was selected from 48h old cultures and mixed in 100μl 1X phosphate-buffered saline (Thermo Fisher Scientific). The inoculum was streaked on a Mueller Hinton agar plate, supplemented as above, and incubated microaerobically at 41°C for 24-48 h. All of the bacterial culture was removed from each plate and placed into individual barcoded Microbank tubes supplied by MicrobesNG (Birmingham, UK), who were subsequently sent the isolates for WGS by HiSeq Illumina next-generation sequencing.

All 50 isolates were analysed using the bioinformatic pipeline ‘Campype’, as previously described *(30)*. Quality control (QC) metrics for all 50 sequenced isolates are available in Table S1.

**Results**

**Sampling Strategy & Study Population.** From February to May 2017, 405 Halal chickens were purchased from 44 individual independent and supermarket retail outlets in England. Convenience sampling was used to select retailers; 295 chickens were purchased from 33 retail premises located in the Northwest of England, 49 chickens from six retailers in the East of England and 61 chickens from five online retailers located in England. Sixty-four chickens were purchased from five supermarkets belonging to three of the nine supermarket retailers with the largest market shares; three branches of two supermarkets were visited in person and chickens were purchased from two online supermarket services. The other 39 retail premises were specialist Halal retailers; 305 chickens were purchased from 36 Halal butchers and 36 chickens were purchased from three online Halal retailers. A median number of ten chickens were purchased from each retail outlet (Minimum = 2, Maximum = 16). Approval codes, identifying the processing slaughterhouse and/or cutting plant, were obtained for 347 chickens.

**Types of Halal Retailer and Halal Packaging Characteristics.** There were major differences in the packaging of chicken from different retailers. Chickens were purchased either from a butcher’s counter in a Halal shop and then supplied to the consumer loose in a plastic carrier bag and/or cardboard box or chickens were purchased from an online Halal retailer or from a major supermarket and were packaged in sealed plastic packaging. Chickens were either supplied to the consumer as individual chickens or in batches of multiple chickens. Overall, 13 different variations in packaging were identified (Figure 1). For analysis, chickens were categorised as ‘Chickens Sold in Packaging’ and ‘Chickens Retailed Loose’ (i.e. chickens supplied loose in cardboard boxes and carrier bags). Extended analysis with further packaging and retailer categorisations can be found in Table S2.

**Prevalence and number of *Campylobacter* in chicken neck skin samples and packaging.** *Campylobacter* spp. were isolated and confirmed by PCR from 265 neck skin samples (65.4%; 95% confidence interval (CI): 60.8-70.1%). Of these, 252 samples (62.2%; 95% CI: 57.5-66.9%) were positive for *C. jejuni* and 36 samples (8.9%; 95% CI: 6.1-11.7%) for *C. coli*. Twenty-three samples (5.7%; 95% CI: 3.4-7.9%) were positive for both *C. jejuni* and *C. coli*. Of 405 chicken neck skin samples, it was possible to enumerate *Campylobacter* spp. from 204 samples. The highest count detected was 39,718 cfu of *Campylobacter* per gram of chicken neck skin and 13.8% (95% CI: 10.5-17.2%) of the neck skin samples had counts >1000 cfu/g. Due to neck skin trimming in the processing plants (FSA, 2017c) and difficulty obtaining 25g of neck skin from smaller-sized chickens, 25g of chicken neck skin was not available from each chicken. Therefore, further skin was collected from the breast skin of the chicken to make a 25g chicken skin sample. An average of 12.02g of chicken neck skin was present in each 25g chicken skin sample.

A comparison of the total number of samples, the number of *Campylobacter*-positive chicken neck skin samples and the percentage of chicken neck skin samples with each level of *Campylobacter* contamination is shown in Table 2. There was no significant difference in the number of *Campylobacter*-positive chicken neck skin samples or in detection of >1000 cfu/g between packaged birds and birds retailed loose (Positive: Χ2=0.2, df=1, p=0.6; >1000 cfu/g: Χ2=1.2, df=1 p=0.3).

Chickens were packaged by retailers individually and in batches and 164 packaging sample swabs were collected from the packaging containing the 405 sampled chickens (Table S3). *C*. *jejuni* was isolated and confirmed by PCR from 28 packaging swabs (17.1%; 95% CI: 11.3-22.8%), which contained a total of 99 chickens. No other *Campylobacter* spp. were identified from packaging swabs. There was a statistically significant difference in packaging testing positive for *Campylobacter* between packaging from chickens retailed loose and that from packaged birds (Χ2=9.1, df=1, p=0.003).

**Descriptive Statistics.** Chickens were assigned into three weight categories defined by the arbitrary weight ranges used by the FSA in their retail surveys (17, 18, 20). ‘Small’ chickens weighed <1400g, ‘medium’ chickens weighed 1400-1750g, and ‘large’ chickens weighed >1750g (Table 3). Large birds were not significantly more likely to be Campylobacter-positive; however, large birds did have a statistically significant higher number of samples with >1000 cfu/g (Χ2=17.7, df=1, p<0.001). There was a trend that as bird size increased, the likelihood of samples being contaminated with the highest levels of contamination also increased (Χ2 for Trend=9.0, df=2, p=0.003). Campylobacter-positive birds (Mann-Whitney U Test: W=15828, p=0.02) and those be contaminated with >1000 cfu/g (W=7780, p=0.01) were significantly heavier in weight compared to Campylobacter-negative birds and those with <1000 cfu/g, respectively.

Chicken neck skin samples with visible liquid in the packaging were significantly less likely to be *Campylobacter*-positive than those without visible liquid in the packaging. There was a significant difference between the level of visible liquid in the packaging and whether the chicken neck skin samples were from chickens sold in packaging or chickens retailed loose (Χ2=83.7, df=1, p<0.001), with more visible liquid present in the packaging of packaged chickens than that of the chickens retailed loose (Tables S4-S6).

There was a significant association between the slaughterhouse approval code displayed on the packaging and total number of *Campylobacter-*positive chicken neck skin samples (Fisher’s Exact Test: p<0.001), detection of >1000 cfu/g (Fisher’s Exact Test: p<0.001) and level of *Campylobacter* contamination (Fisher’s Exact Test: p<0.001) (Table S7). However, some approval codes were only sampled on one day, and in some cases only from one shop on one day, which may reflect the *Campylobacter* status of one flock of chickens, or batches of chickens, being processed in the slaughterhouse on the same day, and would not be representative of the approval code from which they originate. For these reasons, statistical differences detected between samples collected in different months were disregarded as truly significant. However, when approval codes sampled on more than one day were compared, there is a significant association between approval code and total number of positive *Campylobacter* chicken neck skin samples (Fisher’s Exact Test: p<0.001), number of chicken neck skin samples with the highest level of contamination (>1000 cfu/g) (Fisher’s Exact Test: p<0.001) and level of *Campylobacter* contamination (Fisher’s Exact Test: p<0.001).

**Phenotypic Antimicrobial Resistance.** Of the 265 chicken neck skin samples from which Campylobacter spp. were isolated, 252 samples were positive for C. jejuni (N=405; 62.2%; 95% CI: 57.5-66.9%) and 36 samples were positive for C. coli (N=405; 8.9%; 95% CI 6.1-11.7%). The highest prevalence of resistance was seen to tetracycline (205/405; 50.6%; 95% CI: 45.7-55.5%; Table 4) with high prevalences of resistance to ampicillin (199/405; 49.1%; 95% CI: 44.3-54.0%; Table S8) and ciprofloxacin (170/405; 42.0%; 95% CI: 37.3-46.8%; Table 4). The prevalence of resistance to the macrolide, erythromycin, was 2.47% (10/405; 95% CI: 0.96-3.98%; Table 4).

Of 202 *C. jejuni* isolates collected from 156 chicken neck samples resistant to ciprofloxacin, 150 isolates were selected for determination of their MIC to ciprofloxacin. One hundred and thirty-eight isolates had an MIC above the EUCAST CIP MIC breakpoint >0.5μg/ml; 81.3% (95% CI: 75.1-87.6%) had an MIC >32μg/ml.

Twenty packaging swabs (N=164; 12.2%; 95% CI: 7.19-17.2%) contained at least one AMR *C. jejuni* isolate and 17 (N=164; 10.4%; 95% CI: 5.70-15.0%) of these were MDR. The highest prevalences of resistance were seen to the quinolones, ciprofloxacin and nalidixic acid, (N=164; 11.6%; 95% CI: 6.69-16.5%), tetracycline (N=164; 11.0%; 95% CI: 6.19-15.8%) and ampicillin (N=164; 11.0%; 95% CI: 6.19-15.8%).

Extended data of the total number of chicken neck skin and packaging samples containing at least one *Campylobacter* spp. isolate resistant to the tested antimicrobials and MIC to ciprofloxacin are available in Tables S8-S10.

A total of 1031 *Campylobacter* isolates; 938 *C. jejuni* (848 chicken isolates, 90 packaging isolates)and93 *C. coli* (93 chicken isolates only) were isolated from the chicken neck skin and packaging samples. A total of 487 unique *Campylobacter* isolates were identified from the antimicrobial resistance profiles of 941 *Campylobacter* isolates collected from 265 chickens (N=405). Seventy-one chicken neck skin samples were found to be carrying more than one unique AMR *Campylobacter* isolate with different AMR profiles. A total of 44 unique *C. jejuni* isolates were identified from the antimicrobial resistance profiles of 90 *C. jejuni* isolates from 28 packaging swabs (N=164). The percentage of unique *Campylobacter* spp., *C. jejuni* and *C. coli* isolates from 405 chicken neck skin samples and *C. jejuni* isolates from 164 packaging swabs demonstrating resistance to at least one of the tested antimicrobials is included in the Tables S11 and S12. The most common resistance profiles for *Campylobacter* spp. isolated from both neck skin and packaging samples were tetracycline-ampicillin-ciprofloxacin-nalidixic-acid resistance and tetracycline-ampicillin resistance (Table S13). This was also the most common resistance profile for both *C. jejuni* and *C. coli* isolates.

**Multivariable Analysis.** Univariable logistic regression analysis is available in Tables S14 and S15. Four significant mixed-effects logistic regression models were constructed for the outcomes investigated (Table 5). For every kilogram increase in chicken weight (i.e. as chicken carcasses increase in size, and are therefore older birds), the chicken neck skin sample was significantly more likely (OR=8.3; 95% CI: 6.7-10.0; p=0.01) to be Campylobacter-positive and significantly less likely to carry AMR and MDR Campylobacter spp. (AMR: OR=0.997, 95% CI: 0.995-0.999; MDR: OR=0.998, 95% CI: 0.997-0.9998). The presence of visible liquid in the packaging was also protective against the chicken being positive for Campylobacter (OR=0.03; 95% CI: 0.02-0.4).

The estimated ICC suggested that 28.7% of the variation in the risk of a sample being *Campylobacter-*positive was due to packaging-level effects, 35.7% was due to shop-level effects, 10.2% was due to slaughterhouse-level effects and 25.4% was due to other effects. The fixed effects in the final model explained very little of the variation; only 1.4% of the sample-level variation, 4.3% of the packaging-level variation, 2.6% of the shop-level variation and 11.7% of the slaughterhouse-variation were explained by the fixed-effects. The estimated ICC for the outcome carriage of AMR *Campylobacter* spp. suggested that 29.6% of the variation in the risk of a sample carrying AMR *Campylobacter* spp. was due to packaging-level effects, 26.2% was due to shop-level effects, 11.1% was due to slaughterhouse-level effects and 33.1% was due to other effects. The estimated ICC for the model for the outcome carriage of MDR *Campylobacter* spp. suggested that 14.1% of the variation in the risk of a sample carrying MDR *Campylobacter* spp. was due to packaging-level effects, 30.8% was due to shop-level effects, 4.4% was due to slaughterhouse-level effects and 50.8% was due to other effects.

## **Whole Genome Sequencing: MLST Types and Characterisation of Resistance Genes.** Fifty isolates were selected for further investigation of MDR resistance profiles, including ten isolates from different packaging samples and 40 isolates from different chicken skin samples. All isolates resistant to four or more antimicrobial classes (n=4) were selected for investigation. All sequenced isolates were from samples purchased from a Halal butcher or a supermarket, none of the isolates were from samples purchased from online Halal retailers. All 50 isolates were re-confirmed as *C. jejuni* (all primary hits >99%) (Table S16).

WGS revealed a range of 17 different MLST types in the 50 isolates; five MLST types were found in only one isolate, whilst 12 were found in multiple isolates. The phylogenetic tree of the 50 isolates (Figure S1) shows that there was similarity between isolates from chickens purchased from a supermarket and those purchased from a Halal butcher. Core genome genealogies were visualised using Interactive Tree of Life (iTOL) v3 *(35)* and shared with associated metadata at: <http://itol.embl.de/tree/138253218159255561537277855>.

Examination of the 50 isolates revealed resistance genes conferring resistance to tetracycline, beta-lactams and aminoglycosides, with resistance mechanisms including tetracycline resistance ribosomal protection proteins (*tetM*, *tetW*, *tetO*, and *tetS*) (TetR RPP), resistance-nodulation-cell division (RND) antibiotic efflux pump, class A and D beta-lactamases, aminoglycoside nucleotidyltransferase (*ant(6)*), *macB* (subunit of antibiotic efflux pump), the aminoglycoside acetyltransferase (*aac(3)*), and an ATP-binding cassette (ABC) antibiotic efflux pump. There was not a great deal of variation between the resistance genes carried by the isolates. All of the isolates carried genes for at least five of the resistance mechanisms listed and the vast majority of isolates carrying TetR RPP, RND, Class D beta-lactamases, *macB* and ABC (Table S16). There was very little variation in the virulence genes carried by the isolates, with similarity grouped within the MLST types and clonal complexes of the isolates.

**Discussion**

This study estimates the prevalence of *Campylobacter* in UK Halal chicken to be 65.4% (95% CI: 60.8-70.1%). Concerningly, high prevalences of resistance were seen to the fluoroquinolone ciprofloxacin (170/405; 42.0%; 95% CI: 37.3-46.8%). However, macrolide resistance was low, with <2.5% of chicken neck skin samples (10/405; 95% CI; 1.0-4.0%) carrying a *Campylobacter* isolate positive for erythromycin-resistance.

In 2010, the Food Standards Agency (FSA) and the UK poultry industry set up a joint target to reduce the prevalence of the most *Campylobacter-*contaminated chickens (>1000 cfu/g) to below 10% post-slaughter by the end of 2015. The FSA has conducted four annual retail surveys since 2014, estimating a reduction in the prevalence of *Campylobacter* in raw chicken meat from 73.2% to 41.2% and a significant decrease in the percentage of chickens with the highest levels of *Campylobacter* (>1000 cfu/g) from 19.7% to 3.5% *(15)*. The third year of the FSA’s retail survey (August 2016-July 2017) coincided with the sampling time-frame of this study *(17)*. In the design of this study, the same sampling technique and laboratory methodology was used as in the FSA retail surveys to allow comparisons of the prevalence and levels of *Campylobacter* contamination between Halal and non-Halal chickens. This study found a 65.4% prevalence of *Campylobacter-*positive chicken neck skin samples and 13.8% of samples had the highest level of contamination (>1000 cfu/g). Both of these values are considerably higher than the FSA’s findings of 54% and 6.5% *(17)* (Table S17). The last two quarters of the third year of the FSA’s retail survey (January-March 2017 and April-July 2017) found prevalences of 48.8% *(16)* and 56.9% *(17)* with the highest level of *Campylobacter* contamination in 6.5% and 5.9%, samples, respectively. When compared with these last two quarters to account for possible seasonal variation, this study still found much higher prevalences and levels of *Campylobacter* contamination. In the first two years of the FSA’s retail survey, packaging samples were also tested for *Campylobacter*. In the first year, 6.8% of packaging samples were *Campylobacter*-positive *(20)*, decreasing to 5.5% (N=3002) in the second year *(18)*. These prevalences are lower than the 17.1% of *Campylobacter-*positive packaging samples found in this survey. It should be noted that it was not possible to sample the same geographical area as the FSA in this study. Sampling for the FSA surveys was stratified with retail outlets sampled based on market share data and spread across the entirety of the UK to reflect population sizes. Due to funding limitations, this study focussed sampling to the local region in areas with high densities of Muslim residents and utilised online shopping resources. As sample collection was not identical between this study and those of the FSA, this may have introduced a level of sampling bias and should be taken into consideration when comparing the studies’ prevalence estimates.

The results of this study, when compared to those of the FSA, are consistent with Halal chicken having higher levels of contamination than non-Halal chicken. This difference could arise at one or more stages of production and/or retail, including at rearing, in the processing plant or at point-of-retail-sale. Specific hypotheses for differences between Halal and non-Halal chicken meat include an increased number of partial flock depopulation (or ‘thinning’) events, and therefore biosecurity breaches, on broiler farms rearing Halal broilers, and differences in the way meat is retailed, including less plastic packaging, which may decrease *Campylobacter* survival in chicken meat but increase cross-contamination between chickens. In the UK, Halal consumers have two main options for purchasing Halal meat; major supermarkets and independent Halal butchers *(10)*. WGS demonstrated the similarity between isolates purchased from supermarkets and those purchased from Halal butchers. It is worth noting that the isolates selected for sequencing had an AMR or MDR phenotype. This resulted in the selection of proportionally more isolates from samples collected from Halal butchers than supermarkets, which were also clustered within two slaughterhouse approval codes (Approval Codes 4 and 8), which are specific to a processing plant. This may have resulted in the introduction of selection bias and impacted the results of the WGS. Thus, the clustering of these isolates may also account, at least in part, for the genetic relatedness demonstrated by WGS of isolates originating from supermarkets, halal butchers and/or specific slaughterhouses. However, there was similarity between isolates from chickens processed in particular slaughterhouses. This was indicated in the repeated occurrence of ST-354 and ST-573 amongst isolates from Approval Code 4 and demonstrates genetic relatedness between batches of chickens processed in a particular processing plant.

There were major differences in the packaging of chicken from different retailers. Chickens were purchased either from a butcher’s counter in a Halal butcher’s shop and then supplied to the consumer loose in a plastic carrier bag and/or cardboard box or chickens were purchased from an online Halal retailer or from a major supermarket and were packaged in sealed plastic packaging. Loose chickens are at risk of cross-contamination during transport to the retailer and whilst on sale; for example, during close-contact on the butcher’s counter. During handling, preparation and sale of the meat, cross-contamination of the chicken or packaging may occur from the butcher’s hands, utensils or work surfaces, particularly if the meat is prepared or portioned for the customer. Further cross-contamination may occur during transport if the chicken is in contact with other purchased raw chicken or meat products and during storage in the consumer’s home. Additionally, packaging of loose chickens is often in used cardboard boxes, which may be contaminated, or carrier bags, which are not sterile. During sampling for this study, chickens purchased from Halal butchers were supplied in carrier bags that were branded with the logos of other retailers, indicating that these bags were being re-used and may not be sanitary. There is much less risk of cross-contamination between packaged chickens purchased in supermarkets and from online retailers provided the process by which they are packaged minimises cross-contamination.

Other studies comparing the *Campylobacter* contamination of chicken meat from different retailers have found varying levels of difference. Meldrum and Wilson *(38)* did not find a statistically significant difference between the prevalence of *Campylobacter* in chicken purchased from supermarkets and butchers in the UK. However, Harrison *et al.* *(33)* found a significantly (p<0.05) higher prevalence of *Campylobacter* in three supermarkets’ chicken compared to chicken purchased from three local butchers’ shops. Most notably, in the third year of the FSA’s retail survey *(14, 17)*, non-major retailers had significantly more samples with levels of *Campylobacter* >1000 cfu/g (17.1%) than the average among the nine named supermarkets (5.6%). Furthermore, in the fourth year of the FSA’s retail survey *(14)*, *Campylobacter* was detected in 75% of chicken skin samples obtained from non-major retail shops and 15% had counts >1000 cfu/g.

The UK poultry industry is highly integrated, with the top four integrator companies, who supply the major supermarket retailers, accounting for over 80% of total UK production *(9, 36)*. The remaining 20% of production is supplied by independent companies, one of whose main markets is the Halal sector *(36)*. Recently, due to retailer pressure and responding to the FSA’s target to reduce *Campylobacter* in chicken, the largest integrator companies have overhauled their approach to biosecurity along the whole farm to fork chain. This has included reducing the number of thinning events on broiler farms to usually no more than one per flock and improving adherence to biosecurity protocols. Whilst this behaviour change has been widely seen within these largest integrator companies, the pressure to change may not have been felt as widely on farms producing chicken for the Halal market. This may contribute to why this survey has found a higher prevalence of *Campylobacter*-positive chicken samples and more samples with the highest level of contamination than the corresponding FSA survey *(17)*, which predominantly surveyed those retailers supplied by the largest integrators.

This retail survey did not find a statistically significant difference in *Campylobacter-*positive chickens or in detection of >1000 cfu/g between packaged birds and birds retailed loose (Positive: p=0.6; >1000 cfu/g: p=0.3). Therefore, the higher prevalence and levels of *Campylobacter* in this study compared to other UK retail surveys is unlikely to be due to differences in the way Halal meat is supplied to consumers in the UK. However, packaging from chickens retailed loose was more likely to test positive for *Campylobacter* than packaged chickens (p=0.003) and 8.7% of packaging samples from packaged chickens were contaminated with *Campylobacter,* whereas 27.8% of chickens retailed loose were *Campylobacter-*positive.

It was investigated if the presence of liquid in the packaging promotes *Campylobacter* survival. *Campylobacter* spp. survive best in wet conditions and at low temperatures *(8)* with survival being extremely poor in dry conditions *(34)*. In this survey, there were mixed results, with visible liquid in the packaging being both a risk and protective factor for *Campylobacter-*positive neck skin samples. In the mixed-effects models for *Campylobacter*-positive chicken neck skin samples, the presence of visible liquid in the packaging was protective against *Campylobacter* positive chicken. However, this protective effect needs to be interpreted with care, as the presence of visible liquid in the packaging may be confounded by the type of packaging. For example, there was significantly more visible liquid present in the packaging of chickens sold in packaging than that of the chickens retailed loose, due to the sealed plastic packaging retaining liquid within the packaging. However, there was also a significant association between the level and presence of visible liquid in the packaging and the total number of positive *Campylobacter* chicken neck skin samples and detection of >1000 cfu/g. This indicates that the presence of liquid in the packaging promotes the survival of *Campylobacter* andpresents a risk of cross-contamination of which consumers should be aware.

Chickens were assigned into the three weight categories used by the FSA in their recent retail surveys 2017 *(17, 18, 20)*. This enabled analysis to determine whether size, which may be linked to the age of the chicken at slaughter and the number of times the flock had been thinned, is associated with the level of *Campylobacter* present. Thus, it can be hypothesised that heavier birds are more likely to be *Campylobacter-*positive. Cost was also used as a proxy for age, as heavier, and therefore older, birds are more expensive. However, due to differences in type of retailer, location and available discounts, cost is not as accurate a proxy for age as weight. Thus, cost variables were only included in the univariable logistic regression analysis and the variable of ‘Weight (g)’ focussed upon instead for the descriptive statistics and multivariable logistic regression analysis. In this study, birds were statistically more likely to be *Campylobacter-*positive (p=0.02) and to be contaminated with >1000 cfu/g (p=0.01) as weight increased. Mixed-effects logistic regression analysis also found a relationship between weight and a sample being *Campylobacter-*positive (OR=8.3 (95% CI: 6.7-10.0); p=0.01) and having the highest level of *Campylobacter* contamination (OR=6.8 (95% CI: 5.9-7.8); p<0.001). This supports the hypothesis that heavier, and therefore older, birds pose a greater risk of *Campylobacter* contamination. These findings are similar to the first two years of the FSA’s retail survey, which found that medium and large birds had a significantly higher number of samples with >1000 cfu/g *(18, 20)*.

WGS of a selection of isolates revealed resistance genes conferring resistance to tetracyclines, beta-lactams and aminoglycosides, which was consistent with the AMR phenotypes demonstrated on antimicrobial susceptibility testing. Phenotypically, the highest prevalence of resistance in chicken neck skin samples was seen to tetracycline (50.6%; 95% CI: 45.7-55.5%). Moreover, a high proportion of samples (57.3%;95% CI: 52.5-62.1%) contained at least one AMR and 38.5% (95% CI:33.8-43.4%) contained at least one MDR (resistance to ≥3 antimicrobial drug classes) *Campylobacter* isolate. In this study, of 427 unique *C. jejuni* isolates and 60 *C. coli* isolates from chicken neck skin samples, 47.3% (95% CI: 42.6-52.0%) of *C. jejuni* and 43.3% (95% CI: 30.8-55.9%) of *C. coli* isolates were ciprofloxacin-resistant. This level of ciprofloxacin-resistance is similar to that reported in the VARSS Reports *(46, 47)*, where resistance to ciprofloxacin was detected in a relatively high proportion of *C. jejuni* isolates from broilers (40.6% and 48.0%, respectively). Similarly, in the second year of the FSA’s retail survey *(19)*, 54.2% of the *C. jejuni* isolates (237/437; 95% CI: 49.4-59.0%) and 48.1% of the *C. coli* isolates (52/108; 95% CI: 38.4-58.0%) isolates examined from 547 chicken neck skin samples were resistant to ciprofloxacin. Of 150 isolates resistant to ciprofloxacin on disc diffusion susceptibility testing; 138 isolates had an MIC above the EUCAST CIP MIC breakpoint >0.5μg/ml and 81.3% (95% CI: 75.1-87.6%) had an MIC >32μg/ml.

The increasing prevalence of ciprofloxacin-resistant *Campylobacter* from poultry meat at retail in the UK over the past decade is particularly interesting given that in 2012 the British Poultry Council (BPC) started an antimicrobial stewardship scheme and have since prohibited the prophylactic use of antibiotics and committed to only use macrolides and fluoroquinolones as a last resort *(47)*. There has been a reported 96% reduction in the use of fluoroquinolones within the broiler industry and, in 2018, only 0.1% of prescribed antibiotics were fluoroquinolones *(46)*. Moreover, in 2014, usage in broilers was 48.75 mg/kg, which was dramatically reduced to 9.85 mg/kg in 2017, a total reduction of 80% *(5)*.

Mutations in *gyrA* in *C. jejuni*, which is associated with changes in DNA supercoiling,have a positive influence on fitness and mutant fluoroquinolone-resistant strains can outcompete wild-type strains in drug free competitive index experiments *(31)*. These strains have also been shown to persist long after removal of the selective pressure on farms *(28)* and it is possible that resistance is persisting despite changes in prescribing practice in the UK.

This study has demonstrated that Halal chicken has a higher prevalence of *Campylobacter* than chicken not produced for the Halal market. In addition, the packaging of chicken that is retailed loose, such as from a butcher’s counter is more likely to be contaminated with *Campylobacter* than chicken packaged in plastic packaging. Consumers should be aware of this increased risk and interventions should be introduced to improve the food safety of Halal chicken to reduce this risk to public health. Further work should be undertaken to understand the reasons behind this increased risk to Halal consumers. Similar levels of antimicrobial resistance in UK-produced Halal chicken were found as other studies sampling chicken at retail in the UK and across the EU. The high levels of resistance to the critically-important antimicrobial ciprofloxacin is of public health concern. Given the restricted use of this drug in the UK poultry flock and the association between cases of human campylobacteriosis and poultry meat and products, this raises concerns about the availability of effective antimicrobial agents for the treatment of severe *Campylobacter* infections and the ultimate impact on human clinical outcomes. Surveillance of antimicrobial resistance in *Campylobacter* from retail chickens must be continued and the poultry industry must endeavour in their efforts to reduce antimicrobial usage.

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**Supplemental Material**

Supplemental Material associated with this article can be found online at: [URL to be completed by the publisher].

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The research materials supporting this publication can be accessed by contacting the corresponding authors.

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**Figure Legends**

Figure 1: Types of packaging from which 405 chicken neck skin and 164 packaging samples were collected. Thirteen types of packaging were grouped into two categories: ‘Chickens Sold in Packaging’ and ‘Chickens Retailed Loose’ (i.e. chickens supplied loose in cardboard boxes and carrier bags).

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Table 1: Details of breakpoints used to ascertain susceptibility of isolates following antimicrobial susceptibility disc diffusion testing.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antimicrobial** | **Concentration of Antimicrobial Disc (μg)** | **Antimicrobial Class** | **Breakpoint (R≤ Diameter in mm)** | **Reference** |
| Erythromycin | 15 | Macrolide | *C. jejuni*: 19;  *C. coli*: 23 | EUCAST CBPs *a* |
| Tetracycline | 30 | Tetracycline | *C. jejuni/C. coli*: 29 | EUCAST CBPs *a* |
| Gentamicin | 10 | Aminoglycoside | *C. jejuni*: 19 | EUCAST ECOFFs *b* |
| Amoxicillin-Clavulanate | 20/10 | β-lactam | *Enterobacteriaceae*:14 | EUCAST CBPs *a* |
| Ampicillin | 10 | β-lactam | *Enterobacteriaceae*: 14 | EUCAST CBPs *a* |
| Nalidixic Acid | 30 | Quinolone | *Campylobacter* spp.: 19 | BSAC CBPs *c* |
| Ciprofloxacin | 5 | Fluoroquinolone | *C. jejuni/C. coli*: 25 | EUCAST CBPs *a* |

*a*European Committee on Antimicrobial Susceptibility Testing (EUCAST) human clinical breakpoints (CBPs) *(11)*

*b* EUCAST epidemiological cut-off values (ECOFFs) *(12)*

*c*British Society for Antimicrobial Chemotherapy CBPs *(6)*

Table 2: Comparison of total number of samples, number of *Campylobacter*-positive chicken neck skin samples confirmed by PCR, and percentage of chicken neck skin samples with each level of *Campylobacter* contamination, from 405 Halal chickens sampled from retailers between February and May 2017. Samples have been categorised as ‘Packaged Chickens’ and’ Chickens Retailed Loose’.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **Overall** | **Packaged Chickens** | **Chicken Retailed Loose** |
| **Total Number of Samples** | | 405 | 100 | 305 |
| **Overall Percentage of *Campylobacter-*Positive Samples**  **(% (95% CI; n))** | | 65.4  (60.8-70.1; 265) | 63.0  (53.5-72.5; 63) | 66.2  (60.9-71.5; 202) |
| **Percentage of Chicken Neck Skin Samples with Each Level of *Campylobacter* Contamination**  **(% (95% CI; n))** | **<10 cfu/g** | 49.6  (44.8-54.5; 201) | 61.0  (51.4-70.6; 61) | 45.9  (40.3-51.5; 140) |
| **10-99 cfu/g** | 14.1  (10.7-17.5; 57) | 12.0  (5.63-18.4; 12) | 14.8  (10.8-18.7; 45) |
| **100-1000 cfu/g** | 22.5  (18.4-26.5; 91) | 17.0  (9.64-24.4; 17) | 24.3  (19.5-29.1; 74) |
| **>1000 cfu/g** | 13.8  (10.5-17.2; 56) | 10.0  (4.12-15.9; 10) | 15.1  (11.1-19.1; 46) |

Table 3: Number and percentage of positive chicken neck skin samples and number and percentage of chicken neck skin samples with each level of Campylobacter contamination (cfu/g) in relation to chicken weight, from 405 Halal chickens sampled from retailers between February and May 2017.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Chicken**  **Weight Category** | **Total** | **Overall Culture Positive for *Campylobacter*** | | **cfu of *Campylobacter* spp. per g chicken neck skin sample** | | | | | | | |
| **<10** | | **10-99** | | **100-1000** | | **>1000** | |
| **n** | **% (95% CI)** | **n** | **% (95% CI)** | **n** | **% (95% CI)** | **n** | **% (95% CI)** | **n** | **% (95% CI)** |
| **Small (<1400g)** | 269 | 176 | 65.4  (59.7-71.1) | 137 | 50.9  (45.0-56.9) | 34 | 12.6  (8.67-16.6) | 66 | 24.5  (19.4-29.7) | 32 | 11.9  (8.03-15.8) |
| **Medium (1400-1750g)** | 83 | 51 | 61.4  (51.0-71.9) | 45 | 54.2  (43.5-64.9) | 16 | 19.3  (10.8-27.7) | 15 | 18.1  (9.79-26.4) | 7 | 8.43  (2.46-14.4) |
| **Large (>1750g)** | 53 | 38 | 71.7  (59.6-83.8) | 19 | 35.8  (22.9-48.8) | 7 | 13.2  (4.09-22.3) | 10 | 18.9  (8.33-29.4) | 17 | 32.1  (19.5-44.6) |

Table 4: Comparison of total number of samples, percentage of Campylobacter-positive chicken neck skin samples and outer packaging samples confirmed by PCR, and percentage of chicken neck skin samples and outer packaging samples containing at least one Campylobacter isolate demonstrating resistance to erythromycin, tetracycline and ciprofloxacin, from 405 Halal chickens and 164 packaging swabs sampled from retailers between February and May 2017. Samples have been additionally categorised as either Chickens Sold in Packaging or Chickens Sold Loose.

|  |  |  |  |
| --- | --- | --- | --- |
|  | | | **Overall** |
| **Total Number of Samples** | **Chicken Neck Skin Samples** | | 405 |
| **Packaging Samples** | | 164 |
| **Overall Percentage of *Campylobacter* Positive Samples (%)** | **Percentage of Positive Chicken Neck Skin Samples (95% CI; N)** | | 65.4  (60.8-70.1; 265) |
| **Percentage of Positive Packaging Samples (95% CI; N)** | | 17.1  (11.3-22.8; 28) |
| **Erythromycin Resistance** | **Percentage of Chicken Neck Skin Samples with a Resistant Isolate (95% CI; N)** | **A *a*** | 2.47 (0.96-3.98; 10) |
| **B *b*** | 3.77 (1.48-6.07; 10) |
| **Percentage of Packaging Samples with a Resistant Isolate (95% CI; N)** | **A** | 0 (0-0; 0) |
| **B** | 0 (0-0; 0) |
| **Tetracycline Resistance** | **Percentage of Chicken Neck Skin Samples with a Resistant Isolate (95% CI; N)** | **A** | 50.6 (45.7-55.5; 205) |
| **B** | 77.4 (72.3-82.4; 205) |
| **Percentage of Packaging Samples with a Resistant Isolate (95% CI; N)** | **A** | 11.0 (6.19-15.8; 18) |
| **B** | 64.3 (46.5-82.0; 18) |
| **Ciprofloxacin Resistance** | **Percentage of Chicken Neck Skin Samples with a Resistant Isolate (95% CI; N)** | **A** | 42.0 (37.2-46.8; 170) |
| **B** | 64.2 (58.4-69.9; 170) |
| **Percentage of Packaging Samples with a Resistant Isolate (95% CI; N)** | **A** | 8.54 (4.26-12.8; 14) |
| **B** | 50.0 (31.5-68.5; 14) |
| **Resistance To ≥1 Tested Antimicrobial Class *c*** | **Percentage of Chicken Neck Skin Samples with a Resistant Isolate (95% CI; N)** | **A** | 57.3 (52.5-62.1; 232) |
| **B** | 87.5 (83.6-91.5; 232) |
| **Percentage of Packaging Samples with a Resistant Isolate (95% CI; N)** | **A** | 12.2 (7.19-17.2; 20) |
| **B** | 71.4 (54.7-88.2; 20) |
| **Resistance To ≥3 Tested Antimicrobial Classes** | **Percentage of Chicken Neck Skin Samples with a Resistant Isolate (95% CI; N)** | **A** | 38.5 (33.8-43.3; 156) |
| **B** | 58.9 (52.9-64.8; 156) |
| **Percentage of Packaging Samples with a Resistant Isolate (95% CI; N)** | **A** | 10.4 (5.70-15.0; 17) |
| **B** | 60.7 (42.6-78.8; 17) |

a A = Percentage of samples with a resistant isolate out of the total number of collected samples of that category

b B = Percentage of samples with a resistant isolate out of the total number of Campylobacter-positive samples of that category.

c Extended data of the total number of chicken neck skin and packaging samples containing at least one Campylobacter isolate resistant to the tested antimicrobials are available in Tables S8 and S9.

Table 5: Final multi-level logistic regression model for the binomial outcomes, Campylobacter-positive, the highest level of Campylobacter contamination (>1000 cfu/g), and carriage of antimicrobial resistance (AMR) and multi-drug resistance (MDR) Campylobacter spp., for 405 Halal chickens sampled from retailers between February and May 2017. B = Estimate (β); SE = Standard Error;OR = Odds Ratio; CI = Confidence Interval.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Outcome** | **Covariates *a*** | **B** | **SE** | **OR** | **Lower CI** | **Upper CI** | **z-value** | **P-Value** |
| ***Campylobacter*-Positive** | **Weight (kg)** | 2.12 | 0.85 | 8.34 | 6.68 | 10.00 | 2.50 | 0.01 |
| **Visible Liquid (Y/N)** | -3.60 | 1.34 | 0.027 | 0.002 | 0.38 | -2.69 | 0.007 |
| **>1000 cfu/g** | **Weight (kg)** | 1.92 | -0.49 | 6.83 | 5.86 | 7.79 | 3.89 | <0.001 |
| **AMR** | **Weight (kg)** | 10.51 | 3.74 | 36711.33 | 36704 | 36718.66 | 2.81 | 0.005 |
| **Weight^2 (kg)** | -0.003 | 0.001 | 0.997 | 0.995 | 0.999 | -2.60 | 0.009 |
| **MDR** | **Weight (kg)** | 6.32 | 2.50 | 553.30 | 548.40 | 558.20 | 2.53 | 0.01 |
| **Weight^2 (kg)** | -0.002 | 0.0008 | 0.998 | 0.997 | 1.00 | -2.16 | 0.03 |

***a*** The continuous variable ‘Weight (g)’ was scaled to aid model convergence and then converted to ‘Weight (kg)’ to aid interpretation. The OR and 95% CI for the unscaled covariate were calculated by dividing the scaled OR and 95% CI by the standard deviation of the unscaled covariate. Further analysis is provided in Tables S14 and S15.

Figure 1



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