A survey of the levels of Campylobacter spp. contamination and prevalence of selected antimicrobial resistance determinants in fresh whole UK-produced chilled chickens at retail sale (non-major retailers)

FSA Project FS102121

Year 5 (2018/19) Report

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Contents

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Abbreviations

AMR	Antimicrobial resistance
ºC	Degrees Celsius
GBRU	Gastrointestinal Bacteria Reference Unit
Cfu	Colony forming units
CI	Confidence Interval
EQA	External Quality Assurance
FSA	Food Standards Agency
FSS	Food Standards Scotland
G	Gram
H	Hour(s)
PHE	Public Health England
IQA	Internal Quality Assurance
ISO	International Organisation for Standardization
l	Litre
LIMS	Laboratory Information Management System
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
mg	Milligram
ml	Millilitre
MRD	Maximum Recovery Diluent
N	Number
SOP	Standard Operating Procedure
spp.	Species
UK	United Kingdom
UKAS **WGS**	United Kingdom Accreditation Service Whole Genome Sequencing

List of tables

List of figures

Executive summary

Campylobacter spp. are the most common bacterial cause of foodborne illness in the UK, with chicken considered to be the most important vehicle for this organism. The UK Food Standards Agency (FSA) agreed with industry to reduce *Campylobacter* spp. contamination in raw chicken and issued a target to reduce the prevalence of the most contaminated chickens (those with more than 1000 cfu per g chicken neck skin) to below 10 % at the end of the slaughter process, initially by 2016. To help monitor progress, a series of UK-wide surveys were undertaken to determine the levels of *Campylobacter* spp. on whole UK-produced, fresh chicken at retail sale in the UK. The data obtained for the first four years was reported in FSA projects FS241044 (2014/15) and FS102121 (2015 to 2018).

The FSA has indicated that the retail proxy target for the percentage of highly contaminated raw whole retail chickens should be less than 7% and while continued monitoring has demonstrated a sustained decline for chickens from major retailer stores, chicken on sale in other stores have yet to meet this target.

This report presents results from testing chickens from non-major retailer stores (only) in a fifth survey year from 2018 to 2019. In line with previous practise, samples were collected from stores distributed throughout the UK (in proportion to the population size of each country). Testing was performed by two laboratories - a Public Health England (PHE) laboratory or the Agri-Food & Biosciences Institute (AFBI), Belfast. Enumeration of *Campylobacter* spp. was performed using the ISO 10272-2 standard enumeration method applied with a detection limit of 10 colony forming units (cfu) per gram (g) of neck skin. Antimicrobial resistance (AMR) to selected antimicrobials in accordance with those advised in the EU harmonised monitoring protocol was predicted from genome sequence data in *Campylobacter jejuni* and *Campylobacter coli* isolates

The percentage (10.8%) of fresh, whole chicken at retail sale in stores of smaller chains (for example, Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop), independents and butchers (collectively referred to as non-major retailer stores in this report) in the UK that are highly contaminated (at more than 1000 cfu per g) with *Campylobacter* spp. has decreased since the previous survey year but is still higher than that found in samples from major retailers.

Whole fresh raw chickens from non-major retailer stores were collected from August 2018 to July 2019 (n = 1009). *Campylobacter* spp. were detected in 55.8% of the chicken skin samples obtained from non-major retailer shops, and 10.8% of the samples had counts above 1000 cfu per g chicken skin. Comparison among production plant approval codes showed significant differences of the percentages of chicken samples with more than 1000 cfu per g, ranging from 0% to 28.1%. The percentage of samples with more than

1000 cfu of *Campylobacter* spp. per g was significantly higher in the period May, June and July than in the period November to April. The percentage of highly contaminated samples was significantly higher for samples taken from larger compared to smaller chickens. There was no statistical difference in the percentage of highly contaminated samples between those obtained from chicken reared with access to range (for example, free-range and organic birds) and those reared under standard regime (for example, no access to range) but the small sample size for organic and to a lesser extent free-range chickens, may have limited the ability to detect important differences should they exist.

Campylobacter species was determined for isolates from 93.4% of the positive samples. *C. jejuni* was isolated from the majority (72.6%) of samples while *C. coli* was identified in 22.1% of samples. A combination of both species was found in 5.3% of samples. *C. coli* was more frequently isolated from samples obtained from chicken reared with access to range in comparison to those reared as standard birds. *C. jejuni* was less prevalent during the summer months of June, July and August compared to the remaining months of the year.

Resistance to ciprofloxacin (fluoroquinolone), erythromycin (macrolide), tetracycline, (tetracyclines), gentamicin and streptomycin (aminoglycosides) was predicted from WGS data by the detection of known antimicrobial resistance determinants. Resistance to ciprofloxacin was detected in 185 (51.7%) isolates of *C. jejuni* and 49 (42.1%) isolates of *C. coli*; while 220 (61.1%) isolates of *C. jejuni* and 73 (62.9%) isolates of *C. coli* isolates were resistant to tetracycline. Three *C. coli* (2.6%) but none of the *C. jejuni* isolates harboured 23S mutations predicting reduced susceptibility to erythromycin. Multidrug resistance (MDR), defined as harbouring genetic determinants for resistance to at least three unrelated antimicrobial classes, was found in 10 (8.6%) *C. coli* isolates but not in any *C. jejuni* isolates. Co-resistance to ciprofloxacin and erythromycin was predicted in 1.7% of *C. coli* isolates.

Overall, the percentages of isolates with genetic AMR determinants found in this study were similar to those reported in the previous survey year (August 2016 to July 2017) where testing was based on phenotypic break-point testing. Multi-drug resistance was similar to that found in the previous survey years. It is recommended that trends in AMR in *Campylobacter* spp. isolates from retail chickens continue to be monitored to realise any increasing resistance of concern, particulary to erythromycin (macrolide).

Considering that the percentage of fresh, whole chicken from non-major retailer stores in the UK that are highly contaminated (at more than 1000 cfu per g) with *Campylobacter* spp. continues to be above that in samples from major retailers more action including consideration of interventions such as improved biosecurity and slaughterhouse measures is needed to achieve better control of *Campylobacter* spp. for this section of the industry. The FSA has indicated that the retail proxy target for the percentage of highly contaminated retail chickens should be less than 7% and while continued monitoring has demonstrated a sustained decline for chickens from major retailer stores, chicken on sale in other stores have yet to meet this target.

1.0 Background

Campylobacter spp., especially *C. jejuni,* is the main cause of human bacterial gastroenteritis in the developed world and it is estimated that there are in excess of half a million cases and 80,000 general practitioner consultations annually in the UK (Strachan *et al*. 2010). In 2019 the UK reported 58,718 cases of campylobacteriosis and this number is known to underascertained by approximately 9.3-fold (EFSA and ECDC 2021 Tam *et al*. 2012). Source attribution studies, outbreak investigations and case-control reports all incriminate raw chicken meat as the key food-borne vehicle for *Campylobacter* spp. infection (Tam *et al*. 2009, Danis *et al*. 2009, Friedman *et al*. 2004, Mullner *et al*. 2009, Sheppard *et al*. 2009). Consumption of undercooked poultry or cross contamination from raw poultry meat is believed to be an important vehicle of infection (EFSA 2009). Raw chicken meat is frequently contaminated with *Campylobacter* spp. and a decrease in the exposure levels from this source is likely to reduce the number of human cases of campylobacteriosis (Rosenquist *et al.* 2003).

The UK Food Standards Agency (FSA) agreed with industry to reduce *Campylobacter* spp. contamination in raw chicken and issued a target for this in order to measure the effectiveness of the FSA Campylobacter Risk Management Programme (FSA 2010, FSA

2013). The target was to reduce the percentage of chickens produced in UK poultry slaughterhouses (sampled at the post-chill stage) that are contaminated with more than 1,000 colony forming units (cfu) per gram (g), from a 2008 baseline of 27% to less than 10% by December 2015; this target was rolled over to 2016 as it had not been achieved by the end of 2015 (FSA 2015b, FSS 2015). Interventions including enhanced biosecurity measures as well as improvements in slaughterhouse hygine were considered.

Such a reduction would be expected to be reflected in the levels found on chicken at retail sale, although fresh chicken sampled at retail may on average have lower levels of *Campylobacter* spp. compared to those present immediately after slaughter (Purnell *et al*. 2004). This is likely to reflect the sensitivity of campylobacters to the oxygen level in our atmosphere as well as an inability to grow below 30 °C resulting in a reduction of *Campylobacter* spp. levels during the shelf-life of retail chicken.

Enumeration

The most important factor known to affect counts of *Campylobacter* spp. on chicken carcasses is the colonisation status of the chicken itself prior to slaughter (EFSA 2010a, Bull *et al*. 2006, Reich *et al*. 2008, Rosenquist *et al*. 2003). Studies have shown that when birds were not colonised at slaughter, *Campylobacter* spp. were not detected or were present in very low numbers on carcasses (Allen *et al.* 2007). Data from an EU survey suggest that, a colonised batch of chickens was 30 times more likely to result in *Campylobacter* spp. contamination of the carcasses than a non-colonised batch (EFSA 2010b). The same EU survey noted a high proportion (70%) of unexplained variance in *Campylobacter*-contamination results and this was to some extent, attributable to slaughterhouse-specific factors for colonised flocks from countries with a high prevalence, which included the UK. Other data has also supported a role of slaughterhouses by detecting different levels of *Campylobacter* contamination on carcasses from different slaughterhouses despite processing carcasses originating from the same house and/or batch of birds (Sampers *et al*. 2008, Figuerosa *et al*. 2009).

The prevalence of *Campylobacter* spp. in raw retail chicken, as determined by the standard ISO 10272-1 enrichment culture detection (presence/absence) method, has been associated with the time of year sampled (Meldrum 2005, CLASSP Project Team 2010, Hutchison *et al*. 2006). The counts of campylobacters in post-chill chickens were not significantly associated with the month of sampling in the 2008 EU survey. The type of sample examined may affect the counts obtained, but there is evidence that counts

from carcass rinse and neck skin samples taken from the same chicken correlate well (Jorgensen *et al*. 2002).

Campylobacter spp. have been enumerated using conventional culture, Enzyme Linked Immunosorbent Assay (ELISA), and methods based on DNA amplification (Jorgensen *et al*. 2002; Borck *et al*. 2002, Oyarzabal *et al*. 2005, Dufrenne *et al*. 2001, Hong *et al*. 2003; Wolffs *et al*. 2005; Fukushima *et al*. 2007). Accurate enumeration data are needed to support effective monitoring and risk assessment of *Campylobacter* spp. contamination in raw chicken meat and depend on the availability of reliable methods. *Campylobacter* spp. are fastidious bacteria with demanding growth requirements and this may challenge accurate and reliable detection and enumeration (Hutchison *et al*. 2006). While it is normally assumed that detection by enrichment culture is more sensitive than detection by direct plating, the EU survey reported instances where *Campylobacter* spp. was detected by enumeration but not by enrichment suggesting that the enrichment method yielded false negative results (EFSA 2010b). This has been reported elsewhere and may be associated with failure to grow *Campylobacter* spp. sufficiently due to over-growth of other bacteria in the enrichment medium (Habib *et al*. 2008, Jasson *et al*. 2009). The EN/ISO 10272-2 method recommended by the International Organisation for Standardisation provides a horizontal method for the enumeration of *Campylobacter* spp. involving direct plating onto modified charcoal cefoperazone desoxycholate agar (mCCDA) and incubation for 48 h at 41.5 °C (Anonymous, 2017). A collaborative study (Rosenquist *et al*. 2007) confirmed that direct plating on mCCDA is an acceptable protocol for the enumeration of thermotolerant *Campylobacter* spp. in raw chicken meat. The study found difficulties in detecting low numbers and variation between laboratories possibly due to difficulties in handling *Campylobacter* spp. Direct spread plating on mCCDA has been shown to be a reliable alternative to the most probable number method (Scherer *et al*. 2006). This method was therefore chosen for this survey.

Campylobacter types and AMR

In the EU survey, approximately two-thirds of the *Campylobacter* spp. isolates from broiler carcasses were identified as *C. jejuni*, while one third were *C. coli* (EFSA 2010b). Speciation data is essential for meaningful epidemiological analysis and to support interpretation of AMR data. Molecular methods targeting specific genes have proven to be quick at determining species (Best *et al*. 2003, Melero *et al*. 2011) and to predict reduced susceptibility to antimicrobial agents (Painset *et al.* 2020), indicating that WGS may be a powerful tool for AMR surveillance programs. In Europe, certain antimicrobials

are mandatory to be tested for under the harmonised methods scheme for the monitoring of AMR in Campylobacter isolates (ECDC 2016).

Findings from previous survey years (Table 1)

In 2014-15, a FSA funded project FS241044 aimed to gather a full year of data on the level of *Campylobacter* spp. contamination on whole raw chicken at retail sale (FSA 2015c). During that survey 4,011 samples of whole, UK-produced, fresh chicken from February 2014 to March 2015 were tested from all types of retail stores. The prevalence of *Campylobacter* spp. in the fresh chicken at retail in the UK was found to be 73.3% (PHE 2015). A considerable percentage (19.4%) of samples had more than 1000 cfu per g chicken skin, with retailer incidence frequencies ranging between 12.9% to 29.9%. Significant differences among retailers could not be explained by differences in remaining shelf-life, chicken weights, time of year sampled or type of chicken rearing. Some production plant approval codes (signifying the slaughter house premises) showed significant differences in the percentage of chickens with more than 1000 cfu per g, ranging from 9.4% to 29.7%. A higher percentage of chickens had a high level of *Campylobacter* spp. during the summer compared to winter. The larger chickens (weighing more than 1750 g), were more likely to be contaminated with more than 1000 cfu per g. There was no evidence of birds with access to range (for example, free-range and organic birds) being more contaminated than birds reared under standard conditions. For the majority of chicken skin samples (76.6%) from which isolates were submitted for speciation, *C. jejuni* was identified. *C. coli* was identified in 13.9% of samples. Both species were found in 4.2% of samples. *Campylobacter coli* was more frequently isolated in the summer compared to winter and spring months and was more frequently isolated from birds with access to range. The FSA continued the monitoring programme over three further years (under project FS102121). The project also was to continue to identify *Campylobacter* spp. present and determine susceptibility of isolates to a defined range of antimicrobial agents.

Table 1 Comparison of percentages of samples with more than 10 or more than 1000 cfu of Campylobacter spp. per g in survey years 2014 to 2019.

Survey period	Sampling strategy	Retailer	Number of samples tested	Percentage of samples with more than 10 cfu per g	Percentage of samples with more than 1000 cfu per g
2014 to 2015	According to marketshare	All	4011	73.0	19.4
2014 to 2015	According to marketshare	Non-major only	589	77.6	23.9
2015 to 2016	Aiming for same number of samples from each of the major retailers and for 7-10% from non-majors	All	2998	61.3	11.4
2015 to 2016	Aiming for same number of samples from each of the major retailers and for 7-10% from non-majors	Non-major only	196	63.8	14.8
2016 to 2017	Aiming for same number of samples from each of the major retailers and for 7-10% from non-majors	All	4268	54.0	6.0
2016 to 2017	Aiming for same number of samples from each of the major retailers and for 7-10% from non-majors	Non-major only	378	70.4	15.6
2017 to 2018	Sampling from major retailers included in the first quarter – samples from non-majors throughout	All	1044	56.0	7.0

2017 to 2018	Sampling from major retailers included in the first quarter – samples from non-majors throughout	Non-major only	207	75.0	15.0

In the second survey year (2015-16), the prevalence of *Campylobacter* spp. in the fresh chicken at retail sale from all types of stores including major retail stores in the UK had declined to from 73.3% to 61.3% and the percentage of samples with more than 1000 cfu per g chicken skin from 19.4% to 11.4% (PHE 2017). There were significant differences in the percentage of highly contaminated chickens (ranging from 6.7% to 17.7%) between retailers that could not be explained by differences in shelf-life remaining, chicken weights, sampling period or the type of bird reared. Comparing production plant approval codes showed a significant difference in the percentage of chickens with more than 1000 cfu per g, ranging from 1.8% to 19.3%, and it was noted that some retailers were predominantly supplied by specific production plant premises. A higher percentage of chickens were highly contaminated with *Campylobacter* spp. during the first summer months compared to the subsequent months. The larger chickens were more likely to be contaminated with more than 1000 cfu per g. There was no evidence of birds with access to range being more contaminated than birds reared under standard conditions but with much fewer free-range and organic birds tested there was limited precision in the comparison made. For the majority of chicken skin samples (83.0%) from which isolates were submitted for speciation, *C. jejuni* alone was identified. *C. coli* alone was identified in 13.5% of samples. Both species were found in 3.4% of samples. *C. coli* was more frequently isolated in the summer months, and more frequently isolated from birds with access to range.

In the third survey year (2016-17), the prevalence of *Campylobacter* spp. in 4268 fresh chicken at retail sale from all types of stores including major retail stores in the UK had declined further to 54% and the percent of samples with more than 1000 cfu per g chicken skin to 6% (PHE 2018). There were differences in the percentage of highly contaminated chickens (ranging from 1% to 18%) between the different retailer groups and between individual production plant approval codes (ranging from 1% to 19%). For the majority of chicken skin samples (87.7%) from which isolates were submitted for speciation *C. jejuni* was identified. *C. coli* was identified in 10.2% of samples. Similar results were obtained from the first quarter of a 4th survey year where 1044 samples obtained from major as well as non-major retailer stores (these were defined as stores

not belonging to any of the 10 major UK retailers) where the prevalence of *Campylobacter* spp. in the fresh chicken at retail in the UK was 56% and the percentage of samples with more than 1000 cfu per g chicken skin was 6% (PHE 2019). In the sample spanning the entire 4th year from August 2017 to July 2018 with only chickens from non-major retailer stores the prevalence of *Campylobacter* spp. in the fresh chicken was 75% and the proportion of samples with more than 1000 cfu per g chicken skin was 15%. This suggested that chickens from non-major retailer stores continued to pose a greater risk compared to chicken from major retailer stores.

In summary, the survey data from the first four years have shown that the percentage of fresh, whole chicken at retail sale in the UK that are contaminated with a high level of *Campylobacter* spp. has decreased considerably but chickens from non-major retailers have remained more highly contaminated (at more than 1000 cfu of campylobacters per g). The current focus on smaller establishments and their suppliers may allow the improvements to be made across their supply chain including any supplies into the catering trade. The purpose of examining numbers of *Campylobacter* spp. in fresh whole chicken on sale in non-major retailer stores in the UK from August 2018 to July 2019 was to determine if any decline in contamination on chickens for these types of stores could be detected.

2.0 Methods

Sampling and testing procedures for the survey was agreed with the FSA. The survey protocol used for the time-period from August 2018 to July 2019 is briefly described (enclosed as Appendix I).

2.1 Sampling

Sampling locations were identified by Hallmark Ltd. across the UK, to reflect population sizes. Fresh whole chickens were sampled from non-major retailer stores only from August 2018 to July 2019. On arrival at the laboratory, the air temperature of the cool boxes was determined using calibrated temperature probes. Samples details were documented and logged onto the laboratory information management system.

Samples were obtained from stores assigned to one of three categories: smaller chains (including stores recognised as being part of smaller retail chains for example, Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop and other similar chains); butchers, and

others (stores recognised as farm shops, markets and independents but excluding stores belonging to the other two categories).

2.2 Detection of Campylobacter spp.

PHE Food, Water and Environmental Microbiology Service Laboratories and the Agri-Food & Biosciences Institute, Belfast carried out testing. All laboratories enumerated *Campylobacter* spp. based on EN/ISO 10272-2 for the enumeration of *Campylobacter* spp. as detailed in the FSA survey protocol (FSA 2016) using modified Charcoal Cefoperazone Deoxycholate Agar as the primary plating medium. Neck-skin samples were prepared as described before (Appendix I) using a 1:9 (w/w) dilution of chicken neck-skin and buffered peptone water. Sample weights were between 2 to 10 g pure neck-skin.

2.3 Determination of species, multi-locus-sequence-type (MLST) and predicted antimicrobial resistance profile

The aim was to investigate one isolate from each positive sample by WGS (although no isolate was available from a small proportion of positive samples due to loss of isolate viability; see results sections below).

Genomic DNA was extracted from bacterial cultures using a QIAGEN QIAsymphony, fragmented and tagged for multiplexing with Nextera XT DNA Sample Preparation Mits, followed by rapid-run paired-end sequencing on an Illumina High-Seq 2500 platform to produce 100bp reads. The 7-loci MLST was determined from WGS data using MOST, a modified MLST typing tool based on short read sequencing (Tewold *et. al.* 2016). Sequences were assembled using the SPAdes genome assembler in the PHE pipeline. Contigs for each isolate were uploaded to the pubmlst.org/campylobacter database, which automatically identified loci, tagged their location and assigned alleles.

Antimicrobial resistance (AMR) was predicted using a validated in-house bioinformatics pipeline in PHE to detect from WGS data those isolates with known antimicrobial resistance determinants, conferring reduced susceptibility to erythromycin (macrolide), ciprofloxacin (fluoroquinolone), gentamicin and streptomycin (aminoglycosides) and tetracycline (Table 2; Painset *et. al*. 2020).

Table 2 Description of genetic determinants for antimicrobial resistance

23s	Erythromycin (macrolide)	• 23s_ [2075:A-G] • 23s_ [2074:A-C; 2075:A-R] • 23s_ [2074:A-M] • 23s_ [2074:A-T]
gyrA	Ciprofloxacin (fluoroquinolne)	• gyrA_ [86:T-I; 90:D-Y] • gyrA_ [86:T-I] • gyrA_ [86:T-I; 104:P-S] • gyrA_ [86:T-I; 90:D-N] • gyrA_ [86:T-R] • gyrA_ [86:T-V]
tet(O)	Tetracycline (tetracycline)	• tet(O) • tet(O)_2[a] • tet(o)-Cc3
aac(6')-aph(2'')	Gentamicin (aminoglycoside)	aac(6')-aph(2'')
aadK	Streptomycin (aminoglycoside)	aadK
ant(6)-Ia (aadE)	Streptomycin (aminoglycoside)	ant(6)-Ia,aadE-Cp2

[a] This gene is also known as tet(O/32/O).

2.4 Quality Assurance

Both laboratories participate in recognised External Quality Assurance schemes (for example, Proficiency testing for food, water and environmental microbiology) including the FSA funded scheme for enumeration of *Campylobacter* species, as well as operating comprehensive internal quality assurance schemes as part of the requirements of their accreditation to ISO 17025/2017 as assessed annually by the United Kingdom Accreditation Service (UKAS). All analyses were performed by trained and competent staff in a UKAS accredited laboratory operating an internal audit and review programme.

2.5 Statistical Analysis

Cross tabulations were analysed by the calculation of Clopper-Pearson exact 95% confidence intervals for the proportion in each cfu per g category. Confidence intervals given for each variable show the likely range of the results allowing for the number of samples taken. The 95% confidence intervals mean that we would expect the true prevalence to fall within the lower and upper confidence limits 95% of the time. In addition, the Pearson chi square test of association has been used to test the null

hypothesis of no association between the measured variable and *Campylobacter* contamination. Fisher's exact test was used for individual comparisons when samples were small.

3.0 Results

Fresh whole UK produced chickens were collected from non-major retail stores across the UK between August 2018 and July 2019. Samples were collected from many different types of stores and details of samples can be found elsewhere (see Appendix 2, Hallmark report). No test result was obtained from three samples in the samplers report as two (sample numbers 7603 and 364743) had insufficient neck-skin available and one was not tested as the laboratory was informed on its arrival that it had expired (for example, it was past its use-by-date; sample number 381268).

3.1 *Campylobacter* spp. counts in whole fresh UK produced chicken

The FSA instructed PHE to test chickens from non-major retail stores only, from August 2018 to July 2019 as chickens from these stores were thought to constitute a greater risk. *Campylobacter* spp. were enumerated from 1009 chicken skin samples and detected in 55.8% of these. The percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g was 10.8%. The highest single count detected was 200 000 cfu of *Campylobacter* spp. per g skin (see Appendix III for full list of sample result details).

3.1.1 Campylobacter spp. counts in relation to retailer

The percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g ranged from 7.2% to 14.0% amongst the retail store categories during the sampling period (Table 3).

Table 3 Campylobacter spp. counts in whole fresh chicken collected from non-major stores from August 2018 to July 2019.

Less than 10 per gram of chicken skin sample:

Retail Store Category	**Number of samples[a]**	**% (95% CI)**
Smaller chains[b]	177	51.2 (45.8-56.5)
Butchers	154	38.4

		(33.6-43.4)
Others[b]	115	43.9 (37.8-50.1)
Total	446	44.2 (41.1-47.3)

[a] These shops included stores thought to be recognised as being part of smaller retail chains (for example, Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop and other similar chains) but not butchers or other types of stores.

[b] Others included stores recognised as farm shops, markets and independent.

10-99 per gram of chicken skin sample:

Retail Store Category	**Number of samples**	**% (95% CI)**
Smaller chains[b]	88	25.4 (20.9-30.4)
Butchers	79	`19.7 (15.9-23.9)
Others[b]	47	17.9 (13.5-23.1)
Total	214	21.2 (18.7-23.9)

[a] These shops included stores thought to be recognised as being part of smaller retail chains (for example, Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop and other similar chains) but not butchers or other types of stores.

[b] Others included stores recognised as farm shops, markets and independent.

100-1000 per gram of chicken skin sample:

Retail Store Category	**Number of samples**	**% (95% CI)**
Smaller chains[b]	25	16.2 (12.5-20.5)
Butchers	56	27.9 (23.6-32.6)

Others[b]	28	27.5 (22.2-33.3)
Total	109	23.8 (21.2-26.5)

[a] These shops included stores thought to be recognised as being part of smaller retail chains (for example, Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop and other similar chains) but not butchers or other types of stores.

[b] Others included stores recognised as farm shops, markets and independent.

More than 1000 per gram of chicken skin sample:

Retail Store Category	**Number of samples**	**% (95% CI)**
Smaller chains[b]	56	7.2 (4.7-10.5)
Butchers	112	14.0 (10.7-17.8)
Others[b]	72	10.7 (7.2-15.1)
Total	240	10.8 (9.0-12.9)

[a] These shops included stores thought to be recognised as being part of smaller retail chains (for example, Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop and other similar chains) but not butchers or other types of stores.

[b] Others included stores recognised as farm shops, markets and independent.

The percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g was not significantly different (p more than 0.05) for the three categories of stores compared to the average of all samples. Butcher shops had a significantly (p less than 0.01) higher percentage of chickens with more than 1000 cfu of *Campylobacter* spp. per g compared to the store category recognised as being part of smaller chains (for example, Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop and other similar chains).

3.1.2 Campylobacter spp. counts in relation to chicken rearing regime

Table 2 summarises the levels of *Campylobacter* spp. counts detected in samples from birds reared as "standard" (without access to range), free-range, or organic. Fewer

samples from free range or organic chickens were examined, reflecting their lower market share. Within this dataset, no significant differences in the percentage of highly contaminated chickens between the three chicken types were found but note the wide confidence intervals.

Table 4 *Campylobacter* spp. counts in whole fresh chicken collected from non-major retail stores from August 2018 to July 2019, in relation to bird rearing regime. Table shows cfu of *Campylobacter* spp. per g chicken skin sample

Less than ten grams per gram of chicken skin sample:

Rearing Regime	**Number of samples[a]**	**% (95% CI)**
Standard	419	44.7 (41.5-48.0)
Free Range	24	35.8 (24.5-48.5)
Organic	3	60.0 (15-94.7)

10-99 per gram of chicken skin sample:

Rearing Regime	**Number of samples**	**% (95% CI)**
Standard	200	21.3 (18.8-24.1)
Free Range	13	19.4 (10.8-30.9)
Organic	1	20.0 (0.5-71.6)

100-1000 per gram of chicken skin sample:

Rearing Regime	**Number of samples**	**% (95% CI)**
Standard	217	23.2 (20.5-26.0)
Free Range	22	32.8 (21.9-45.4)
Organic	1	20.0 (0.5-71.6)

More than 1000 per gram of chicken skin sample:

Rearing Regime	**Number of samples**	**% (95% CI)**
Standard	101	10.8 (8.9-12.9)
Free Range	8	11.9 (5.3-22.2)
Organic	0	0

3.1.3 Campylobacter spp. counts in relation to chicken production plant

There were differences in the percentage of chicken samples that were highly contaminated among the different production plant approval numbers. The number of samples collected from each production plant was limited which meant only large significant differences could have been detected (Table 3).

The percentages of chickens with more than 1000 cfu of *Campylobacter* spp. per g ranged from 0% (approval number 3011) to 28.1% (approval number 5003) among the production plant remises. Production plant numbers 3011 and 5464 produced significantly (p less than 0.01 and p less than 0.05, respectively) fewer highly contaminated chickens compared to the average (10.8%) for all production premises. Compared to the average of all samples, a significantly (p less than 0.01) higher percentage of samples obtained from approval number 5003 were in the highly contaminated category. There were no significant differences in the percentages of highly contaminated chickens for any of the remaining production plant codes compared to the average for all plants.

Table 5 Campylobacter spp. counts in chicken collected from non-major retail stores from August 2018 to July 2019, in relation to production plant. cfu of Campylobacter spp. per gram of chicken skin sample.

Less than 10 grams:

Plant Approval Number	n[a]	% (95% CI)
5007	115	38.7 (33.2-44.5)
5464	39	55.7 (43.3-67.6)
3011	48	80.0 (67.7-89.2)
4017	20	34.5 (22.5-48.1)
1007	27	47.4 (34.0-61.0)
4800	22	47.8 (33.0-63.1)
5003	13	40.6 (23.7-59.4)
2037	11	47.8 (26.8-69.4)
1100	18	81.8 (59.7-94.8)
8013	6	30.0 (11.9-54.3)
4561	9	45.0 (23.1-68.5)
Other codes[b]	109	38.4 (32.7-44.3)
Not available[c]	9	45.0 (23.1-68.5)

10 to 99 grams:

Plant Approval number	n	% (95% CI)
5007	87	29.3 (24.2-34.8)
5464	18	25.7 (16.0-37.6)

3011	9	15.0 (7.1-26.6)
4017	12	20.7 (11.2-33.4)
1007	8	14.0 (6.3-25.8)
4800	4	8.7 (2.4-20.8)
5003	3	9.4 (2.0-25.0)
2037	9	39.1 (19.7-61.5)
1100	3	13.6 (2.9-34.9)
8013	4	20.0 (5.7-43.7)
4561	2	10.0 (1.2-31.7)
Other codes[b]	51	18.0 (13.7-22.9)
Not available[c]	4	44.7 (41.5-48.0)

100 to 1000 grams:

Plant approval number	**n**	**%** **(95% CI)**
5007	63	21.2 (16.7-26.3)
5464	11	15.7 (8.1-26.4)
3011	3	5.0 (1.0-13.9)
4017	24	41.4 (28.6-55.1)
1007	15	26.3 (15.5-40.0)
4800	16	34.8 (21.4-50.3)
5003	7	21.9 (9.3-40.0)

2037	2	8.7 (1.1-28.0)
1100	0	0 (0-15.0)
8013	8	40.0 (19.1-64.0)
4561	4	20.0 (5.7-43.7)
Other codes[b]	84	30.0 (24.3-35.3)
Not available[c]	5	25.0 (8.7-49.1)

More than 1000 grams:

Plant Approval number	**n**	**% (95% CI)**
5007	32	10.8 (7.5-14.9)
5464	2	2.3 (0.4-9.9)
3011	0	0 (0-6.0)
4017	2	3.5 (0.4-11.9)
1007	7	12.3 (5.1-23.7)
4800	4	8.7 (2.4-20.8)
5003	9	28.1 (13.8-46.8)
2037	1	4.4 (0.1-22.0)
1100	1	4.6 (0.1-22.8)
8013	2	10.0 (1.2-31.7)
4561	5	25.0 (8.7-49.1)
Other codes[b]	40	14.1 (10.3-18.7)

Not available[c]	2	10.0 (1.2-31.7)

[a] n = number of samples.

[b] Samples listed within the 'Other codes' category had less than 20 chickens from any single processor tested. The full list of approved premises codes can be found on the FSA website including the details of each license. http://www.food.gov.uk/enforcement/sectorrules/meatplantsprems/meatpremlicence.

[c] Samples lacking the production plant approval number.

3.1.4 Campylobacter spp. counts in relation to sampling period

The percentage of samples with more than 1000 cfu per g of *Campylobacter* spp. tended to be higher in sampling periods with warmer months with a significantly higher percentage of samples tested in period 4 being highly contaminated compared to samples tested in the colder months in period 2 and 3 (Table 6) (p less than 0.05). The percentage of samples with less than 10 cfu per g of *Campylobacter* spp. was highest in the sampling periods with colder months and was significantly higher for samples collected in period 2 compared to periods 1 and 4 (Table 6).

Table 6 Campylobacter spp. counts in whole fresh chicken collected from non-major retail stores, in relation to sampling period. cfu of *Campylobacter* spp. per gram of chicken skin sample

Less than 10 grams:

Sampling period months	**n**	**% (95% CI)**
1 Aug/Sep/Oct 2018	73	32.0 (26.3-38.8)
2 Nov/Dec 2018 & Jan 2019	146	55.6 (49.3-61.6)
3 Feb/Mar/Apr 2019	109	47.0 (40.4-53.6)
4 May/Jun/Jul 2019	114	40.7 (34.9-46.7)

More than 1000 grams:

Sampling period months	n	% (95% CI)
1 **Aug/Sep/Oct 2018**	29	12.8 (8.8-17.9)
2 **Nov/Dec 2018 & Jan 2019**	23	8.8 (5.6-12.8)
3 **Feb/Mar/Apr 2019**	17	7.3 (4.3-11.8)
4 **May/Jun/Jul 2019**	40	14.3 (10.4-18.9)

3.1.5 Campylobacter spp. counts in relation to chicken pack weight

Chickens were assigned into three weight categories ‘small’ (more than 1400 g), ‘medium’ (1400 to 1750 g) or ‘large’ (more than 1750 g). Assignment of a size category to the chicken purchased enabled analysis to determine whether or not size, which may be linked to the age of the chicken at slaughter, was associated with the level of *Campylobacter* spp. present. Comparison of these categories, showed that the proportion of samples with less than 10 cfu per g of *Campylobacter* spp. was significantly higher for chicken weighing less than 1400 g compared to larger chickens (Table 7).

Table 7 Campylobacter spp. counts in chicken from stores not part of major chains from August 2018 to July 2019 in relation to chicken weight. Table shows cfu of cfu of Campylobacter spp. per gram of chicken skin sample.

Less than 10 grams:

Chicken pack weight	n	% (95% CI)
Small (less than 1400 g)	209	51.6 (46.6-56.6)
Medium (1400-1750 g)	170	29.6 (25.0-34.6)
Large (more than 1750 g)	64	26.9 (21.4-33.0)

More than 1000 grams:

Chicken pack weight	n	% (95% CI)
Small (less than 1400 g)	36	8.9 (6.3-12.1)
Medium (1400-1750 g)	37	10.3 (7.3-13.9)
Large (more than 1750 g)	35	14.7 (10.5-19.9)

The percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g tended to be lower for smaller chicken weights, and the proportion of highly contaminated chicken was significantly (p more than 0.05) higher for large chickens compared to chickens in the small weight group.

3.2 Campylobacter types in chicken at retail sale in non-major stores

From all samples tested (n = 1009), a total of 526 isolates were subjected to *C. jejuni/C. coli* speciation testing. *C. jejuni* alone was found in 72.6%, *C. coli* alone in 22.1% and both species in 5.3% of samples where isolates were tested (Table 8). No speciation test was available for 37 samples (6.6%) as the isolates from these sampled died before WGS could be done. *C. coli* was significantly more frequent in samples from chicken reared as free-range compared to samples from chickens reared without access to range (p less than 0.01).

Table 8 C. jejuni and C. coli isolates from retail chicken skin samples in relation to bird rearing regime. % of samples with *Campylobacter* species.

Species detected	All rearing regimes	Standard[a] (n)	Free range (n)	Organic (n)
***C. jejuni* only**	72.6 (382)	74.0 (358)	55.0 (22)	100 (2)
***C. coli* only**	22.1 (116)	20.5 (99)	42.5 (17)	0 (0)
C. jejuni* and *C. coli	5.3 (28)	5.6 (27)	2.5 (1)	0 (0)

[a] In standard rearing no access to range is provided

The percentage of *C. jejuni* in samples from the summer period was significantly lower compared to samples from the rest of the year (p more than 0.001; Table 9) in the chickens tested from the non-major retail stores from August 2018 to July 2019.

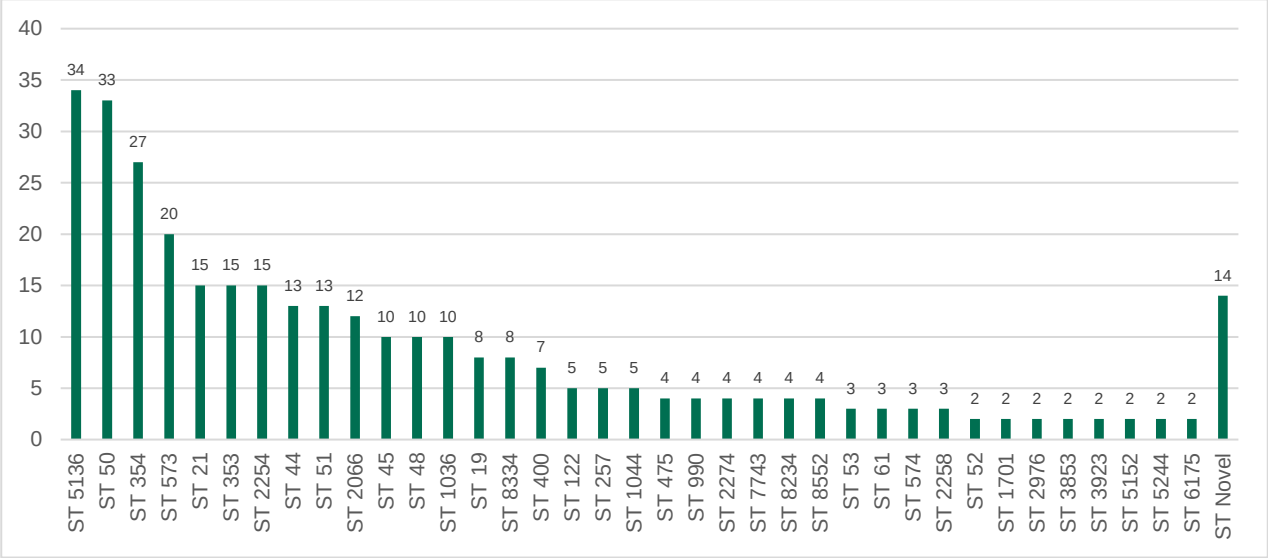
Table 9 C. jejuni and C. coli isolates from chicken skin samples collected from non-major retailer stores in relation to season. Table shows the % of samples with *Campylobacter* species (95% CI).

Species detected	**Summer (August 2018 and June & July 2019);** (n = 149)	**Autumn, Winter and Spring (September-December 2018 & January-May 2019);** (n = 377)
***C. jejuni* only**	59.5 (51.1-67.4)	78.0 (73.5-82.1)
***C. coli* only**	31.8 (24.4-39.9)	18.3 (14.5-22.6)
C. jejuni* and *C. coli	9.5 (5.3-15.4)	3.7 (2.0-6.2)

Sequence types (MLST) based on WGS data was assigned for 476 isolates. WGS was attempted for 506 isolates; 31 were found to be of mixed type and no ST was therefore assigned.

There was considerable diversity with 97 different STs detected and 19 isolates were assigned as novel ST. The most common STs within *C. jejuni* were ST5136, ST50, ST354, ST573, ST21, ST353 and ST2254 together accounting for 44% (Figure 1).

Figure 1: Whole-genome-sequencing-based MLSTs for *C. jejuni* isolates (n = 360).

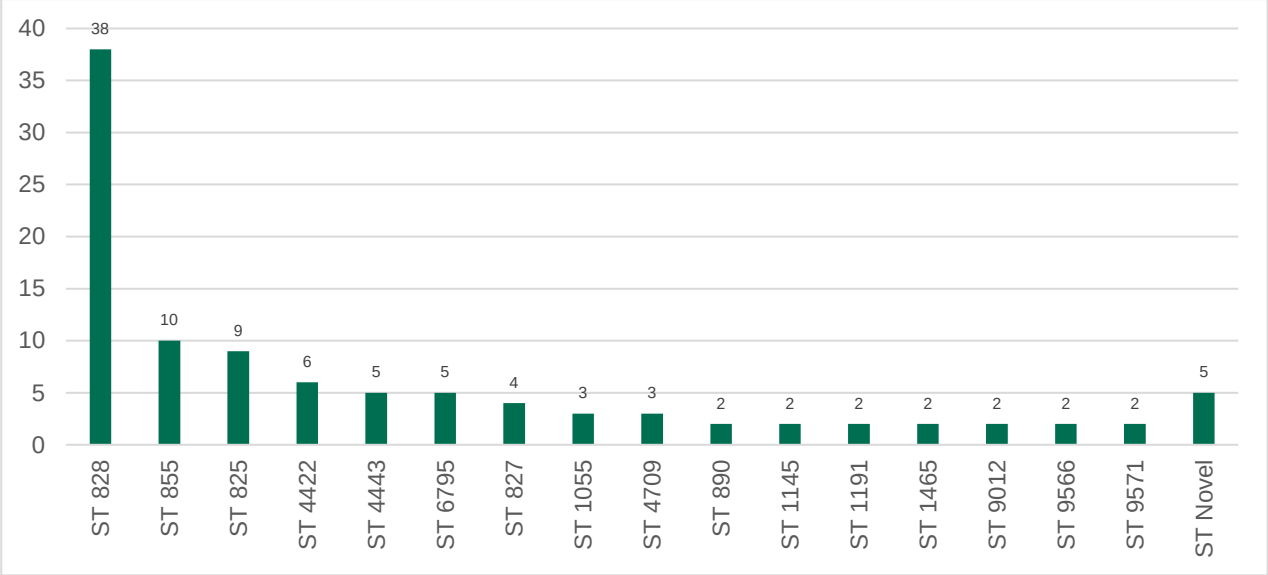


The following 29 *C. jejuni* MLST were detected in a single isolate: ST22, ST42, ST137, ST230, ST262, ST447, ST464, ST904, ST905, ST945, ST1076, ST1301, ST1489,

ST1709, ST2211, ST2314, ST4697, ST5483, ST5805, ST6209, ST6461, ST7435, ST7735, ST7744, ST7749, ST9567, ST9570, ST9572, ST9581.

There was less diversity within *C. coli* and the most common STs were ST828, ST855 and ST825 accounting for 50% of the isolates (Figure 2).

Figure 2: Whole-genome-sequencing-based MLST for *C. coli* isolates (n = 116)



The following 14 *C. coli* MLSTs were detected in a single isolate : ST832, ST962, ST1438, ST1578, ST1585, ST1595, ST1774, ST2195, ST2256, ST2273, ST3077, ST3567, ST4453 and ST4543.

3.3 AMR in C. jejuni and C. coli isolates from chicken in non-major stores

Results of WGS from a total of 360 *C. jejuni* and 116 *C. coli* isolates from 475 samples were tested to establish predicted AMR profiles. In only one sample were AMR profiles obtained from two isolates – both were *C. jejuni* and had identical predicted AMR profile results.

A total of 185 (51.7%) isolates of the *C. jejuni* and 49 (42.2%) isolates of *C. coli* were resistant to ciprofloxacin (fluoroquinolone) as predicted from the detection of known point mutations in *gyrA*. The presence of *tet(O)* variants conferring resistance to tetracycline were detected in 220 (61.1%) isolates of *C. jejuni* and in 73 (62.9%) isolates of *C. coli*. Only 3 (2.6%) isolates of the *C. coli* and none of the isolates of *C. jejuni* were predicted to have resistance to erythromycin (macrolide) by the detection of a known point mutation

(A2075G) in at least 2 out of 3 copies of the 23S rRNA gene. The *aadK* gene which is associated with reduced susceptibility to streptomycin (aminoglycoside) was detected in 14 (12.1%) isolates of *C. coli*, but was not detected in any of the 360 isolates of *C. jejuni*; while the presence of the gene *aac(6')-aph(2")* which is associated with reduced susceptibility to gentamicin (aminoglycoside) was not detected in any of the isolates of *C. jejuni* or *C. coli*.

There were 10 (8.6%) isolates of *C. coli* and none of the isolates of *C. jejuni* classified as multidrug resistant (MDR), for example, harbouring genetic determinants known to have resistance to at least three unrelated antimicrobial classes. Seven ST828 isolates were predicted to have reduced susceptibility to ciprofloxacin (fluoroquinolone), streptomycin (aminoglycoside) and tetracycline; one ST828 to ciprofloxacin, erythromycin (macrolide) and tetracycline; and one ST1438 to ciprofloxacin, erythromycin, streptomycin and tetracycline. One *C. coli* ST890 isolate was predicted to have reduced susceptibility to ciprofloxacin (fluoroquinolone), streptomycin (aminoglycoside) and tetracycline (*tetOCc3*).

Table 10 Antimicrobial resistance in C. jejuni and C. coli isolates (n = 476) from whole fresh chicken at retail sale in 2018 – 2019 predicted from whole genome sequencing.

Antimicrobial class (antimicrobial name)	C.Jejuni (n=360) Number of isolates	C.jejuni (n=360) % resistant (95% CI)	C.coli (n=116) Number of isolates	C.coli (n=116) % resistant (95% CI)	All isolates % resistant (95% CI)
Aminoglycosides (Streptomycin)	0	0	14	12.07 (6.76, 19.42)	2.94 (1.62, 4.89)
Aminoglycosides (Gentamicin)	0	0	0	0	0
Fluoroquinolones (Ciprofloxacin)	185	51.67 (46.37, 56.94)	49	42.24 (33.13, 51.76)	49.16 (44.58, 53.75)
Macrolides (Erythromycin)	0	0	3	2.60 (0.54, 7.37)	0.63 (0.13, 1.83)
Tetracycline	220	61.11 (55.86, 66.18)	73	62.93 (53.47, 71.71)	61.55 (57.02, 65.95)

4.0 Discussion

4.1 Survey results

This report describes results from continued testing of whole fresh chickens from non-major retailer stores only in the UK from August 2018 to July 2019. These chickens are more contaminated than chickens from the major retailers (PHE 2019) and therefore thought to pose a greater risk to consumers if thorough cooking and hygienic handling are not followed.

In this period, *Campylobacter* spp. were detected in 55.5% of the chicken samples and 10.8% had counts above 1000 cfu per g chicken skin. In comparison, a significantly higher level of contamination was found in the previous survey year (2017-2018) where *Campylobacter* spp. were detected in 75% (p less than 0.001) of chicken samples and 15% (p less than 0.05) had counts above 1000 cfu per g chicken skin. These changes suggest improvements and continued monitoring could be used to ascertain if this trend is sustained. A lower percentage of highly contaminated chickens was found for chickens that were sampled from all store types including major retailers from August to October 2018 where 7% of samples had counts of more than 1000 cfu of *Campylobacter* spp. per g.

In the data from August 2018 to July 2019 the proportion of chickens with *Campylobacter* spp. levels at more than 1000 cfu per g ranged from 7.2% to 14% across the types of stores and the proportion of highly contaminated chickens was slightly higher for butcher stores compared to the group of stores recognised as being part of smaller retail chain stores (for example, Iceland, McColl’s, Budgens, Nisa, Costcutter, One Stop). Further studies would be needed to provide a more comprehensive understanding of the extent to which different processors or other factors such as sourcing of birds from specific farms may explain the observed difference in contamination.

There was significant evidence that the processor approval number was associated with the levels of *Campylobacter* spp. found in samples from whole fresh retail chicken. The percentage of chickens with more than 1000 cfu of *Campylobacter* spp. per g ranged from 0 to 28.1% between approval numbers. This could reflect differences in

slaughterhouse hygiene practices and/or differences in the proportion of highly contaminated flocks received by slaughterhouses.

Whilst there was no evidence that free-range or organic chickens were more highly contaminated than conventionally reared birds, this finding should be treated with caution as low numbers of free-range and organic chickens were examined due to their low overall market share. Their corresponding confidence intervals were wide and would therefore only be able to verify very large differences. Nevertheless, a very similar finding was made in the previous survey years (PHE 2017; PHE 2018; PHE 2019).

From the majority (72.6%) of chicken skin samples, where *Campylobacter* spp. were isolated, *C. jejuni* (only) was detected while *C. coli* (only), was identified in 22.1% of samples. Compared to previous survey years the proportion of *C. coli* was higher, and this was probably related to a larger proportion of chickens obtained from non-major stores in the dataset from this fifth year. The proportion of *C. coli* in samples from chickens reared as free-range or organic (all chicken reared as organic are reared with access to range) was higher compared to samples from chickens reared without access to range. The proportion of *C. jejuni* and *C. coli* isolates from human cases in the UK has been reported as approximately 90% and 10%, respectively (CLASSP Project Team 2010).

This data from *Campylobacter* spp. isolates obtained from chickens on retail sale in non-major retailer stores from August 2018 to July 2019 showed similar results for predicted AMR when compared to the data from isolates obtained from the previous survey years (Table 11). This was despite a difference in sampling where samples from major retailers were prominent in the earlier survey years. Comparison of previous and current datasets (2007/2008 FSA retail chicken survey, the 2010 CLASSP survey (FSA 2003; FSA 2009; CLASSP Project Team 2010)) demonstrate significantly higher percentages of isolates with resistance to ciprofloxacin in recent years compared to earlier studies.

The percentage of chicken from non-major retail stores that are highly contaminated is still higher than on chicken obtained from major chains and more needs to be done to achieve better control of *Campylobacter* spp. in sector supplying non-major retail stores. This could include consideration of measures to achieve more consistent biosecurity and improvements in slaughterhouse hygiene.

In summary, the proportion of chicken at retail sale in non-major stores in the UK that are contaminated with the highest level of *Campylobacter* spp. has decreased since 2014/15.

Data from this survey and from the previous survey years has demonstrated a significant decline in the percentage of fresh whole UK chicken that are contaminated with campylobacters at the highest level (for example, with more than 1000 cfu per g) from all store types. The FSA has indicated that the average retail proxy for the proportion of highly contaminated retail chickens should be less than 7% and continued monitoring may establish if this level can be achieved for non-major retail stores.

Table 11 Occurrence of resistance to selected antimicrobials in C. jejuni and C. coli isolates from UK fresh whole retail chicken from 2015 to 2019. All figures (% of isolates)

Antimicrobial	**Break point (mg/l)**	**Species**	**2015-16[a]**	**2016-17[a]**	**2017-18[a]**	**2018-19[b]**
Ciprofloxacin	> 0.5	*C. jejuni*	54	41	52	52
Ciprofloxacin	> 0.5	*C. coli*	48	52	48	42
Erythromycin	> 4	*C. jejuni*	0 [95% CI = 0-1]	0.4	0.6	0 [95% CI = 0-3]
Erythromycin	> 8	*C. coli*	1.9	0 [95% CI = 0-4]	3.1	2.6
Tetracycline	> 2	*C. jejuni*	68	54	52	61
Tetracycline	> 2	*C. coli*	67	62	60	63

[a] AMR profiles based on phenotypic testing.

[b] AMR profiles predicted from WGS data.

4.2 Conclusions

- The percentage of fresh whole chicken on retail sale in minor retail stores in the UK that are contaminated with the highest level (more than 1000 cfu/g) of *Campylobacter* spp. has decreased from 2014, and further in the period from 2017 to 2019. For example, 18% of samples from retail stores in the category termed “Other“, which excluded major retailers but included stores similar to those included in the current survey year, had more than 1000/g in the survey year from August 2016 to July 2017 compared to the 10.8% found in the present survey period.

- Continued monitoring will be required to demonstrate if a sustained decline can be achieved in chickens from non-major retail stores and whether the target prevalence of 7% of chickens falling in the category with highest levels of contamination can be achieved and maintained.

- Additional analysis to understand why there may be differences between production plants would be useful to help achieve the target

- Overall, as predicted from WGS data in the current study year and phenotypically in previous years, there are no major differences in the levels of antimicrobial susceptibility in isolates of *C. jejuni* and *C. coli* over the last five years from chicken across a range of retailers. This includes negligible (0.6%) resistance to erythromycin in *Campylobacter* spp. isolates from this study year, in comparison to those levels in human strains from the EU in 2019 which are reported to harbour 1.5% erythromycin resistance in isolates of *C. jejuni* and 12.9% erythromycin resistance in isolates in *C. coli* (EFSA and ECDC, 2021). Quinolone and tetracycline resistance in isolates obtained from poultry meat and human strains continues to be high and current measures taken are not adequately achieving a reduction in AMR in the food chain. Comparisons of percentages of resistant isolates between the current and the earlier survey years must be treated with some caution, however as the majority of isolates from earlier survey years were obtained from major retailers. While it is possible that this may have influenced the percentages of resistant isolates observed, there is no evidence for any link between the type of retailer and the extent of AMR in campylobacter from chicken

- It is recommended that trends in antimicrobial resistance in *Campylobacter* spp. isolates from retail chickens continue to be monitored

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6.0 Appendices

6.1 Appendix I Survey protocol

Appendix I.docx

6.2 Appendix II Sample data year 5 data

HallmarkdataY5final .xls

6.3 Appendix III Survey year 5 microbiological data

See attachment

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