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 and 6 (2018 to 2020) Final Report

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Abbreviations

Acronym	Definition
AMR	Antimicrobial drug resistance
ºC	Degrees Celsius
GBRU	Gastrointestinal Bacteria Reference Unit
cfu	Colony forming units
CI	Confidence Interval
EQA	External Quality Assurance
EU	European Union
FSA	Food Standards Agency
FSS	Food Standards Scotland
G	Gram
H	Hours
PHE	Public Health England
MRD	Maximum Recovery Diluent
N	Number
SOP	Standard Operating Procedure
spp.	Species
WGS	Whole Genome Sequencing
MRD	Maximum Recovery Diluent
N	Number
UK	United Kingdom
µL	Microlitre

UKAS	United Kingdom Accreditation Service
WGS	Whole Genome Sequencing

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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 of transmission for this organism. The UK Food Standards Agency (FSA) agreed with poultry industry to reduce *Campylobacter* spp. contamination in raw chicken and set a target to reduce the prevalence of the most contaminated chickens (those with more than 1000 colony forming units (cfu) per gram (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 to 2015) and FS102121 (2015 to 2018). The FSA has recommended 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 in a sixth survey year from 2019 to 2020 and summarises the data for both the fifth and sixth survey year together presenting data from 2018 to 2020. 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 - the Public Health England (PHE), Porton Food, Water and Environmental laboratory and 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 cfu per g of chicken 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 of *Campylobacter jejuni* and *Campylobacter coli* isolates. A proportion of isolates were also subjected to AMR testing to determine the minimum inhibitory concentration to selected antimicrobials.

In the sixth survey year, 1008 test results were determined from chicken skin samples collected from non-major retailer stores between August 2019 to October 2020. *Campylobacter* spp. were detected in 59.6% of these samples, and 12.8% of these had counts above 1000 cfu per g chicken skin (referred to as highly

contaminated samples hereafter). There was no significant difference in the percentage of highly contaminated samples with counts above 1000 cfu per g chicken skin between the fifth and sixth survey year and the average for both years was 11.8%. While the average percentage of highly contaminated samples from fresh, whole chicken at retail sale in UK stores of smaller chains (for example Iceland, McColl's, Budgens, Nisa, Costcutter, One Stop), independents and butchers (these are collectively referred to as non-major retailer stores in this report) has decreased since the previous survey years (2014 to 2018) it is still higher than in samples from major retailers. The results from survey years 5 and 6 were combined and comparison among processing plant approval codes showed significant differences in the percentages of chicken samples with more than 1000 cfu per g, ranging from 0% to 34.9%. The percentage of samples with less than 10 cfu of *Campylobacter* spp. per g was significantly lower for samples collected in the months of June, July and August compared to the other calendar months. The percentage of highly contaminated samples was significantly higher in samples taken from larger chicken (those weighing more than 1750 g) compared to smaller ones. There were no statistical differences in the percentage of highly contaminated samples between those obtained from free-range and organically reared birds and those reared under a standard regime (these have 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 93.3% of the positive samples. *C. jejuni* was isolated from the majority (71.9%) of these samples while *C. coli* was identified in 23.8% of samples. A combination of both species was found in 4.3% of samples. *C. coli* was more frequently isolated from samples obtained from chicken reared with access to range than from standard birds. Compared to *C. coli*, detection of *C. jejuni* was less frequent 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 whole genome sequencing (WGS) data by the detection of known antimicrobial resistance determinants. In total 751 *C. jejuni* and 252 *C. coli* isolates were analysed by WGS. Resistance to ciprofloxacin was detected in 52.9% of *C. jejuni* isolates and in 43.7% of *C. coli* isolates. Resistance to tetracycline was detected in 61.5% of *C. jejuni* and in 66.3% of *C. coli* isolates. Five

C. coli (2%) isolates harboured the 23S mutations predicting reduced susceptibility to erythromycin whilst this was not detected in any *C. jejuni* isolates. Multidrug resistance (MDR), defined as harbouring genetic determinants for resistance to at least three unrelated antimicrobial classes, was found in 13 (5.2%) *C. coli* isolates but not in any *C. jejuni* isolates. Co-resistance to ciprofloxacin and erythromycin was predicted in 1.6% of *C. coli* isolates. Additional phenotypic AMR using minimum inhibitory concentration (MIC) testing was performed for 128 isolates and results were in agreement with those obtained by detection of AMR determinants from analysis of WGS data for 99.4%. Three isolates (0.3% of the total) expressed phenotypic resistance to streptomycin but none of the genetic determinants included in the analysis predicted this; one isolate did not express MIC-based phenotypic resistance to streptomycin but predicted to be so from a genetic determinant detected. Overall, the percentages of isolates with AMR determinants detected by WGS found in this study were similar to those reported in previous survey years (2015 to 2018) but it should be noted that testing was based on phenotypic break-point testing in the earlier survey years and that the majority of isolates were from major retailers in those years. It is recommended that trends in AMR for *Campylobacter* spp. isolates from retail chickens continue to be monitored to detect any increasing resistances of concern, particularly to erythromycin as this antimicrobial is of clinical importance.

This survey has found that 11.8% of fresh, whole UK chicken from non-major retailer stores were contaminated with high levels (meaning more than 1000 cfu per g) of *Campylobacter* spp. and that this continues to be above the levels found in chicken samples from major retailers (according to the *Campylobacter* data published on the nine major retailers' websites). The FSA has indicated that the target for the percentage of highly contaminated retail chickens should be less than 7% across all retailers. Whilst continued monitoring, according to data published on the nine major UK retailers' websites, show that chicken from major retailer stores have met the target, chicken on sale in other stores have yet to meet this target. More action, for example, consideration of interventions such as improved biosecurity on farms and slaughterhouse measures, is needed to achieve better control of *Campylobacter* spp. for this section of the retail industry.

1.0 Background

Campylobacter spp., especially *C. jejuni,* is the main cause of human bacterial gastroenteritis in the higher income countries and it has been estimated that there are in excess of half a million cases per year, leading to 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 be an underestimate by approximately 9.3-fold (EFSA and ECDC 2021, Tam *et al*. 2012). Source attribution studies, outbreak investigations and case-control reports all identify 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 pathway for 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 2015a and FSA 2015b, FSS 2015). Interventions including enhanced biosecurity measures as well as improvements in slaughterhouse hygiene 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. than samples taken immediately after slaughter (Purnell *et al*. 2004). This is likely to reflect the sensitivity of *Campylobacter* spp. to the oxygen level in air 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.

1.1 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. This was, to some extent, attributable to slaughterhouse-specific factors for colonised flocks from countries with a high prevalence, including the UK. Other data has also supported a role of slaughterhouses by detecting different levels of *Campylobacter* spp. contamination on carcasses from different slaughterhouses, despite the processed 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 finding a higher proportion of contaminated product during the warmer summer months compared to the rest of the year (Meldrum 2005, CLASSP Project Team 2010, Hutchison *et al*. 2006). The counts of *Campylobacter* spp. in post-chilled 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 depends 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 (ISO) provides a horizontal method for the enumeration of *Campylobacter* spp. involving direct plating onto modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubation for 48 h at 41.5 °C (ISO, 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). The EN/ISO 10272-2 method was therefore chosen for this survey.

1.2 Campylobacter types and AMR

In an 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 predicting reduced susceptibility to antimicrobial agents (Painset *et al.* 2020), indicating that WGS is a powerful tool for AMR surveillance programs. In Europe, certain antimicrobials must be tested for under the harmonised methods scheme for the monitoring of AMR in *Campylobacter* spp. isolates (ECDC 2016).

1.3 Findings from previous survey years

Table 1 provides a summary of the Campylobacter findings obtained in the previous years (2014 to 2019) of the survey.

Table 1 Percentages of samples with more than 10 or more than 1000 cfu of *Campylobacter* spp. per gram in the survey years from 2014 to 2019.

Survey period (year)	Sampling strategy	Retailer, number of samples[a]	Number of samples with more than 10 cfu per gram (%)	Number of samples with more than 1000 cfu per gram (%)
2014 to 2015 (1)	According to market share	Major, 3760	2758 (73.4)	707 (18.8)
2014 to 2015 (1)	According to market share	Non-major, 251	184 (73.3)	73 (29.1)
2015 to 2016 (2)	Aiming for same number of samples from each major retailer	Major, 3089	1849 (59.9)	314 (10.2)
2015 to 2016 (2)	Aiming for 7-10% of samples from non-majors	Non-major, 325	197 (60.6)	49 (15.1)
2016 to 2017 (3)	Aiming for same number of samples from each major retailer	Major, 3890	2036 (52.3)	193 (5.0)
2016 to 2017 (3)	Aiming for 7-10% of samples from non-majors	Non-major, 378	266 (70.4)	59 (15.6)
2017 to 2018 (4)	Sampling from major retailers in August to October 2017	Major, 955	500 (52.4)	50 (5.2)
2017 to 2018 (4)	Sampling from from non-majors throughout the survey year	Non-major, 814	614 (75.4)	120 (14.7)

2018 to 2019 (5)	Samples from non-majors only	Non-major, 1008	562 (55.8)	109 (10.8)

[a] Retailers were categorised as either belonging to one of the nine major UK retailers (major retailers) or not (non-major).

In 2014-15 (survey year 1), the 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 year, samples from 4,011 packs of whole, fresh, UK chicken collected (according to market share, from all types of retail stores) were tested between February 2014 and March 2015. The overall prevalence of *Campylobacter* spp. in these samples of fresh chicken at retail in the UK was 73.3% (PHE 2015). A considerable percentage (19.4%) of samples had more than 1,000 cfu of *Campylobacter* per g chicken skin, with percentages ranging between 12.9% to 29.9% among retailers.

Significant differences among retailers could not be explained by remaining shelf-life, chicken weights, time of year sampled or type of chicken rearing. Some production plant approval codes (signifying the slaughterhouse premises) showed significant differences in the percentage of chickens with >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 (here defined as chicken packs 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 (meaning chicken sold as free-range and organic) being more contaminated than birds reared under standard conditions. *C. jejuni* was detected in 76.6% the chicken skin samples from which isolates were speciated. *C. coli* was identified in 13.9% of samples and both species were found in 4.2% of samples. *Campylobacter coli* was more frequently detected in the summer months than in winter and spring months and was more frequently detected in chicken reared with access to range. The FSA continued the monitoring programme over three further years (under project FS102121). This project also continued to identify *Campylobacter* spp. present and to determine susceptibility of the *Campylobacter* spp. isolates obtained to a defined range of antimicrobial agents.

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 retailer stores in the UK was observed to decline from 73.3% to 61.3% and the percentage of samples with more than 1000 cfu per g chicken skin declined from 19.4% to 11.4% (PHE 2017). There were statistically significant differences in the percentages of highly contaminated chickens (ranging from 6.7% to 17.7%) among retailers that could not be explained by remaining shelf-life, chicken weights, sampling period or the type of bird reared. Comparison of production plant approval codes identified significant differences 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 (those weighing more than 1750 g) were more likely to be contaminated with more than 1000 cfu per g. There was no evidence of free-range and organic birds being more contaminated than birds reared under standard conditions, but the small numbers of free-range and organic birds tested prevented robust statistical analysis. 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 isolated more frequently 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 percentage of samples with more than 1000 cfu per g declined to 6% (PHE 2018). There were differences among the percentages of highly contaminated chickens from the different retailer groups (ranging from 1% to 18%) and among individual production plant approval codes (ranging from 1% to 19%). *C. jejuni* was identified in the majority of chicken skin samples (87.7%) from which isolates were submitted for speciation, *C. coli* was identified in 10.2%. Similar results were obtained from the first quarter of a 4th survey year, where 1044 samples were obtained from major as well as non-major retailer stores (the latter defined as stores not belonging to any of the major UK retailers).

The prevalence of *Campylobacter* spp. in fresh chicken at retail in the UK was approximately 56% and the percentage of samples with more than 1000 cfu per g chicken skin was approximately 6% (PHE 2019). In the sample spanning the entire 4th survey year from August 2017 to July 2018, with chickens only from non-major retailer stores, the prevalence of *Campylobacter* spp. was 75.4% and the proportion of samples with more than 1000 cfu per g chicken skin was 14.7%. In samples collected from non-major stores during the 5th survey year (from August 2018 to July 2019) the prevalence of *Campylobacter* spp. was 55.8% and the proportion of samples with more than 1000 cfu per g chicken skin was 10.8% (FSA 2021). While this could suggest some improvement in this sector of the market, chickens from non-major retailer stores continued to pose a greater risk than chicken from major retailer stores.

In summary, the survey data from the first four survey years showed 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 since the first survey period that started in 2014. A higher percentage of chickens from non-major retailers have remained contaminated with high levels of *Campylobacter* (with more than 1,000 cfu per g). The current focus on smaller establishments and their suppliers may allow 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 October 2020 was to determine if any decline in contamination of chickens for these types of stores could be detected and to monitor trends in the percentage of isolates with AMR.

2.0 Methods

Sampling and testing procedures for the survey were agreed with the FSA. The survey protocol used for the time-period from August 2018 to October 2020 is briefly described (enclosed as Appendix I).

2.1 Sampling

Sampling locations were identified by Hallmark Meat Hygiene Ltd. across the UK, to reflect population sizes. Fresh whole chickens were sampled from non-major retailer stores from August 2018 to October 2020. On arrival at the laboratory, the air temperature of the cool boxes was determined using calibrated temperature probes. Sample details were documented and logged onto the laboratory information management system. Samples were obtained from non-major 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). These stores are collectively referred to as non-major retailer stores in this report.

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/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 antimicrobial resistance profile

The aim was to investigate one isolate from each positive sample by WGS (although no isolates were available from a small proportion of the 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 Kits, followed by rapid-run paired-end sequencing on an Illumina High-Seq 2500 platform to produce 100 base pair 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.

Table 2 Description of the genetic determinants for antimicrobial resistance in *Campylobacter* spp. tested for survey years 5 and 6.

Gene affected	Antimicrobial	Resistance determinant
23s	Erythromycin (macrolide)	• 23s_ [2075:A-G] • 23s_ [2074:A-C; 2075:A-R] • 23s_ [2074:A-M] • 23s_ [2074:A-T]
gyrA	Ciprofloxacin (fluoroquinolone)	• 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).

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 (referred to as resistance hereafter) to erythromycin (macrolide), ciprofloxacin (fluoroquinolone), gentamicin and streptomycin (aminoglycosides) and tetracycline (Table 2). A very high level of agreement (97.5%) was found between genotype and phenotype in the PHE validation study (Painset *et. al*. 2020).

For a sub-set of isolates (84 *C. jejuni* and 44 *C. coli* isolates), AMR testing was also done by determining the Minimum Inhibitory Concentration (MIC) towards selected antimicrobials. Isolates were chosen to include any predicted multi-drug resistant isolate as well as all isolates predicted to have resistance to erythromycin or streptomycin, then choosing one isolate from each ST representing fully sensitive as well as resistant isolates. Each isolate for MIC testing was prepared by inoculating on to CCDA then Blood agar to achieve single colony growth and the plates were incubated at 41.5 ± 1ºC for 24 ± 2 hours in micro-aerophilic conditions. Using a nephelometer, the opacity was checked by inoculating 5 mL of distilled water with a little growth transferred with a 1 µL loop from the pure colonies. The distilled water bottle and inoculum were mixed by gently inverting the bottle 8-10 times before 100 µL of the inoculum was transferred to an 11 mL Cation Adjusted Mueller-Hinton Broth with N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid with 5% lysed horse blood. This was then mixed by inverting the bottle 8-10 times. The inoculated broth was used to inoculate 100 µL into each of the 96 plate wells using Senititre AIM (automated inoculation delivery system). The plates were covered with the adhesive seal provided, ensuring that all wells are covered and sealed. The plates intended for CO_2 incubation were covered with a perforated seal. To avoid growth of the inoculum, no more than 15-20 minutes passed from suspension preparation to plate inoculation and sample incubation. The plate was incubated at 42 ± 1 °C in micro-aerophilic conditions for 24 ± 3 hours ensuring that the plate was not shaken or stacked more than three plates high. After incubation, the plates were read in VIZION. Each plate was assigned a unique bar code. Plates were read for determining MIC values and interpreted for resistance based on EUCAST ECCOF (Epidemiological cut-off points) values specific for Campylobacter as described in Annex, Part A, Table 2 of Decision EU/2013/652. *C. jejuni* ATCC 33560, the recommended control organism, was tested with each batch of tests as detailed above.

2.4 Quality Assurance

Both laboratories participate in recognised External Quality Assurance schemes for example the 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.

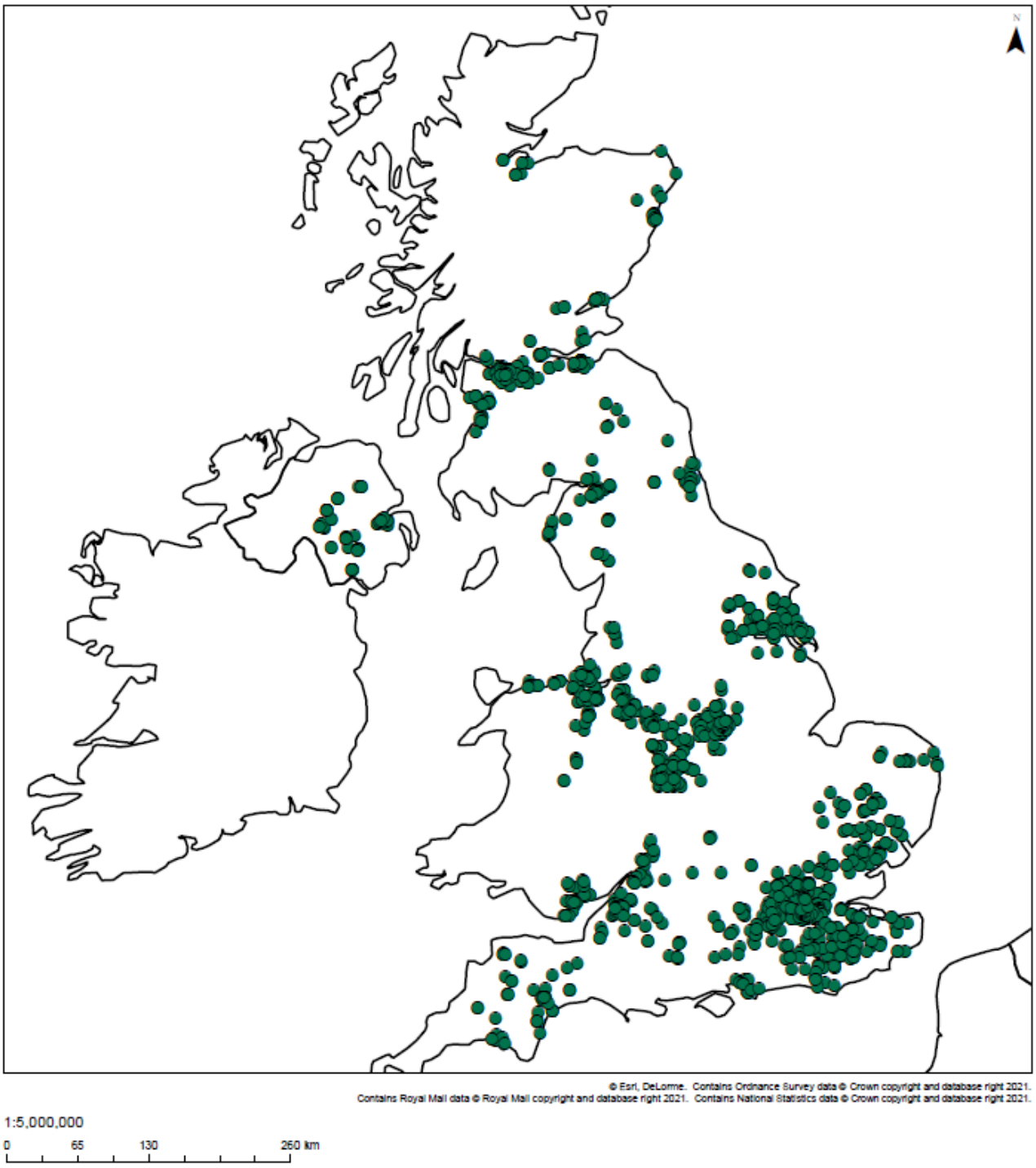
2.5 Statistical Analysis

Cross tabulations were analysed by the calculation of Clopper-Pearson exact 95% confidence intervals (CI) for the proportion in each cfu per g category. Confidence intervals given for each variable show the likely range of results allowing for the number of samples taken. The 95% CIs 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* spp. contamination. Fisher's exact test was used for individual comparisons when samples were small.

3.0 Results

Results are presented for the entire sampling period from August 2018 to October 2020 and also separately for Year 5 and 6 in the comparison of the different categories of non-major retailer stores. Fresh whole UK produced chickens were collected from non-major stores across the UK between August 2018 and October 2020, although sampling was disrupted due to the Covid19 pandemic and no samples were collected in April and May 2020 (Figure 1). Samples were collected from many different types of stores and details of samples can be found elsewhere (see accompanied data, Hallmark report). In year 6, no test results were obtained from 21 samples in the sampler's report as they either had insufficient neck-skin or exceeded the recommended transport time (received more than 48 hours after sampling) or did not meet the survey criteria (for example if a non-UK chicken).

Figure 1: Map of store locations of chicken samples tested in year 5 (August 2018 - July 2019) and 6 (August 2019 - October 2020).



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

In year 6 of the survey (from August 2019 to October 2020) *Campylobacter* spp. were enumerated in 1008 chicken skin samples from non-major retail stores and

detected in 601 (59.6%) of these of which 129 (12.8%) had counts above 1000 cfu per g. Chickens from non-major retailer stores were tested as these chickens were thought to constitute a greater risk than chicken from major retailer stores (FSA 2021). The highest single count detected was 89000 cfu of *Campylobacter* spp. per g skin (see accompanied data for full list of sample result details). There was no significant ($p < 0.05$; fishers exact test) difference in the percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g between year 6 and year 5. In total for survey years 5 and 6 the percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g was 11.8% and *Campylobacter* spp. were detected in 57.7% of samples (if present at more than or equal to 10 cfu per g).

3.1.1 *Campylobacter* spp. counts in relation to non-major retailer store category

In year 6, the proportion of chickens with *Campylobacter* spp. levels at more than 1000 cfu per g ranged from 5.0% to 17.3% amongst the groups of retail stores (Table 3). In year 5, the percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g ranged from 7.5% to 14.0% amongst the retail store categories. There were no significant differences between survey years 6 and year 5 in terms of the percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g for each of the three categories of non-major retailer stores. In total for survey years 5 and 6 the percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g ranged from 6.3 % to 15.2 % amongst the non-major retail store categories. The percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g was not significantly different (p more than 0.05) for stores categorised as “other” or “butcher” compared to the average for all samples. Butcher shops had a higher percentage (p less than 0.01) of chickens with more than 1000 cfu of *Campylobacter* spp. per g compared to the smaller chains store category, recognised as being part of smaller chains (for example, Iceland, McColl’s, Budgens, Nisa, Costcutter, One Stop and other similar chains). In year 5, the percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g ranged from 7.5% to 14.0% amongst the retail store categories.

Table 3 *Campylobacter* spp. counts in whole fresh UK chicken from non-major stores in year 5 and 6.

Retail store category	Survey Year	Number of samples	Percentage of samples with less than 10 cfu of Campylobacter per gram (95% CI[a])	Percentage of samples with 10 to 1000 cfu of Campylobacter per gram (95% CI)	Percentage of samples with more than 1000 cfu of Campylobacter per gram (95% CI)
Smaller chains[b]	6	338	55.0 (49.6-60.4)	39.9 (34.7-45.4)	5.0 (3.0-7.9)
Butchers	6	415	31.6 (27.1-36.3)	52.1 (47.1-57.0)	16.4 (13.0-20.3)
Others[c]	6	255	35.3 (29.4-41.5)	47.5 (41.2-53.8)	17.3 (12.8-22.5)
All	6	1008	40.4 (37.3-43.5)	46.8 (43.7-50.0)	12.8 (10.8-15.0)
Smaller chains	5	360	50.8 (45.5-56.1)	41.7 (36.5-47.0)	7.5 (5.0-10.7)
Butchers	5	401	38.4 (33.6-43.4)	47.6 (42.7-52.7)	14.0 (10.7-17.8)
Others	5	247	44.1 (37.8-50.6)	45.3 (39.0-51.8)	10.5 (7.0-15.0)
All	5	1008	44.3 (41.2-47.4)	44.9 (41.8-48.1)	10.8 (9.0-12.9)
Smaller chains	5 and 6	698	52.9 (49.1-56.6)	40.5 (36.9-44.3)	6.3 (4.6-8.4)
Butchers	5 and 6	816	34.9 (31.7-38.3)	49.9 (46.4-53.4)	15.2 (12.8-17.9)
Others	5 and 6	502	39.6 (35.3-44.1)	46.4 (42.0-50.9)	13.9 (11.0-17.3)
All	5 and 6	2016	42.3 (40.1-44.5)	45.9 (43.7-48.1)	11.8 (10.4-13.3)

[a] Confidence intervals (CI) show the likely range of results allowing for the number of samples taken; the 95% CI means that we would expect the true prevalence to fall within the lower and upper limits 95% of the time.

[b] These shops included stores 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.

[c] Others included independents, farm shops, markets but not butchers or chains or any of the major retailer stores.

3.1.2 *Campylobacter* spp. counts in relation to chicken rearing regime

In Table 4 the levels of *Campylobacter* spp. counts detected in samples from birds reared as “standard” (reared without access to range), free-range, or organic are summarised. There were fewer samples from free range or organic chickens examined, reflecting their lower market share. Within this dataset, no significant differences in the percentage of highly contaminated chickens between the three types of chicken 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 October 2020, in relation to chicken rearing regime.

Chicken rearing regime	Number of samples	Percentage of samples with less than 10 cfu of campylobacters per gram (95% CI)	Percentage of samples with 10 to 1000 cfu of campylobacters per gram (95% CI)	Percentage of samples with more than 1000 cfu of campylobacters per gram (95% CI)
Standard	1873	43.4 (41.0-45.5)	45.2 (43.0-47.5)	11.5 (10.1-13.1)
Free Range	134	29.1 (21.6-37.6)	54.5 (45.7-63.1)	16.4 (10.6-23.9)
Organic	9	44.4 (13.7-78.8)	55.6 (21.2-86.3)	0.0 (0.0-33.6)

3.1.3 *Campylobacter* spp. counts in relation to processing plant

Table 5 summarises the *Campylobacter* spp. levels obtained from the non-major retailers by processing plants. There were differences in the percentage of chicken samples that were highly contaminated among the different processing plants. The number of samples collected from each processing plant was limited which meant only large differences could be statistically significant (Table 5). The percentages of chickens with more than 1000 cfu of *Campylobacter* spp. per g ranged from 0 (for approval number 3011) to 34.9% (for approval number 4561) among the processing plants. Processing plants 2037, 2750, 3011 and 5464 produced significantly fewer highly contaminated chickens compared to the average (11.8%) for all samples.

Compared to the average, a significantly larger percentage of samples obtained from processing plant 4561 were highly contaminated. 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 samples.

Table 5 *Campylobacter* spp. counts in chicken collected from non-major retail stores from August 2018 to October 2020, in relation to processing plant for survey years 5 and 6.

Processing plant approval number	Number of samples	Percentage of samples with less than 10 cfu of campylobacters per gram (95% CI)	Percentage of samples with 10 to 1000 cfu of campylobacters per gram (95% CI)	Percentage of samples with more than 1000 cfu of campylobacters per gram (95% CI)
1007	111	39.6 (30.5-49.4)	43.2 (33.9-53.0)	17.1, (10.6-25.4)
1100	26	80.8 (60.7-93.5)	15.4 (4.4-34.9)	3.8, (0.1-19.6)
2023	141	33.3 (25.6-41.8)	53.9 (45.3-62.3)	12.8, (7.7-19.4)
2037	126	55.6 (46.4-64.4)	39.7 (31.1-48.8)	4.8, (1.8-10.1)
2653	167	46.7 (39.0-54.6)	40.1 (32.6-48.0)	13.2, (8.4-19.3)
2750	116	69.0 (59.7-77.2)	27.6 (19.7-36.7)	3.4, (1.0-8.6)
3011	61	78.7 (66.3-88.1)	21.3 (11.9-33.7)	0.0, (0.0-5.9)
4017	111	28.8 (20.6-38.2)	62.2 (52.5-71.2)	9.0, (4.4-15.9)
4561	43	30.2 (17.2-46.1)	34.9 (21.0-50.9)	34.9, (21.0-50.9)
4800	60	43.3 (30.6-56.8)	35.0 (23.1-48.4)	21.7, (12.1-34.2)
5003	82	31.7 (21.9-42.9)	48.8 (37.6-60.1)	19.5, (11.6-29.7)
5007	367	36.8 (31.8-42.0)	52.0 (46.8-57.3)	11.2, (8.1-14.9)
5464	165	50.9 (43.0-58.8)	44.2 (36.5-52.2)	4.9, (2.1-9.3)
8013	30	20.0 (7.7-38.6)	66.7 (47.2-82.7)	13.3, (3.8-30.7)
9554	78	21.8 (13.2-32.6)	57.7 (46.0-68.8)	20.5, (12.2-31.2)
Other codes[a]	274	35.8 (30.1-41.8)	50.4 (44.3-56.4)	13.9 (10.0-18.5)
Not available[b]	58	48.3 (35.0-61.8)	39.7 (27.1-53.4)	12.1 (5.6-23.3)

[a] Samples listed within the 'Other codes' category had less than 25 chickens from any single processor tested. The full list of approved processing plant premises numbers can be found on the FSA website including the details of each license.

[b] Samples lacking the processing plant approval number.

3.1.4 *Campylobacter* spp. counts in relation to sampling period

For all samples tested in the period from August 2018 to October 2020, the proportion of samples with more than 1000 cfu of *Campylobacter* spp. per g was 13.6% for samples collected in June, July and August compared to 11.2% for samples from the remaining months, although this difference was not statistically significantly different (Table 6). The percentage of samples with less than 10 cfu per g of *Campylobacter* spp. was significantly (Fishers exact test) lower in June, July and August than in the remaining sampling months (Table 6).

Table 6 *Campylobacter* spp. counts in whole fresh chicken collected from non-major retail stores, in relation to sampling months for survey years 5 and 6.

Sampling months	Number of samples	Percentage of samples with less than 10 cfu of campylobacters per gram (95% CI)	Percentage of samples with 10 to 1000 cfu of campylobacters per gram (95% CI)	Percentage of samples with more than 1000 cfu of campylobacters per gram (95% CI)
June, July and August	516	35.7 (31.5-39.9)	50.8 (46.4-55.2)	13.6 (10.7-16.8)
September to May	1500	44.6 (41.1-47.2)	44.2 (41.7-46.8)	11.2 (9.7-12.9)

3.1.5 *Campylobacter* spp. counts in relation to chicken pack weight

Chickens were assigned into three weight categories which were 'small' (less than 1400 g), 'medium' (1400 to 1750 g) or 'large' (more than 1750 g); no weight was available for eight samples. Assignment of a size category to the chicken 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 1750 g compared to larger chickens (Table 7). The percentage of samples with more than 1000 cfu of *Campylobacter* spp. per g was significantly higher for large chickens compared to pack weights of less than or equal to 1750 g.

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 for the combined data for survey years 5 and 6.

Chicken pack weight	Number of samples	Percentage of samples with less than 10 cfu of Campylobacter per gram (95% CI)	Percentage of samples with 10 to 1000 cfu of Campylobacter per gram (95% CI)	Percentage of samples with more than 1000 cfu of Campylobacter per gram (95% CI)
Small (less than 1400 gram)	702	49.0 (45.2-52.8)	40.5 (36.8-44.2)	10.5 (8.4-13.1)
Medium (1400-1750 gram)	741	46.0 (42.4-49.7)	43.7 (40.1-47.4)	10.3 (8.2-12.7)
Large (more than 1750 gram)	565	28.7 (25.0-32.6)	55.9 (51.7-60.1)	15.4 (12.5-18.6)

[a] no weight data was available for eight chickens (no exact weight from the store was available).

3.2 *Campylobacter* spp. in chicken at retail sale in non-major stores

From all samples tested in survey years 5 and 6 (n = 2016), *C. jejuni/C. coli* speciation testing was performed for 1085 of the 1163 samples where *Campylobacter* spp. were detected. No speciation test was available for the remaining samples as the isolates from these samples died before speciation could

be completed. *C. jejuni* alone was detected in 71.9%, *C. coli* alone in 23.8% and both species in 4.3% of samples (Table 8). The occasions where both species were detected were as a result of either mixed or hybrid strains and this was not investigated further.

Table 8 Number of samples where of *Campylobacter jejuni (C. jejuni)* or *Campylobacter coli (C. coli)* or both *C. jejuni* and *C. coli* were detected in fresh chicken from non-major retailer stores for survey years 5 and 6.

Survey year	Number of samples with *C. jejuni*	Percentage of samples where *C. jejuni* was detected	Numbero samples with *C. coli*	Percentage of samples where *C. coli* was detected	Number of samples with *C. jejuni* and *C. coli*	Percentage of samples where both *C. jejuni* and *C. coli* were detected
Year 5	381	72.6	117	22.3	27	5.1
Year 6	399	71.3	141	25.2	20	3.6
Total	780	71.9	258	23.8	47	4.3

The total of 781 *C. jejuni* and 258 *C. coli* isolates were tabulated in relation to the different chicken rearing types (in one sample two isolates were characterised hence 781 *C. jejuni* isolates but 780 samples with *C. jejuni*; Table 9). *C. coli* was detected significantly more frequently in samples from chicken reared as free-range compared to samples from chicken reared in a standard regime (p less than 0.001) (Table 9).

Table 9 Number and percentages of *Campylobacter jejuni (C. jejuni)* and *Campylobacter coli (C. coli)* isolates in fresh chicken from non-major retail samples in relation to the chicken rearing regime for survey years 5 and 6.

Rearing	Total number of *C. jejuni* and *C. coli* isolates	Number of *C. jejuni* isolates	Percentage of isolates that were *C. jejuni*	Number of *C. coli* isolates	Percentage of isolates that were *C. coli*
Standard	948	733	77.3	215	22.7
Free range	85	43	50.6	42	49.4
Organic	5	5	80.0	1	20.0

The percentage of *C. jejuni* was significantly lower in the summer months than in the remaining months; conversely the percentage of *C. coli* was higher in summer months (p less than 0.001; Table 10).

Table 10 *Campylobacter jejuni (C. jejuni)* and *Campylobacter coli (C. coli)* isolates from chicken skin samples collected from non-major retailer stores in relation to season for survey years 5 and 6.

Months	Total number *of C. jejuni* and *C. coli* isolates	Percentage of isolates that were *C. jejuni*, 95% CI	Percentage of isolates that were *C. coli*, 95% CI
June, July August	303	69.6, 64.1-74.8	30.4, 25.2-35.9
September to May	941	80.2, 77.5-82.7	19.8, 17.3-22.5

Figure 2a: Whole-genome-sequencing-based MLSTs of *Campylobacter jejuni* isolates from survey years 5 and 6.

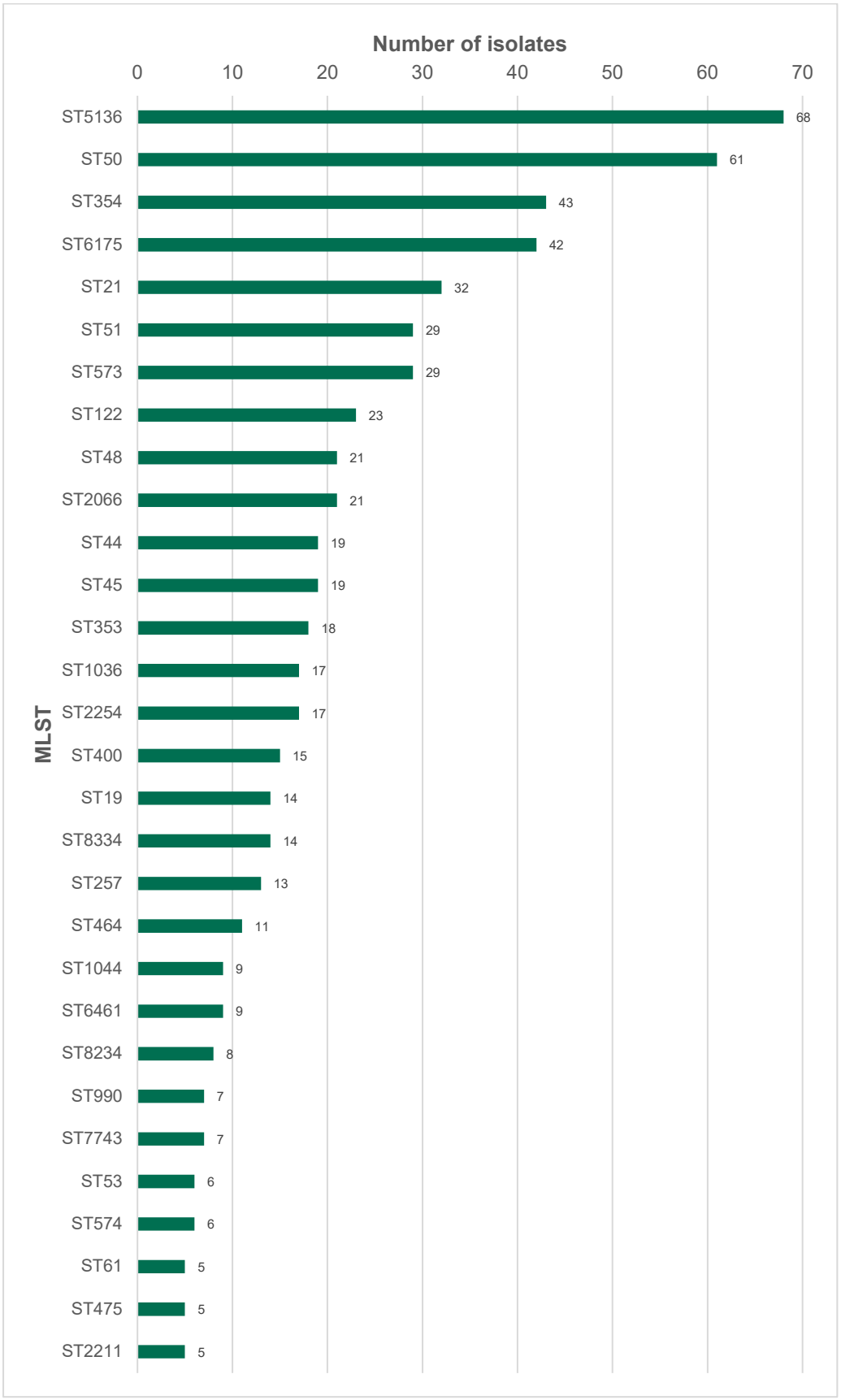
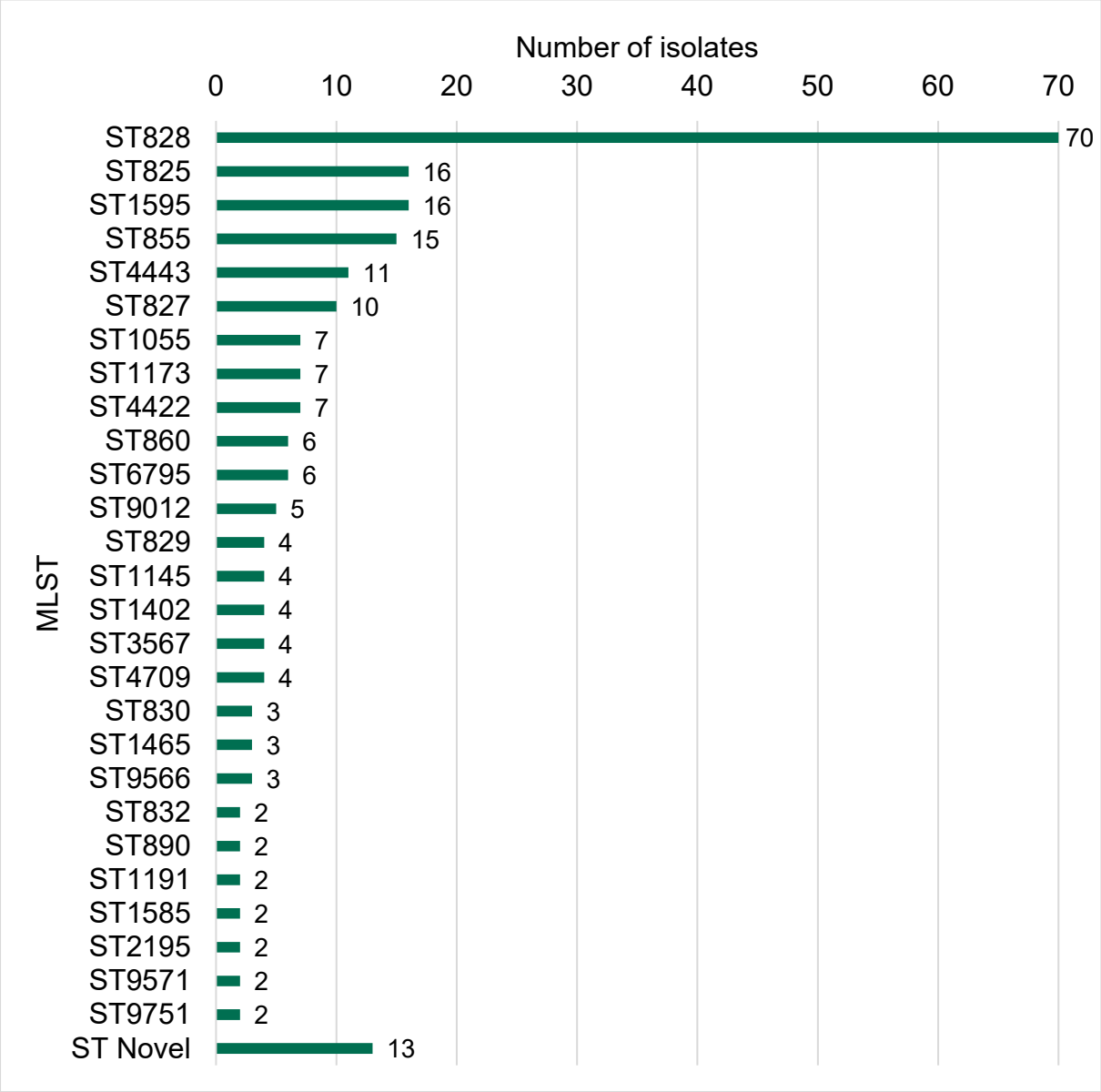


Figure 2b: Whole-genome-sequencing-based MLSTs of *Campylobacter jejuni* isolates from survey years 5 and 6.

Sequence types (MLST) based on analysis of WGS data was assigned for 1003 isolates. No ST could be assigned to 47 isolates that were found to be of mixed type. There was considerable diversity with 135 different STs detected and 59 isolates were assigned as novel ST. The ten most common STs within the 752 *C. jejuni* isolates were ST5136, ST50, ST354, ST6175, ST 21, ST 51, ST 573, ST122, ST48 and ST2066, together accounting for 49% of the *C. jejuni* isolates (Figure 2a and 2b). The following 33 *C. jejuni* MLST were detected in a single isolate: ST5, ST11, ST 22, ST230, ST267, ST447, ST461, ST577, ST699, ST814, ST904, ST905, ST945, ST 996, ST1034, ST1076, ST1268, ST1489, ST1709, ST1900, ST2314, ST3895, ST4425, ST5805, ST6209, ST7420, ST7749, ST8395, ST8461, ST9401, ST9570, ST9572 and ST9581.

There was less diversity within the 252 *C. coli* isolates and the most common STs were ST828, ST825, ST1595 and ST855 accounting for 46% of the *C. coli* isolates (Figure 3). The following 20 *C. coli* MLSTs were detected in a single isolate each: ST853, ST962, ST1107, ST1438, ST 1578, ST1749, ST1774, ST2256, ST2273, ST2733, ST3077, ST3404, ST4149, ST4304, ST4425, ST4433, ST4453, ST4543, ST8053 and ST10042.

Figure 3: Whole-genome-sequencing-based MLST for *Campylobacter coli* isolates for survey years 5 and 6.



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

Predicted AMR determinants as derived from analysis of WGS data were obtained from a total of 751 *C. jejuni* and 252 *C. coli* isolates from 1002 samples in the survey period from August 2018 to October 2020. In one sample, AMR profiles were obtained from two isolates – both were *C. jejuni* and no AMR determinants were detected in these two isolates (Table 11).

Table 11 Frequencies of antimicrobial resistance determinants based on WGS, in *Campylobacter jejuni (C. jejuni)* isolates from chicken at retail sale (non-major stores) in survey years 5 and 6.

Time period	Number of isolates	Percentage of *C. jejuni* isolates with predicted resistance to ciprofloxacin (95% CI)	Percentage of *C. jejuni* isolates with predicted resistance to tetracycline (95% CI)
Year 5	359	51.0 (45.7-56.3)	61.0 (55.7-66.1)
Year 6	392	54.6 (49.5-59.6)	62.0 (57.0-66.8)
Total	751	52.9 (49.2-56.5)	61.5 (57.9-65.0)

There were no significant differences in the percentages of isolates with AMR determinants as determined by analysis of WGS data between the survey year 5 and 6 isolates except for resistance determinants to streptomycin in *C. coli* where the percentage of isolates with such determinants was lower in year 6 compared to year 5 (Table 12).

Table 12 Percentages of antimicrobial resistance determinants based on WGS, in *Campylobacter coli (C. coli)* isolates from chicken at retail sale (non-major stores) in survey years 5 and 6.

Survey year	Number of isolates	Percentage of *C. coli* isolates with predicted resistance to ciprofloxacin (95% CI)	Percentage of *C. coli* isolates with predicted resistance to tetracycline (95% CI)	Percentage of *C. coli* isolates with predicted resistance to erythromycin (95% CI)	Percentage of *C. coli* isolates with predicted resistance to streptomycin (95% CI)
Year 5	116	42.2 (33.1-51.8)	62.1 (52.6-70.9)	2.6 (0.5-7.4)	12.1 (6.8-19.4)
Year 6	136	44.1 (35.6-52.9)	69.9 (61.4-77.4)	1.5 (0.2-5.2)	1.5 (0.2-5.2)
Total	252	43.7 (37.4-50.0)	66.3 (60.1-72.1)	2.0 (0.7-4.6)	6.4 (3.7-10.1)

For survey years 5 and 6, a total of 397 (52.9%) isolates of the *C. jejuni* and 109 (43.3%) isolates of *C. coli* harboured genetic determinants for resistance to ciprofloxacin (fluoroquinolone) as predicted from the detection of known point mutations in *gyrA* (Table 11 and 12). The presence of *tet(O)* variants conferring resistance to tetracycline were detected in 461 (61.2%) *C. jejuni* isolates and in 167 (66.3%) *C. coli* isolates. Five (2.0%) 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 two out of three copies of the 23S rRNA gene. The *aadK* gene, which is associated with reduced susceptibility to streptomycin was detected in 16 (6.3%) *C. coli* isolates, but was not detected in any of the *C. jejuni* isolates tested. The presence of the gene *aac(6')-aph(2")* which is associated with reduced susceptibility to gentamicin was not detected in any *C. jejuni* or *C. coli* isolates. Of the 128 isolates where phenotypic MICs were also determined there was an extremely high correlation with the predicted AMR profile derived from the analysis of the WGS data; with the correct prediction of sensitive/resistant AMR phenotypes in 636 out of 640 (99.4%) isolate/antimicrobial combinations. Only four discrepancies between AMR predicted from WGS and phenotype testing were identified and these were all observed for streptomycin. One discrepant result was streptomycin resistance predicted according to detection of the *ant(6)-Ia* gene but phenotypic resistance to streptomycin was not confirmed by MIC; in three isolates phenotypic resistance to streptomycin was detected by MIC but not predicted by any aminoglycoside determinants included in the PHE pipeline (Painset *et. al*. 2020). Thirteen (5.2%) of the *C. coli* isolates, all belonging to ST828, were classified as multidrug resistant (MDR), i.e. harbouring genetic determinants known to confer resistance to at least three unrelated antimicrobial classes (Table 13). None of the *C. jejuni* isolates were classified as MDR.

Combined resistance to ciprofloxacin and erythromycin was detected in 1.6% of *C. coli* isolates but not in any *C. jejuni* isolates.

Table 13 Multidrug resistant isolates of *Campylobacter coli* from whole fresh chicken at retail sale from non-major stores from survey years 5 and 6.

Sample number	MLST	Streptomycin (amino-glycoside)	Ciprofloxacin (fluoro-quinolone)	Erythromycin (macrolide)	Tetracycline (tetracycline)
364768	828	R[a]	R	S[b]	R
364766	828	R	R	S	R
364904	828	R	R	S	R
364650	828	R	R	S	R
364674	828	R	R	S	R
364735	828	R	R	S	R
364573	828	R	R	S	R
540420	828	S	R	R	R
343625	828	R	R	S	R
560952	828	S	R	R	R
364887	890	R	R	S	R
343262	8053	S	R	R	R
2447932	1438	R	R	R	R

[a] R denotes resistant isolates according to EUCAST breakpoints

[b] S denotes isolates susceptible to the antimicrobial

4.0 Discussion

4.1 Survey results

This report presents results from continued testing of whole fresh chickens from non-major retailer stores in the UK from August 2018 to October 2020. Such chickens have been found to be more contaminated with higher levels of campylobacters (meaning more than 1000 cfu of Campylobacter per g) than chickens from major retailers (PHE 2019). These chickens are, therefore thought to pose a greater risk to consumers if thorough cooking and hygienic handling procedures are not followed.

There was no significant difference in the percentage of samples with counts above 1000 cfu of campylobacters per g chicken skin between samples from survey Year 5 (August 2018 to July 2019) and survey Year 6 (August 2019 to October 2020); the

average percentage for both years was 11.8%. In comparison, a significantly higher level of contamination was found in survey year 4 (2017-2018) where the percentage of chicken samples with counts above 1000 cfu per g chicken skin was 15% (p less than 0.05). This could suggest improvement (for example in biosecurity on farms or improved hygiene during processing), 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 Year 6 survey, the proportion of chickens with *Campylobacter* spp. levels at more than 1000 cfu per g chicken skin ranged from 6.3% to 15.2% across the types of stores and the proportion of highly contaminated chickens was higher for butcher stores compared to the group of stores recognised as being part of smaller retail chain stores. Further studies would be needed to provide a more comprehensive understanding of the extent to which different processing plants or other factors such as sourcing of birds from specific farms may explain the observed difference in levels of contamination. Retailers may be able to use knowledge about processing plant performance to influence their sourcing of chicken suppliers.

By tracking chickens to their processing plant through the plant approval number, there was significant evidence of a link between processing plants and the levels of *Campylobacter* spp. found in whole fresh retail chicken samples. The percentage of chickens with more than 1000 cfu of *Campylobacter* spp. per g ranged from 0 to 34.9% among approval numbers/processing plant. This range could reflect differences in slaughterhouse hygiene practices and/or differences in the proportion of highly contaminated chicken flock batches received by slaughterhouses. The proportion of highly contaminated chicken batches received at slaughterhouses in turn may relate to differences in the likelihood of supplying farms rearing (and supplying) chicken that are, or are not, colonised with *Campylobacter* (Bull et al. 2006).

Whilst there was no statistical evidence that free-range or organic chickens were more highly contaminated than birds reared in a standard regime with no access to range, 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. The corresponding confidence intervals were wide and would therefore only be able to verify very large differences. Nevertheless, very similar findings were made in the previous survey years (PHE 2017; PHE 2018; PHE 2019).

The percentage of samples with more than 10 cfu of *Campylobacter* spp. per g was significantly higher for samples collected in June, July and August compared to the other calendar months. The tendency to have a higher percentage of samples testing positive for *Campylobacter* spp. during the summer months was also found in samples tested during the first two survey years.

The percentage of highly contaminated samples was significantly higher for samples from larger chickens than from smaller chickens. This could relate to the age of the chicken and / or the likelihood of originating from a previously partly depopulated chicken house. The age of the birds at slaughter as well (as a chicken house previously partly depopulated) has been associated with an increased likelihood of testing positive for campylobacters at slaughter (Bull et al. 2006; EFSA 2010b).

C. jejuni was isolated from the majority (71.9%) of the samples detailed in this report and *C. coli* was identified in 23.8% of samples, whilst 4.3% of samples were contaminated with both species. In agreement with findings from the previous survey years, *C. coli* was more frequently detected in samples from chickens reared as free-range compared to samples from chickens reared without access to range. It is possible this could relate to the generally older age of free-range/organic birds and/or the breed/rearing in free-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). Detection of *C. jejuni* was less common during the summer months of June, July and August compared to the remaining months of the year in agreement with findings from previous survey years.

4.2 Antimicrobial resistance (AMR) results

The data from *Campylobacter* spp. isolates obtained from chickens on retail sale in non-major retailer stores from survey years 5 and 6 showed similar results for predicted AMR when compared to the data from the previous survey years (Table 14). This was despite a difference in sampling where samples from major retailers were much more prominent in the earlier survey years and a difference in the

methodology used to ascertain rates of AMR. Comparison of previous and current datasets (i.e., 2007/2008 FSA retail chicken survey, the 2010 CLASSP survey (FSA 2003; FSA 2009; CLASSP Project Team 2010)) demonstrate higher percentages of isolates with resistance to ciprofloxacin in recent years compared to earlier studies from 2001 to 2007. In survey years 5 and 6, MDR was only found in *C. coli* isolates (5.2%) but this percentage was low compared EU data from 2019 (EFSA and ECDC 2021). Combined resistance to ciprofloxacin and erythromycin was significantly lower in the chicken isolates from all years compared to EU data on isolates from broilers in 2019 where this co-resistance was detected in 6.5% of *C. coli* and in 1.2% of *C. jejuni* isolates.

Table 14 Percentages of resistance to selected antimicrobials in *C. jejuni* and *C. coli* isolates from UK fresh whole retail chicken from 2015 to 2020.

Antimicrobial	Break point (mg/l)	Species	2015 to 2016[a] (95% CI)	2016 to 2017[a] (95% CI)	2017 to 2018[a] (95% CI)	2018 to 2020[b] (95% CI)
Ciprofloxacin	> 0.5	*C. jejuni*	54 (40-68)	41 (36-46)	52 (47-58)	53 (49-57)
Ciprofloxacin	> 0.5	*C. coli*	48 (38-58)	52 (42-62)	48 (35-60)	44 (37-50)
Erythromycin	> 4	*C. jejuni*	0 (0-1)	0.4 (0-2)	0.6 (0-2)	0 (0-0.5)
Erythromycin	> 8	*C. coli*	1.9 (0-6.5)	0 (0-4)	3.1 (0-11)	2.6 (0-7)
Tetracycline	> 2	*C. jejuni*	68 (63-72)	54 (49-58)	52 (47-58)	62 (58-65)
Tetracycline	> 2	*C. coli*	67 (57-75)	62 (51-71)	60 (47-72)	66 (60-72)

[a] AMR profiles based on phenotypic testing.

[b] Ciprofloxacin, erythromycin and tetracycline profiles predicted from WGS data and phenotypic MIC in agreement for all isolates tested.

In summary, the percentage of fresh whole chicken sold in non-major retailer stores in the UK that are contaminated with the highest level of *Campylobacter* spp. has decreased since 2014/15 but it is still higher than on chicken obtained from major retailers. More needs to be done to achieve better control of *Campylobacter* spp. in

the sector supplying non-major retail stores and this could include consideration of measures to achieve more consistent biosecurity and improvements in slaughterhouse hygiene.

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 *Campylobacter* spp. at the highest level (samples with more 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.

4.3 Conclusions and recommendations

- The percentage of fresh whole chicken on retail sale in non-major retailer stores in the UK that is contaminated with the highest level of more than 1000 cfu of *Campylobacter* spp. per gram has decreased since 2014 and has decreased further between 2017 and 2020. For example, the percentage of high-level contamination in these stores has declined from 18% of samples collected from 2016 to 2017, to 11.8% from 2018 to 2020.
- Continued monitoring will be required to confirm that a sustained decline can be achieved in chickens from non-major retail stores, and whether or not the FSA target of no more than 7% of chickens in the high contamination category can be achieved and maintained. Further action needs to be taken to compel non-major stores to achieve the target as this has been achieved by a large majority of retail stores.
- Additional analysis to understand and reduce the factors which cause some processing plants to supply highly contaminated chicken would help in achieving the FSA target. Improvements for some of the processing plants supplying non-major retailer stores may be facilitated by implementation of measures that resulted in improvements in plants supplying major retailers.
- Further research to better understand how colonisation of flocks on farms may be reduced is also needed, including determining any role of supply from breeders.
- Overall, as predicted from AMR determinants based on WGS data in survey years 5 and 6 and phenotypically in the previous survey years since 2014,

there have been no major differences in the levels of antimicrobial susceptibility in isolates of *C. jejuni* and *C. coli* from retail chicken. This includes negligible (0.5%) resistance to erythromycin in *Campylobacter* spp. isolates from the study years 2018 to 2020, in comparison to levels in isolates from human cases in the EU in 2019 which are reported to harbour erythromycin resistance in 1.5% of *C. jejuni* and 12.9% of *C. coli* (EFSA and ECDC, 2021).

- Quinolone and tetracycline resistance in isolates obtained from poultry meat and human cases continues to be high and current measures are not achieving an adequate reduction of AMR in campylobacters in the food chain. Comparisons of percentages of resistant isolates between the current and the earlier survey years must be treated with some caution. The majority of isolates examined from earlier survey years were obtained from major retailers, but this is no longer the case as in the recent years only samples from non-major retailers were tested. While it is possible that this and other changes may have influenced the percentages of resistant isolates observed, there is no evidence for any link between types of retailers and the extent of AMR in *Campylobacter* spp. isolates from UK chicken.
- It is recommended that trends in antimicrobial resistance in *Campylobacter* spp. isolates from retail chickens continue to be monitored with particular emphasis on strains with co-resistance to ciprofloxacin and erythromycin.

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6.0 Appendices

6.1 Appendix I Survey protocol

Appendix I.docx

6.2 Appendix II Sample data year 5 and year 6 data

HallmarkdataY5final .xls

Ye6samplingddata.xlsx

6.3 Appendix III Survey year 5 and 6 campylobacter enumeration data

[doc to be hyperlinked by FSA]

FinalY5&6_campycfuappendixv4.xlsx

6.4 Appendix IV Survey year 5 and 6 campylobacter AMR data

[doc to be hyperlinked by FSA]

FinalY5&6_ST&AMR appendixv2.xlsx

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