Final Report

Surveillance of Antimicrobial Resistance (AMR) in *E. coli* and *Campylobacter* from retail turkey meat and *E. coli* from retail lamb in 2020/21 - FS102109

26th January 2022

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https://doi.org/10.46756/sci.fsa.hlo814

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1. Liability statement

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2. Executive summary

Background

This report presents results of the surveillance of antimicrobial resistance (AMR) in specific bacteria, i.e., *Campylobacter* and *Escherichia coli (E. coli*) from lamb and turkey meats on retail sale in the UK between October 2020 and February 2021. The aim was to test by culture approximately 200 samples each of lamb and turkey meat for *E. coli*, and also to test the turkey samples for *Campylobacter.* The FSA requested testing of lamb and turkey meat as the majority of AMR surveys on UK retail meats have focused on beef, chicken and pork. As such there is an evidence gap for AMR in lamb and turkey meat.

E. coli is a normal inhabitant of the mammalian and avian gut and most isolates do not cause observable clinical disease in healthy animals and humans. Therefore, *E. coli* isolates can be useful “indicators” of AMR in gut bacteria. *Campylobacter* is frequently present in the gut of healthy poultry, and thermophilic species (*Campylobacter jejuni* and *Campylobacter coli*) typically cause food poisoning in humans.

The monitoring of lamb and turkey meat for AMR is not mandatory as part of the European Directive **2003/99/EC**, but the methodology used in this survey was broadly based on the current EU methodologies for the testing of retail beef, chicken and pork. These methodologies involve culture of *E. coli* on selective agar media containing the antimicrobial drug cefotaxime. Growth of *E. coli* on such plates indicate resistance to third generation cephalosporin antimicrobial drugs, including extended-spectrum beta lactamase (ESBL) and AmpC type resistance. Such isolates should be further tested for susceptibility to a panel of antimicrobials by determining minimum inhibitory concentration (MIC) values using a broth dilution method based on EN ISO 20776-1:2006.

As recommended by the EU, additional selective cultures were performed on samples to isolate any *E. coli* resistant to carbapenem antimicrobials. Carbapenems are termed ‘last resort’ drugs, used to treat severe infections when other treatment options are ineffective because of multiple resistances in the target Gram negative bacteria.

At the request of the FSA (non-harmonised testing outside the remit of Decision 2013/652/EU) further screening was performed for *E. coli* strains resistant to colistin (another 'last resort' human antimicrobial drug) and those specifically producing ESBL resistance enzymes. Colistin-resistant strains may harbour *mcr* resistance genes, which are located on plasmids that can transfer between bacteria.

Sampling

Samples were collected from retail premises across England, Scotland, Wales and Northern Ireland. To compensate for potential missing sample data, 105% of the required number of samples was incorporated into the sampling plan. Proportionate stratified sampling was used to allocate turkey and lamb samples to county and local authority areas determined by the Nomenclature of Territorial Units for Statistics (NUTS-3) regions, with the number of samples being distributed in proportion to population size.
A total of 210 samples each of eligible fresh lamb and turkey meat were collected from October 2020 to February 2021. The samples came from 25 and 13 different butchers for lamb and turkey samples respectively plus twelve and nine different supermarket companies for lamb and turkey, respectively.

Culture and analysis methods

Methods were in line with EU protocols and APHA internal Standard Operating Procedures.
Sub-samples weighing 27 ± 0.5 grams were added in a 1 to 9 ratio by weight to Buffered Peptone Water (BPW, a liquid bacterial recovery medium), and were homogenised. Skin was used if present (mainly just for turkey), with surface muscle being used additionally or instead, if skin was insufficient or absent in the submitted sample. Samples to make up the 27 g were taken from multiple areas of the meat samples.

E. coli

A 20 ml aliquot of the sample/BPW homogenate was taken from each (270 mls) of homogenate, directly applied in 100 µl aliquots onto a range of agar based selective culture media, incubated and examined to estimate the numbers of *E. coli* in the original meat samples. The remaining 250 ml of the homogenate was incubated for 18 to 22 hours aerobically at 37◦C ± 0.5◦C, to allow physiological recovery and multiplication of bacteria (pre-enrichment), before being inoculated onto the selective culture plates which were incubated aerobically at 37◦C ± 0.5◦C for 18 to 22 hours. The method after using pre-enrichment has the theoretical potential to detect one *E. coli* cell or cluster (colony-forming unit; cfu) in 25 grams of meat.
The media employed were: MacConkey (for overall *E. coli* counts), MacConkey plus cefotaxime or colistin (for strains resistant to third-generation cephalosporin antimicrobials or to colistin), chromID® CARBA and chromID® OXA-48 (for carbapenem-resistant strains), and CHROMagar™ ESBL (for strains expressing ESBL resistance enzymes). *E. coli* isolated on this last agar were tested for the presence and sequence type of $bla_{\text{CTX-M}}$, bla_{OXA}, bla_{SHV} and bla_{TEM} ESBL genes by whole-genome sequencing (WGS).
MIC values for isolates from MacConkey plus cefotaxime agar were determined for a panel of antimicrobials using standard methods according to Commission Implementing Decision 2013/652/EU.

Campylobacter

Sample of the sample/BPW homogenate were spread on to *Campylobacter*-selective agar and incubated in a low oxygen atmosphere following the ISO 10272 method for detection and enumeration of *Campylobacter* (colony forming units per gram of meat sample)**.** For each isolate, the species of *Campylobacter* isolates was determined by analysis of the mass spectrum of subcellular fragments resulting from laser irradiation (matrix-assisted laser desorption ionisation – time of flght mass spectroscopy, MALDI-TOF MS). All isolates were whole genome sequences and analysed to determine multi-locus sequence type (MLST) for assigning to clonal complexes, plus AMR gene profiles.

MIC values were determined for a panel of antimicrobials using standard methods according to Commission Implementing Decision 2013/652/EU.

Results

E. coli

Homogenate samples cultured on MacConkey agar without pre-enrichment in BPW had a detection limit of 3000 cfu per gram (cfu/g). Only seven samples (all originating from ‘turkey mixed other pieces’ which included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks) yielded *E. coli* with this method, giving counts of 3,000 to 17,000 cfu/g. None of these were from cefotaxime-containing agar.

Post-enrichment, two (0.95% [95% confidence interval 0.12% to 3.40%]) of the lamb samples and 24 (11.43% [95% confidence interval 7.46% to 16.53%]) of the turkey samples yielded *E. coli* on cefotaxime-containing MacConkey agar.

For these isolates from cefotaxime-supplemented MacConkey agar, the patterns of MIC values allowed their classification as expressing ESBL- or AmpC-type resistance. One each of the two lamb isolates was classified as AmpC- and ESBL-type resistance, whereas all 24 of the turkey isolates expressed an ESBL resistance pattern. All *E. coli* isolated from cefotaxime media were microbiologically resistant (according to epidemiological cut-off MIC values) to cefotaxime, ceftazidime and ampicillin; most were also resistant to cefepime, and the AmpC-type isolates showed characteristic resistance to cefoxitin.

None of these cefotaxime-resistant isolates showed resistance to the ‘last resort’ carbapenem antimicrobials imipenem and meropenem, or to colistin, azithromycin, temocillin or tigecycline. Resistance was observed in more than half of these isolates to both of the quinolone antimicrobials (nalidixic acid and/or ciprofloxacin). Also, about half of the isolates were resistant to tetracycline and most of these were also resistant to both of the quinolones. Most isolates were sensitive to sulfamethoxazole and trimethoprim and only one (lamb ESBL-type) showed resistance to gentamicin. Most of the turkey isolates were sensitive to chloramphenicol.

No carbapenem-resistant *E. coli* were isolated on carbapenem-selective agars. On CHROMagar ESBL one lamb sample (0.48% [95% confidence interval 0.01% to 2.62%]) and 25 turkey samples (11.9% [95% confidence interval 7.85% to 17.07%]) yielded isolates after pre-enrichment. For these lamb (L) and turkey (T) samples, genome sequencing revealed the following extended-spectrum beta-lactamase genes: CTX-M 15 (1x L and 13x T), CTX-M 55 (6x T), CTX-M 27 (1x T), CTX-M 65 (1x T) and SHV-134 (2x T).

Three pre-enriched turkey samples (1.43% [95% confidence interval 0.30% to 4.12%]), two from the UK and one from Germany yielded isolates on colistin-supplemented MacConkey agar with the *mcr-1* transferable colistin resistance gene being detected using molecular genetic techniques. This is the first time that *mcr* plasmid-mediated colistin resistant *E. coli* have been isolated from retail turkey meat in the UK.

Campylobacter

Campylobacter was isolated from 22 of 210 turkey samples (10.5% [95% confidence interval 6.4% to 14.6%]); of these, 9.5% (95% confidence interval 5.6% to 13.5%) yielded *Campylobacter jejuni* (*C. jejuni*) and 3.3% (95% confidence interval 0.9% to 5.8%) yielded *Campylobacter coli* (*C. coli*). Of the 80 samples that contained turkey skin, *Campylobacter* was detected in 21 samples (26.3% [95% confidence interval 16.7% to 35.9%]). The percentage of positives was lower in the 130 samples without skin, with only one was positive (0.8% [95% confidence interval 0.0% to 2.3%]). *Campylobacter* contamination in excess of 1000 cfu/g is considered to represent a higher exposure risk for consumers,[1] and this was found in only one sample (0.5% [95% confidence interval 0.0% to 1.5%] of samples overall). The majority of *Campylobacter*-positive turkey samples (6.2% [95% confidence interval 2.9% to 9.4%] of samples overall) contained between 10 and 99 cfu/g.

All isolates of *C. jejuni* (n=49) and a sub-set of *C. coli* (n=4), from a total of 20 turkey meat samples, were subjected to further characterisation. A high percentage (79%) of *Campylobacter* isolates was resistant to at least one antimicrobial, but no isolate exhibited multiple drug resistance (MDR), defined as resistance to three or more different classes of antimicrobials.

Considering *C. jejuni* positive samples only (n=20), a high proportion (75% [95% confidence interval 56% to 94%]) yielded one or more isolates resistant to at least one antimicrobial. Resistance to ciprofloxacin, nalidixic acid and tetracycline was identified in 60%[95% confidence interval 38.5% to 81.5%], 55% [95% confidence interval 33.2% to 55.0%] and 65%[95% confidence interval 44.1% to 85.9%], respectively of such samples. Resistance to all three antimicrobials was found in 50% of *C. jejuni* isolates. Resistance to erythromycin, gentamicin and streptomycin was not detected in any of the turkey samples tested in this study.

All four analysed *C. coli* isolates were resistant to at least one antimicrobial: three (75%) were resistant to ciprofloxacin, three (75%) were resistant to nalidixic acid and two (50%) were resistant to tetracycline. It is not possible to report resistance at the sample level for *C. coli* as not all of the detect isolates were characterised.

Whole genome sequences were obtained for 48 *C. jejuni* and four *C. coli* isolates. Twenty one different MLST profiles were identified, and at least 14 of these have previously been seen in clinical cases in humans via the PubMLST website.[2] Most of the samples yielded a single MLST sequence type (ST). In six samples, multiple STs were detected. ST573 was the most widespread, being detected in four samples.

MLST subtypes can be assigned to clonal complexes (CCs), sharing sequences in at least four of the seven genetic locations used by the MLST system.[2] Clonal complex 21 and CC573 were most widespread, each being present in four different meat samples, whilst CC354 was present in three samples and CC828 was detected in two samples.

The presence of antimicrobial resistance genes in whole genome sequences generally correlated with resistance types expressed as raised MIC values, with few anomalies found. For quinolone/fluoroquinolone resistance, the majority (90%) of resistant isolates had a single mutation in the *gyrA* gene in the quinolone resistance determinant region. The $bla_{\text{OXA-61}}$ gene, which encodes resistance to beta-lactam antimicrobials such as ampicillin (not included in the harmonised phenotypic MIC panel for *Campylobacter*), was detected in 14 of the 20 samples. Putative multiple drug resistant isolates were identified by combining genetic data with MIC profiles. Single isolates of *C. jejuni* (ST5136) and *C. coli* (ST1089) were candidate MDR strains, showing elevated MIC values to quinolones and tetracycline alongside the presence of beta-lactam resistance genes. Additionally, one *C. jejuni* isolate (ST2836) had MIC values indicating resistances to ciprofloxacin and tetracycline, alongside genetic determinants for resistance to streptomycin and the sulphonamide antimicrobial class, and also for reduced susceptibility to quaternary ammonium disinfectant compounds. There was thus an extensive putative MDR profile for this last isolate.

Summary

The present survey includes an initial (i.e., baseline) assessment of AmpC/ESBL resistant *E. coli* in UK retail lamb and turkey meat, yielding prevalence values of 2/210 (1%) and 24/210 (11%) samples, respectively. Further screening of the current samples was performed for *E. coli* showing carbapenem resistance (not detected) and *mcr-1*-mediated transmissible colistin resistance, the latter being observed in three of 210 (1.4%) turkey samples.

Ten percent of the 210 turkey meat samples were contaminated with *Campylobacter* species (*C. jejuni* and *C. coli*) that are relevant from a public health perspective, albeit at low concentrations in the large majority of cases. Legs, crowns and whole birds were more commonly contaminated than breast, and mixed other pieces. *Campylobacter* contamination was much more frequent on turkey meat samples containing skin relative to skinless turkey meat samples. A high proportion of the *C. jejuni*-positive turkey meat samples (75%) contained *C. jejuni* isolates with resistance to at least one antimicrobial. The most frequent resistance detected in the *C. jejuni* isolates was to ciprofloxacin (66%), whilst 43% of *C. jejuni* isolates exhibited resistance to three tested antimicrobials: ciprofloxacin, nalidixic acid plus tetracycline. No resistance to erythromycin, gentamicin or streptomycin was observed.

3. Glossary

- **AmpC phenotype** – A phenotype of resistance to cephalosporin antimicrobials such as cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor-β-lactam combinations.
- **AmpC enzyme** – Enzyme conferring AmpC type resistance
- **AMR** – Antimicrobial resistance
- **APHA** – Animal and Plant Health Agency
- **BPW** – Buffered Peptone broth, a liquid media widely used to grow bacteria
- **CRL** – Community Reference Laboratory
- **CTX-M** – A group of ESBL enzymes that give bacteria resistance to cephalosporin antimicrobials.
- **Enterobacteriaceae** – Family of bacteria including many common gut bacteria such as *Escherichia coli* or *E. coli*
- **CA-ESBL** - CHROMagar™ ESBL, for isolation of ESBL-producing *E. coli*
- **CARBA** - ChromID® CARBA agar, for isolation of carbapenemase resistant *E. coli*
- **CC** – Clonal complex (MLST)
- **COL** - Colistin
- **CTX** – Cefotaxime
- **ECOFF** – Epidemiological Cut Off value (with respect to antimicrobial resistance)
- **EN** - Norme Européenne /Europäische Norm (European Standard)
- **ESBL** – Extended Spectrum β-lactamase. Enzymes that are capable of breaking down many penicillin type antimicrobials, including cephalosporin antimicrobials
- **EU** – European Union
- **EUCAST** - European Committee on Antimicrobial Susceptibility Testing
- **FSA** – Food Standards Agency
- **HCCA** - α-Cyano-4-hydroxycinnamic acid
- **ISO** - International Organisation for Standardisation
- **MALDI ToF** – Matrix-Assisted Laser Desorption / Ionization Time-of-Flight
- **MCA** – MacConkey agar
- **MCA-COL** – MacConkey agar + 2 mg/L colistin

- **MCA-CTX** - MacConkey agar + 1 mg/L cefotaxime
- **mCCDA-** modified Charcoal Cefoperazone Deoxycholate agar
- **MIC** – Minimum Inhibitory Concentration
- **MLST** – Multilocus sequence typing
- **MS** – Member States
- **NUTS** - Nomenclature of Units for Territorial Statistics
- **OXA-48** - ChromID® OXA-48 agar, for isolation of carbapenemase resistant *E. coli*
- **PBS** – Phosphate Buffered saline
- **QC** – Quality control
- **SOP** – Standard Operating Procedure
- **ST** – Sequence type (MLST)
- **WGS**- Whole Genome Sequencing

4. Materials and Methods

Sampling criteria

For comparability, the sampling frame development for the additional AMR work for lamb and turkey meat was based on the instructions the FSA prescribed for the previous AMR surveys for chicken, pork and beef.

As a brief:

- The sampling period was from October 2020 to February 2021.
- The number of samples included 200 lamb samples and 200 turkey samples, plus 5% contingency, totalling 420 samples (210 of each meat type).
- Similar to the previous designed AMR survey, the types of samples included fresh/chilled meats only. Frozen, cooked, processed, pre-prepared, ready-based, marinated, seasoned, herbed, stuffed, cook in the bag, breaded and battered turkey and lamb meat were excluded from this survey.
- The lamb samples included chops, shoulders, legs, diced/cubed, steaks, loins (similar to pork & beef AMR survey).
- The turkey samples included whole birds, breasts (including sliced & diced) and other cuts, (e.g., joints & portions like quarters, legs, thighs, drumsticks, etc), keeping as close to the chicken AMR survey cuts as possible. It also included cuts with or without skin. For the chicken AMR survey we had approximately 50% whole birds, 25% breasts and 25% other cuts, so again this was kept as similar as possible for turkey.
- The Kantar market share data provided by FSA was used. This data was provided in a spreadsheet and dated as covering the year to 6th September 2020.
- The same supermarkets as used in the 2020 chicken survey were used for comparability.
- The number of samples in each NUTS3 region was proportional to the population size. In addition, Kantar's regions codes were used to determine the market share percentage among these regions.

Work performed at APHA Weybridge – General methods and *E. coli*

For meat samples in general and specifically for *E. coli,* the methodology with respect to the work performed is detailed in eight internal APHA Standard Operating Procedures (SOPs, not included in this report).

These SOPs are:-

- Isolation of background (indicator commensal) and antimicrobial resistant Enterobacteriales from meats and caecal contents according to EU and / or APHA protocols (CBU 0278, version 9 – 20-05-2020).
- Microbank -70ºC Bacterial Storage System (CBU 0155).
- Identification of Bacteria by Oxidase (BA 050) and Indole Spot Test – a rapid method for bacteria (BA0130) and by MALDI ToF (BAC 0334).
- Minimum inhibitory concentration (MIC) – The Sensititre Method (BA0604).
- Oxidase (BA 050).
- Indole Spot Test – a Rapid Method for Bacteria (BA 0130).
- Identification of bacteria by MALDI ToF (BAC0334).
- Real Time PCR for plasmid mediated colistin resistance genes *mcr-1*, *mcr-2* and *mcr-3* (BAC0415).

The methodology for each of these aspects is summarised briefly below.

Isolation of background (indicator commensal) and antimicrobial- resistant Enterobacteriales from meats and caecal contents according to EU and / or APHA protocols

The methodology follows that outlined in EU documents, and the SOP CBU 0278 is based on these EU methods as below for the work outlined in this report:-

- **EU method -** Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from fresh meat – Version 7, December 2019.

- **EU method -** Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AmpC-producing *E. coli* in meat and animals – Version 3, November 2017.
- **EU method** – Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli* – Version 2, January 2015.
- **EU method -** Quantification of ESBL/AmpC-producing *E. coli* in caecal content and fresh meat samples – Version 1, December 2017.

Pdf files of the most recent versions of the above EU methods can be found on-line.

In brief, 27 ± 0.5 grams of the retail meat sample collected, transported and stored under conditions as stipulated by the EU protocols, was homogenised in ~ 100 ml (from 243 ml) of sterile chilled BPW, before adding this homogenate to the remaining BPW and gently mixing, providing 270 ml of BPW homogenate. In line with EFSA guidance as outlined in the APHA internal SOP, the 27 grams of meat was taken as skin if possible. If less than 27 grams of skin was available, then this was supplemented with surface muscle and surface muscle was used entirely for skinless samples.

From this 270 ml BPW homogenate, 20 mls was taken for the viable bacterial counts. Viable counts were performed according to the EU protocol with slight variation. This variation was homogenisation of one meat portion per sample in chilled BPW only, not one portion for counts in chilled saline and another portion for enrichment in chilled BPW. The full rationale and validation of this variation, which was approved by the FSA and the Danish Technical University (DTU), has been provided in a previous report.

For counts, the method involved plating 100 µl BPW homogenate prior to incubation to MacConkey agar containing ± 1 mg/L cefotaxime. These two agars are used to enumerate the number of presumptive *E. coli* and the number of presumptive AmpC/ESBL-producing *E. coli* on the meat samples. The EU method states that at least 30 colonies must be counted to give an accurate estimate of the viable counts and this limits the detection level to 3,000 cfu/g of meat. Because of the low

numbers of *E. coli* in the meat samples, in general it is not necessary to further dilute the initial BPW homogenate for counts beyond the initial tenfold dilution.
The remaining 250 mls of BPW homogenate (e.g., 25 grams of meat and 225 mls of BPW as per EU protocols) was incubated at 37 ± 1ºC for 18-22 hours.
The incubated BPW / meat homogenate was used to inoculate (10µl) MacConkey agar containing 1 mg/L cefotaxime (MCA-CTX), chromID® CARBA (CARBA) and chromID® OXA-48 (OXA-48).

Samples were also plated to CHROMagar™ ESBL (CA-ESBL), for specific detection of ESBL-producing *E. coli* and to MacConkey agar containing 2 mg/L colistin (MCA-COL), for detection of colistin resistant *E. coli*, and these were additional non-EU stipulated screening agars added at the request of the FSA (**UK non-harmonised tests**).

All plates were QC tested prior to use, according to EU or APHA methods as appropriate, as outlined in the SOP.
MCA-CTX and MCA-COL plates were incubated for 18-22 hours at 44 ± 0.5 °C before checking for lactose fermenting colonies. Other media were incubated at 37 ± 1ºC for 18-22 hours, before checking for presumptive *E. coli.*

Lactose fermenters from MCA-CTX were assumed to be presumptive AmpC / EBSL-producing *E. coli,* red/purple colonies from CA-ESBL were assumed to be presumptive ESBL-producing *E. coli* and pink to burgundy colour colonies from CARBA and OXA-48 agars were assumed to be presumptive carbapenem-resistant *E. coli.* Three single presumptive *E. coli* colonies from each of these agars were plated again to the agar of origin to ensure purity prior to confirming one of the isolates as *E. coli,* and then storing this isolate pending further tests.
Overall, this method post enrichment in BPW has the theoretical potential to detect one *E. coli* of interest per 25 grams of meat.

From MCA-COL plates, a sweep of ~ 10 to 20 lactose fermenters (based on SOP BAC 0415) was used to prepare a crude DNA sample for detection of *mcr-1, mcr-2* and *mcr-3* plasmid-mediated colistin resistance genes by real time PCR. A sweep was taken to increase the sensitivity of detection of the *mcr* genes.

Storage of purified *E. coli* isolates of interest prior to further tests

Isolates from MCA-CTX agar and if present from CARBA and OXA-48 agars will be stored for up to five years to comply. Isolates were stored in duplicate, on “beads” (frozen in cryogenic material at -70ºC).
For “beads,” purified bacterial culture was aseptically transferred using a 10 µl loop from the pure culture on agar to a commercial “beads” tube. The cryogenic liquid and bacterial growth were mixed in the tube, before removing most of the supernatant cryogenic liquid, and then storing the tube at - 70ºC.

Identification of bacteria by MALDI ToF or confirmation of lactose fermenters as *E. coli* using oxidase and indole tests

For lactose fermenters isolated from MCA-CTX at 44ºC, combined use of oxidase and indole tests as described by in-house SOPs, was used to confirm isolates as *E. coli.* Presumptive *E. coli* from other agars, such as CA-ESBL, CARBA and OXA-48, were first streaked to MCA and incubated for 18-22 hours at 44 ± 0.5 °C to confirm isolates as lactose fermenters. If isolates were lactose fermenters, they were then identified as *E. coli* by combined use of oxidase and indole tests as described by in-house SOPs.
For the oxidase and indole tests, a single well isolated colony was taken from MCA or MCA-CTX agar, plated onto blood agar and incubated overnight at 37ºC. Growth from the blood agar was then used to perform oxidase and indole tests.
For the oxidase test, in-brief, a portion of bacterial colony to be tested was taken with a sterile plastic loop and rubbed onto filter paper impregnated with oxidase reagent. A deep purple colour developing within 10 seconds was taken to be “oxidase positive". The indole test was performed in the same way but using filter paper impregnated with James reagent (BioMerieux). Within 10 seconds, a positive reaction was indicated by the presence of a colour change to pink/red. Lactose fermenter colonies from MCA-CTX that grew aerobically at 44ºC were confirmed as *E. coli* if oxidase negative and indole positive.

MALDI ToF was used for identification of problem isolates giving equivocal results by other tests only if required, and was used as described by an in-house SOP and based on that previously described.[3] For MALDI ToF identifications if required, isolates were also grown on blood agar. A small amount of bacterial growth was applied to the metal target plate. Growth on the target plates was overlaid with 1 µl of 70% formic acid to perform a partial protein extraction and allowed to dry. Each spot was then overlaid with 1 µl of HCCA matrix, and again this was allowed to dry before the target plate was loaded into the MALDI ToF machine. Using Biotyper software, resulting spectra from the MALDI ToF run were searched against the Bruker database of spectra, and if the resulting score was ≥ 2.000, this was taken as reliable identification to the species level, dependant also on consistency score and caveats that might apply for some bacteria species.

Determination of Minimum Inhibitory Concentrations (MICs) by broth micro dilution.

MICs were performed as described in our in-house SOP (BA0604), based on EN ISO 20776-1:2006.
E. coli isolates were inoculated into Mueller Hinton broth at a suitable dilution for application to commercially prepared plates containing two fold dilution series of antimicrobial compounds in accordance with Decision 2013/652/EU. After incubation at 37^{o}C for 18 hours, the plates were examined, and growth end points established for each antimicrobial to provide MIC’s. Microbiologically resistant and susceptible interpretation for the MIC’s were obtained by comparison with ECOFF’s published by EUCAST based Decision 2013/652/EU.
It should be noted that a new EU Decision 2020/1729 repealing the EU decision 2013/652/EU was issued on the 17th November 2020. This decision affects the ECOFFs for some antibiotics, such as nalidixic acid and meropenem. So that results are consistent with previous reports and for comparability with the EFSA monitoring, the 2013/652/EU ECOFFs have been applied to MICs in this study.
For *E. coli*, the presence of carbapenemase producing strains, Extended Spectrum Beta-Lactamase producers (ESBL) or AmpC enzyme producers was determined initially by assessing isolate MIC’s against the microbiological breakpoints for

meropenem, cefotaxime and ceftazidime. Any isolates showing meropenem MIC's greater than 0.125mg/l, cefotaxime MICs greater than 0.25mg/l or ceftazidime MIC's greater than 0.5mg/l were tested against a further panel of antimicrobials containing cefotaxime, ceftazidime, cefotaxime / clavulanate, ceftazidime / clavulanate, imipenem, ertapenem, temocillin, cefoxitin, cefepime and meropenem. Consequently, isolates have results reported for all of these confirmatory antimicrobials where an MIC greater than the cut off values stated above was observed for any of the screening compounds (cefotaxime, ceftazidime or meropenem) included in the first panel of antimicrobials.

Isolates confirmed resistant to meropenem were to be considered to carry a carbapenemase.

The presence of ESBL-producing *E. coli* strains was determined as follows: Isolates resistant to one or both of cefotaxime and ceftazidime that also had an MIC of greater than 0.125mg/l against cefepime and also showed a reduction in MIC of ≥ 8-fold against combined cefotaxime / clavulanate or ceftazidime / clavulanate when compared with the cephalosporin alone were considered to carry an ESBL.

Isolates resistant to cefotaxime or ceftazidime that also had an MIC of greater than 8mg/l against cefoxitin and showed no reduction to MIC's or a reduction of less than three dilution steps for cefotaxime or ceftazidime in the presence of clavulanate were considered to be carrying an AmpC enzyme.

Detection and sequencing of bla_{CTX-M}, bla_{OXA}, bla_{SHV} and bla_{TEM}

Presence of bla_{CTX-M}, bla_{OXA}, bla_{SHV} and bla_{TEM} from CA-ESBL and subsequent sequencing was performed by Illumina whole genome sequencing (WGS). Resulting FASTQ files were assembled using "SPAdes - St Petersburg aligner"[4] and analysed using DTU pipelines "ResFinder 4.1."[5]

Real time PCR for plasmid mediated *mcr-1, mcr-2* and *mcr-3* genes

Samples that gave rise to lactose fermenting colonies on MCA-COL were tested for the presence of plasmid-mediated colistin resistance genes *mcr-1, mcr-2* and *mcr-3* by real time (RT) PCR, according to an in-house SOP (BAC0415). To make detection more sensitive, a “sweep” of ~ 10 to 20 colonies was taken to prepare the crude DNA for RT-PCR.

If the initial “sweep” was PCR positive, multiple individual suspect *E. coli* colonies (up to 10 as available) were further examined by PCR for *mcr-1, 2* and *3* genes.

It should be noted that only lactose fermenters with an *E. coli* phenotype were investigated. As such it is possible that *mcr* if detected in the original “sweep” but not in isolated colonies, could be present in other bacterial genera. This might include non-target lactose fermenters such as *Klebsiella* and *Citrobacter* [6] as well as non-lactose fermenters.

Up to four (per meat sample) individual suspected *mcr E. coli* were, at the request of the FSA, subjected to illumina whole genome sequencing (WGS).

Resulting FASTQ files were assembled using “SPAdes - St Petersburg aligner”[4] and analysed using DTU pipelines “MLST,”[7] “SpeciesFinder,”[8] “ResFinder 4.1,”[5] “VirulenceFinder,”[9] and “PlasmidFinder.”[10]

Statistical Analysis

95% confidence intervals were calculated for the proportion of *Campylobacter*- and *E. coli*- positive results. In addition, the Pearson Chi-square test of association has been used to test the null hypothesis of no association between the skin on samples for AmpC/ESBL *E. coli* and *Campylobacter* contamination. Fisher’s exact test was used for individual comparisons when samples were small.

Work performed at APHA Weybridge – *Campylobacter*

The initial processing of meat samples for *Campylobacter* was identical to that used for *E. coli.*

To allow for detection and enumeration of *Campylobacter*, a procedure based on ISO10272-1,2:17 was used. The suspensions were inoculated onto modified charcoal cefoperazone deoxycholate agar (mCCDA). A 1ml volume of homogenate was plated across three standard sized mCCDA plates. An additional mCCDA plate was inoculated with 100 µl of homogenate. All plates were incubated in an microaerobic atmosphere at 41.5 ± 1ºC for at least 44 hours.

Putative *Campylobacter* colonies were counted and up to five colonies were picked and subcultured onto 7% sheep blood agar and incubated in a microaerobic atmosphere at 41.5 ± 1ºC. Confirmation of *Campylobacter* genus and the identification of species was determined by MALDI-ToF as described previously for *E. coli*. If isolates were found not to be *Campylobacter* then the enumeration count was adjusted. The minimum detectable level of *Campylobacter* was 10 colony forming units (cfu) per g of sample. It was possible to quantify the number of *Campylobacter* in a sample when levels were greater than 45 cfu/g and less than 15,100 cfu/g.

Confirmed *Campylobacter* isolates were stored in 10% glycerol broth at -80ºC until MIC testing and WGS could be performed. Up to five isolates per sample were stored for further analysis.

For WGS, FASTQ files were assembled using "SPAdes - St Petersburg aligner"[4] and analysed using DTU pipelines "MLST"[7] and "ResFinder 4.1."[5]

MICs were performed using commercially prepared plates as for *E. coli*, but just one plate (EUCAMP2) per isolate. The EUCAMP2 plates contain doubling dilutions of the antibiotics ciprofloxacin erythromycin, gentamicin, nalidixic acid, streptomycin and tetracycline. The interpretative criteria used for the MIC resistance data were EUCAST ECOFFs according to Commission Implementing Decision 2013/652/EU, which were CIP– ciprofloxacin (R>0.5mg/l); ERY- erythromycin (R>4 mg/l, or >8 mg/l coli); GEN- gentamicin (R>2 mg/l); NAL-nalidixic acid (R>16 mg/l); STR-streptomycin (R>4 mg/l); TET- tetracycline (R>1 mg/l, or >2 mg/l *C. coli*).

5. Results

General considerations

An excellent working partnership continued with HallMark Veterinary and Compliance Services, which was the company contracted by FSA to collect and deliver the meat samples. Communication between the two organisations and all other aspects of the partnership were highly satisfactory.

Sampling

The number of samples collected per area is shown in Table 1 (lamb) and Table 2 (turkey).

Table 1 – Number of lamb samples per area

Taken from Hallmark report with permission.

Areas	Total Lamb planned	Total Lamb completed	Deviations
East	21	16	5
London	29	20	9
Midlands	33	36	-3
North	47	48	-1
Northern Ireland	4	4	0
Scotland	17	13	4
South	50	61	-11
Wales	9	12	-3
Total	**210**	**210**	**0**

Table 2 – Number of turkey samples per area

Taken from Hallmark report with permission.

Areas	Total Turkey planned	Total Turkey completed	Deviations
East	21	26	-5
London	29	38	-9
Midlands	33	30	3
North	47	46	1
Northern Ireland	4	4	0
Scotland	17	21	-4
South	50	39	11
Wales	9	6	3
Total	**210**	**210**	**0**

The retailer type from which the samples were obtained in the UK are shown below. The samples came from 25 and 13 different butchers for lamb and turkey samples respectively plus twelve and nine different supermarket companies for lamb and turkey, respectively (Table 3 and 4)**.**

Table 3 – Lamb samples per retail chain, per UK region country

Retailer code	England	Wales	Scotland	Northern Ireland	United Kingdom
A	7	1	0	0	8
B	8	0	1	0	9
C	11	1	1	0	13
D	41	3	4	0	48
E	31	0	2	2	35
F	21	1	0	1	23
G	0	0	0	1	1
H	18	3	3	0	24
I	16	0	0	0	16
J	1	0	0	0	1

Retailer code	England	Wales	Scotland	Northern Ireland	United Kingdom
K	6	0	2	0	8
L	21	3	0	0	24
Total	**181**	**12**	**13**	**4**	**210**

Table 4 –Turkey samples per retail chain, per UK region country

Retailer code	England	Wales	Scotland	Northern Ireland	United Kingdom
A	0	0	0	0	0
B	6	0	0	0	6
C	24	2	2	0	28
D	35	0	2	1	38
E	31	0	3	2	36
F	24	3	4	0	31
G	0	0	0	0	0
H	25	0	3	0	28
I	17	0	2	1	20
J	0	0	0	0	0
K	6	0	3	0	9
L	11	1	2	0	14
Total	**179**	**6**	**21**	**4**	**210**

Retailers A, G and J provided lamb, but not turkey samples

Whole turkey could not be easily obtained due to its seasonality. To replace this, the other turkey cuts were collected as follows:-

- As many fresh whole turkeys as available were purchased.
- Where there was not enough fresh whole turkeys to make up the numbers required, these were replaced with turkey crowns with skin on, so these could be as comparable as possible to whole turkeys.

- Failing this, other fresh turkey cuts were purchased to make up the numbers.

The different types of meats collected are shown in tables 5 (lamb) and 6 (turkey).

Table 5 – Total number of lamb samples tested per food category

Lamb Cubed / Diced or Lamb Stewing	Lamb Frying/Grilling Chops	Lamb Frying/Grilling Steak	Lamb Leg Roasting Joint	Lamb Shoulder Roasting Joint	Total lamb samples tested
25	39	31	91	24	210

Table 6 – Total number of turkey samples tested per food category

Turkey Breast	Turkey Crown joint	Turkey Leg	Turkey Mixed Other Pieces†	Turkey Whole Bird	Total turkey samples tested
28	38	14	125	5	210

† Turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks.

See Appendices 1 and 2 for details of all lamb and turkey samples collected respectively.

Results specific for *E. coli*

Samples positive for presumptive AmpC/ESBL resistant *E. coli* on MCA-CTX – EU harmonised test

Post enrichment, two (0.95%, 95% confidence interval 0.12% to 3.40%) of the lamb samples (Table 7) and 24 (11.43%, 95% confidence interval 7.46.% to 16.53%) of the turkey samples (Table 8) gave rise to *E. coli* on MCA-CTX – See also Figure 1.

Table 7 – Lamb meat samples† positive for presumptive AmpC/ESBL phenotype *E. coli* on MCA-CTX

The country of origin of the above samples was stated to be the UK.

Sample Number	Scheduled Sampling Date	Food Category	Retailer code
L00462793	17/11/2020	Lamb Frying/Grilling Steak	I
L00511945	08/02/2021	Lamb Frying/Grilling Chops	A

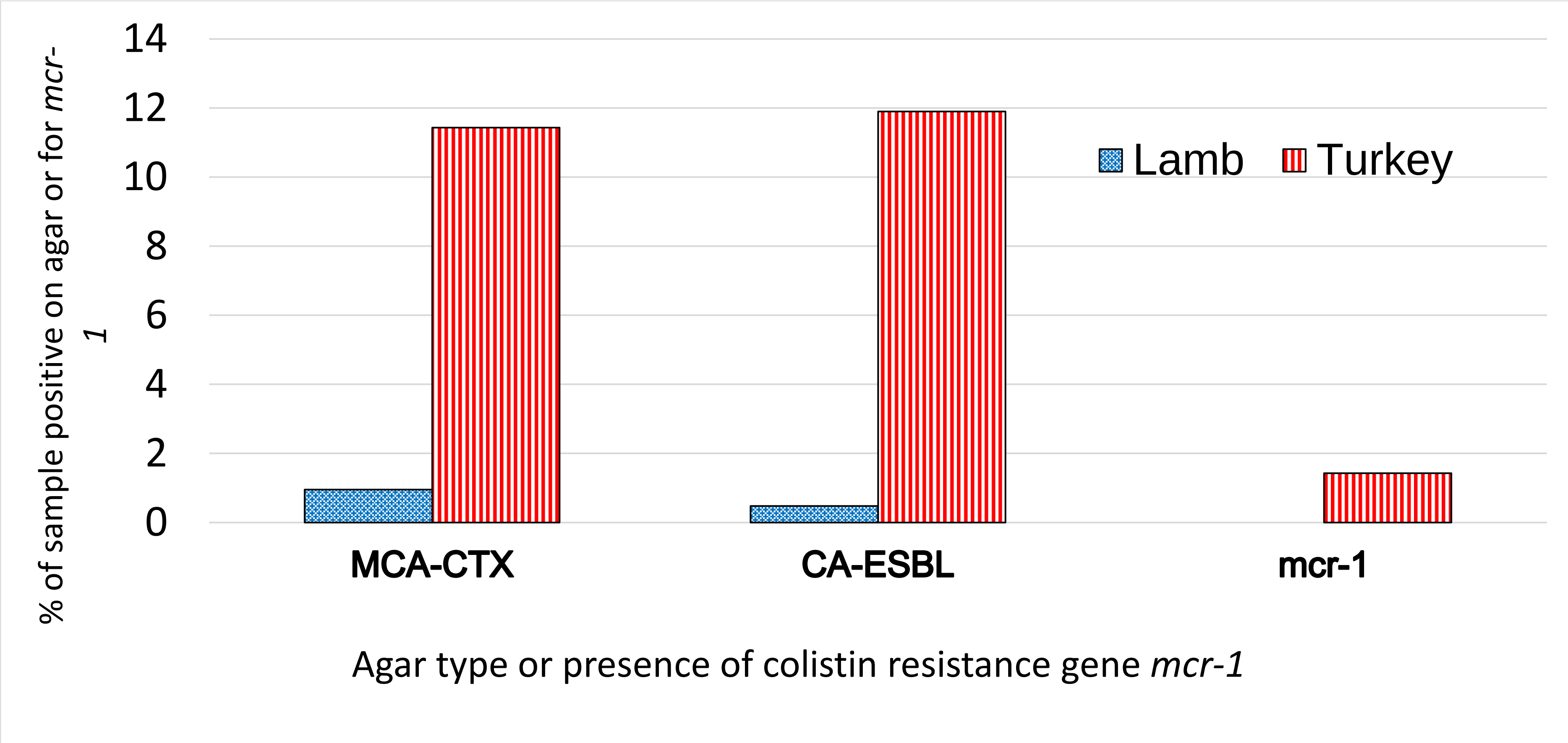
Table 8 – Turkey meat samples positive for presumptive AmpC/ESBL phenotype *E. coli* on MCA-CTX

The country of origin of the above samples was stated to be the UK.

Sample Number	Scheduled Sampling Date	Food Category *	Retailer code
T00462378	20/10/2020	Turkey Mixed Other Pieces	F
T00462414	20/10/2020	Turkey Mixed Other Pieces	F
T00462448	15/12/2020	Turkey Whole Bird	L
T00462505	21/10/2020	Turkey Mixed Other Pieces	F
T00462506	21/10/2020	Turkey Mixed Other Pieces	E
T00462507	21/10/2020	Turkey Mixed Other Pieces	E
T00462771	11/12/2020	Turkey Leg	E
T00462772	11/12/2020	Turkey Whole Bird	B
T00462773	15/12/2020	Turkey Mixed Other Pieces	E
T00462805	18/11/2020	Turkey Crown joint	H
T00511964	08/02/2021	Turkey Mixed Other Pieces	B
T00512045	16/12/2020	Turkey Breast	F
T00512100	09/12/2020	Turkey Mixed Other Pieces	H
T00512110	10/12/2020	Turkey Mixed Other Pieces	F
T00512111	10/12/2020	Turkey Leg	E
T00512133	09/12/2020	Turkey Crown joint	L
T00512177	10/12/2020	Turkey Leg	E
T02664387	19/10/2020	Turkey Breast	F
T02664388	19/10/2020	Turkey Mixed Other Pieces	E
T02664390	19/10/2020	Turkey Mixed Other Pieces	I
T02672449	09/12/2020	Turkey Leg	E
T02797779	09/12/2020	Turkey Leg	E
T02797792	09/12/2020	Turkey Breast	L
T02797996	19/10/2020	Turkey Mixed Other Pieces	I

* Turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks.

Figure 1. Percentages of lamb and turkey meat samples positive on MCA-CTX and CA-ESBL or for plasmid mediated colistin resistance gene *mcr-1*



Results for *E. coli* from MCA-CTX for skin on and skin off samples

Of the 210 turkey meat samples tested, 80 had skin on and 130 were skinless. Of these, 12 (15%) of the turkey skin on and 12 (9.2%) of the skinless turkey samples were positive on MCA-CTX however using Chi-Square test the difference observed was not significant.

Of the 210 lamb samples, 23 had skin on and 187 had skin off. Only 2 (1.1%) skinless lamb samples were positive for MCA-CTX whilst there was no positive detected for lamb containing skin. The Chi-Square test for lamb samples was invalid as the number of the samples was too small, but the Fisher Exact test showed that the differences for lamb for skin off and skin on samples was not significant.

MIC results for isolates from MCA-CTX – EU harmonised test

It should be noted that a new EU Decision 2020/1729 repealing the EU decision 2013/652/EU was issued on the 17th November 2020. This decision affects the ECOFFs for some antibiotics, such as nalidixic acid and meropenem. To ensure that results were consistent with previous reports and for comparability with the EFSA monitoring, the 2013/652/EU ECOFFs have been applied to MIC results in this study.

Based on MICs for isolates from MCA-CTX (Tables 9 and 11, lamb and 12 and 11, turkey), one of the lamb isolates had an AmpC-phenotype *E. coli* (0.48%, 95% confidence interval 0.01% to 2.62%) whilst the other lamb isolate had an ESBL-phenotype *E. coli* (0.48%, 95% confidence interval 0.01% to 2.62%). All of the 24 turkey isolates from MCA-CTX were ESBL-phenotype *E. coli* (11.43%, 95% confidence interval 7.46.% to 16.53%).

As would be expected, as the isolates were obtained from agar with 1 mg/L cefotaxime, all lamb and turkey isolates were microbiologically resistant (when ECOFFs were applied to the MIC results) to the β-lactam antimicrobials ampicillin, cefotaxime and ceftazidime and most were also resistant to cefepime (Tables 9 and 11, lamb and 12 and 11, turkey). The AmpC-phenotype lamb isolate was resistant to cefoxitin, as resistance to this antibiotic is what defines isolates as AmpC phenotype.

None of the 2 lamb isolates or the 24 turkey isolates from MCA-CTX were resistant to the ‘last resort’ carbapenem antimicrobials imipenem and meropenem or to colistin (Tables 9 and 11, lamb and 12 and 11, turkey). Additionally, none of the isolates were resistant to the antibiotics azithromycin, temocillin or tigecycline (Table 11).

Only one lamb ESBL-phenotype *E. coli* isolate was resistant to gentamicin, whilst most of the turkey isolates were sensitive to chloramphenicol (Table 11). Over half of the isolates were resistant to the quinolone antibiotics ciprofloxacin or nalidixic acid or to chloramphenicol and to tetracycline (Table 11), but most isolates were sensitive to sulfamethoxazole and trimethoprim.

Table 9 - MIC results of 19 antimicrobials against lamb AmpC/ESBL *E. coli* isolates from MCA-CTX - Resistant (R) or Sensitive (S) for different antimicrobials

Isolate Ref	Phenotype	AMP	AZI	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ERT	IPM	MEM	GEN	TMC	TET	TGC	SUL	TMP
L00462793	AmpC	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
L00511945	ESBL	R	S	R	R	S	R	R	R	R	S	S	S	S	R	S	R	S	R	R

R – Resistant; S – Sensitive. Any isolates with an ESBL phenotype would have shown synergy with cefotaxime and or ceftazidime + clavulanic acid – not shown in above.

AMP – ampicillin (R > 8 mg/L); AZM – azithromycin (R > 16 mg/L); FEP – cefepime (R > 0.125 mg/L); CTX – cefotaxime (R > 0.25 mg/L); FOX – cefoxitin (R > 8); CAZ – ceftazidime (R > 8 mg/L); CHL – chloramphenicol (R > 16 mg/L; CIP –

ciprofloxacin (R > 0.064 mg/L); NAL - nalidixic acid (R > 16 mg/L); CST – colistin (R > 2 mg/L); ETP – Ertapenem (R > 0.064 mg/L); IPM – Imipenem (R > 0.5 mg/); MEM – Meropenem (R > 0.125 mg/L); GEN – gentamicin (R > 2 mg/L); TMC - temocillin (R > 32mg/L); TET – tetracycline (R > 8); TGC - tigecycline (R > 0.5); SUL – sulfamethoxazole (R > 64 mg/L); TMP - trimethoprim (R > 2 mg/L).
Interpretative criteria according to tables 1and 4 in Commission Implementing Decision 2013/652/EU.

Table 10 - MIC results of 19 antimicrobials against turkey ESBL *E. coli* isolates from MCA-CTX- Resistant (R) or Sensitive (S) for different antimicrobials

Isolate Ref	Phenotype	AMP	AZI	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ERT	IPM	MEM	GEN	TMC	TET	TGC	SUL	TMP
T00462505	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T00462506	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T00462507	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T002664390	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T002664388	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T002664387	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T002797996	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T00462414	ESBL	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
T00462378	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T00462805	ESBL	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
T002672449	ESBL	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
T002797792	ESBL	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
T002797779	ESBL	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S

Isolate Ref	Phenotype	AMP	AZI	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ERT	IPM	MEM	GEN	TMC	TET	TGC	SUL	TMP
T00512133	ESBL	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
T00512100	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T00512177	ESBL	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
T00512110	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T00512111	ESBL	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
T00462772	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	S	S	R	R
T00462771	ESBL	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
T00462448	ESBL	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	S	R	R
T00462773	ESBL	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	S	S
T00512045	ESBL	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
T00511964	ESBL	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	S	S	S

R – Resistant; S – Sensitive. Any isolates with an ESBL phenotype would have shown synergy with cefotaxime and or ceftazidime + clavulanic acid – not shown in above.

AMP – ampicillin (R > 8 mg/L); AZM – azithromycin (R > 16 mg/L); FEP – cefepime (R > 0.125 mg/L); CTX – cefotaxime (R > 0.25 mg/L); FOX – cefoxitin (R > 8); CAZ – ceftazidime (R > 8 mg/L); CHL – chloramphenicol (R > 16 mg/L; CIP – ciprofloxacin (R > 0.064 mg/L); NAL - nalidixic acid (R > 16 mg/L); CST – colistin (R

> 2 mg/L); ETP – Ertapenem (R > 0.064 mg/L); IPM – Imipenem (R > 0.5 mg/); MEM – Meropenem (R > 0.125 mg/L); GEN – gentamicin (R > 2 mg/L); TMC - temocillin (R > 32mg/L); TET – tetracycline (R > 8); TGC - tigecycline (R > 0.5); SUL – sulfamethoxazole (R > 64 mg/L); TMP - trimethoprim (R > 2 mg/L).
Interpretative criteria according to tables 1and 4 in Commission Implementing Decision 2013/652/EU.

Table 11 - Summary of resistance phenotypes for turkey (and lamb) *E. coli* from MCA-CTX - No. resistant[a] / No. tested (results for lamb)

Antimicrobial	ESBL	ESBL	AmpC	AmpC
Ampicillin	24/24	(1/1)	0	(1/1)
Azithromycin	0/24	(0/1)	0	(0/1)
Cefepime	24/24	(1/1)	0	(0/1)
Cefotaxime	24/24	(1/1)	0	(1/1)
Cefoxitin	0/24	(0/1)	0	(1/1)
Ceftazidime	24/24	(1/1)	0	(1/1)
Chloramphenicol	2/24	(1/1)	0	(0/1)
Ciprofloxacin	19/24	(1/1)	0	(0/1)
Colistin	0/24	(0/1)	0	(0/1)
Ertapenem	0/24	(0/1)	0	(0/1)
Gentamicin	0/24	(1/1)	0	(0/1)
Imipenem	0/24	(0/1)	0	(0/1)
Meropenem	0/24	(0/1)	0	(0/1)
Nalidixic Acid	14/24	(1/1)	0	(0/1)
Sulfamethoxazole	4/24	(1/1)	0	(0/1)
Temocillin	0/24	(0/1)	0	(0/1)
Tetracycline	15/24	(1/1)	0	(0/1)
Tigecycline	0/24	(0/1)	0	(0/1)
Trimethoprim	4/24	(1/1)	0	(0/1)

Orange highlight denotes the four different cephalosporin antimicrobials which were tested.

Grey highlight denotes the three carbapenem antimicrobials ertapenem, imipenem and meropenem and colistin (all ‘last resort’ antimicrobials).

[a] Microbiologically-resistant using EUCAST ECOFFS

Counts of presumptive *E. coli* on MCA and MCA-CTX agars pre-enrichment – EU harmonised test

Using the EU method “Quantification of ESBL/AmpC-producing *Escherichia coli* in caecal content and fresh meat samples” none of the lamb meat samples pre-enrichment gave rise to background *E. coli* on MCA or to presumptive ESBL/AmpC-producing *E. coli* on MCA-CTX above the limit of detection (3,000 cfu/gram). The same applied pre-enrichment to presumptive ESBL/AmpC-producing *E. coli* for turkey samples.

For background *E. coli* on MCA for turkey samples, 7 samples pre-enrichment gave rise to viable counts that ranged from 3,100 to 17,100 cfu/gram (Table 12). These samples were all “Turkey mixed other pieces” that all had the UK as their stated country of origin. Five of the samples were obtained in December 2020, and all 7 samples were obtained from retailers in different parts of the UK. One sample (T00512110) also gave rise to ESBL-phenotype *E. coli* post-enrichment on MCA-CTX.

Table 12 – Viable counts of turkey presumptive *E. coli* on MCA pre-enrichment

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin	Cfu/g MCA
T00511943	08/02/2021	TMOP	E	Suffolk	UK	3,800
T00462748	18/11/2020	TMOP	F	North Yorkshire CC	UK	5,000
T00512179	10/12/2020	TMOP	F	Hackney and Newham	UK	17,100
T00512110	10/12/2020	TMOP	F	East Lothian & Midlothian	UK	5,900
T00512078	14/12/2020	TMOP	H	Barnet	UK	10,500
T00512079	14/12/2020	TMOP	K	Harrow and Hillingdon	UK	3,100
T00512042	15/12/2020	TMOP	I	Berkshire	UK	4,000

TMOP – Turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks.

Presumptive ESBL-producing *E. coli* from CA-ESBL and results for $\mathit{bla}_{\mathrm{CTX\text{-}M}}$, $\mathit{bla}_{\mathrm{OXA}}$, $\mathit{bla}_{\mathrm{SHV}}$, $\mathit{bla}_{\mathrm{TEM}}$ genes - UK non-harmonised additional test

For the non-EU stipulated agars post enrichment, a total of one lamb (Table 13) sample (0.48%, 95% confidence interval 0.01% to 2.62%) and 25 turkey (Table 14) samples (11.9%, 95% confidence interval 7.85% to 17.07%) gave rise to growth on CHROMagar™ ESBL – see also Figure 1.

The isolate from lamb and about half the isolates from turkeys (n=13) were positive for CTX-M 15 gene (Tables 13 and 14). Additionally, six isolates from turkeys were positive for CTX-M 55 gene, one was positive for CTX-M 27 gene, one was positive for CTX-M 65 gene and two were positive for SHV-134 gene (Table 14).

CTX-M 15 gene in *E. coli* can be associated with the O25:H4-ST131 human pandemic *E. coli.*[11] For this reason, the serotype and ST (MLST) of *E. coli* isolates harbouring the CTX-M 15 gene was checked from the WGS data. The lamb CTX-M 15 isolate was O33:H4-ST1640 (predicted serotype from WGS data). Additionally, none of the CTX-M 15 isolates from turkey meat were ST31, most were ST1163. None of the ESBL-phenotype *E. coli* isolates harbouring the CTX-M 15 gene therefore were the O25:H4-ST131 human pandemic strain.

Table 13 – $\mathit{bla}_{\mathrm{CTX\text{-}M}}$, $\mathit{bla}_{\mathrm{OXA}}$, $\mathit{bla}_{\mathrm{SHV}}$, $\mathit{bla}_{\mathrm{TEM}}$ genes in presumptive ESBL *E. coli* from CHROMagar™ ESBL for lamb samples.

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	CTX-M, OXA, SHV and TEM gene
L00511945	08/02/2021	Lamb Frying/Grilling Chops	A	CTX-M-15, TEM-1b

Table 14 – blaCTX-M, blaOXA, blaSHV, blaTEM genes in presumptive ESBL E. coli from CHROMagar™ ESBL for turkey samples

Turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks.

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	CTX-M, OXA, SHV and TEM gene
T00462378	20/10/2020	Turkey Mixed Other Pieces	F	CTX-M-15
T00462414	20/10/2020	Turkey Mixed Other Pieces	F	CTX-M-15
T00462448	15/12/2020	Turkey Whole Bird	L	CTX-M-15, TEM-1b
T00462505	21/10/2020	Turkey Mixed Other Pieces	F	CTX-M-15
T00462506	21/10/2020	Turkey Mixed Other Pieces	E	CTX-M-15
T00462507	21/10/2020	Turkey Mixed Other Pieces	E	CTX-M-15
T00462771	11/12/2020	Turkey Leg	E	CTX-M-55
T00462805	18/11/2020	Turkey Crown joint	H	CTX-M-15
T00462809	09/12/2020	Turkey Mixed Other Pieces	H	CTX-M-15
T00511964	08/02/2021	Turkey Mixed Other Pieces	B	SHV-134, TEM-1b
T00512020	18/01/2021	Turkey Mixed Other Pieces	B	SHV-134
T00512045	16/12/2020	Turkey Breast	F	CTX-M-55
T00512098	09/12/2020	Turkey Breast	L	CTX-M-65, TEM-1b
T00512100	09/12/2020	Turkey Mixed Other Pieces	H	CTX-M-55
T00512110	10/12/2020	Turkey Mixed Other Pieces	F	CTX-M-15
T00512111	10/12/2020	Turkey Leg	E	ND
T00512133	09/12/2020	Turkey Crown joint	L	CTX-M-27
T00512177	10/12/2020	Turkey Leg	E	CTX-M-55
T02664387	19/10/2020	Turkey Breast	F	CTX-M-15
T02664388	19/10/2020	Turkey Mixed Other Pieces	E	CTX-M-15
T02664390	19/10/2020	Turkey Mixed Other Pieces	I	CTX-M-15
T02797779	09/12/2020	Turkey Leg	E	CTX-M-55
T02797792	09/12/2020	Turkey Breast	L	CTX-M-55
T02797927	18/11/2020	Turkey Mixed Other Pieces	B	Neg
T02797996	19/10/2020	Turkey Mixed Other Pieces	I	CTX-M-15

ND – Not determined

Plasmid mediated colistin resistance genes *mcr-1, mcr-2* and *mcr-3* - UK non-harmonised additional test

Three (1.43%, 95% confidence interval 0.30% to 4.12%) turkey meat samples originating from the UK (n=2) or Germany (n=1) were positive for the *mcr-1* transferable colistin resistance gene post enrichment (Tables 16, 17, 18 and Figure 1).

For the three samples from which *mcr-1* positive *E. coli* were recovered, multiple colonies (three per meat sample) were characterised by PCR and short read WGS (Table 15, 16, 17).

For sample T00512133 (Table15), all three colonies examined had the identical predicted serotype, MLST, resistance genes and plasmids. These three colonies all had the predicted serotype O9/O9a:H10 with MLST 8778 and just two plasmids, Col(pHAD28), IncX1/4. Apart from the colistin resistance gene *mcr-1,* these isolates also had resistance genes potentially conferring resistance to antibiotics such as ampicillin, trimethoprim, quinolones and sulphonamides.

For sample T00512101 (Table 16), two of the colonies had identical predicted serotype (O101:H25) and MLST (ST58), whilst the third colony was predicted serotype O9/O9a:H25 and ST889. However, all three colonies had the same mutation in *gyrA* and a similar set of resistance genes and plasmids. These three colonies had a considerably higher number of resistance genes and plasmids than the three colonies from meat sample T00512133, potentially conferring additional resistances to antibiotics such as aminoglycosides, chloramphenicol and tetracycline.

For the final meat sample T00512003 (Table 17) that was positive for *mcr-1 E. coli,* as from sample T00512133, all three colonies examined had the identical predicted O serotype O101 (with H9 or H9/H21), mutations in *gyrA* and *parC* and MLST (ST744) and similar resistance genes and plasmids.

Although the colonies from this meat sample had fewer resistance genes than the colonies from sample T00512101, most of the resistance gene types from both samples were identical, with notable differences such as the presence of *cmlA1* and *blaOXA-1* resistance genes in all three isolates from sample T00512101.

For five further turkey retail meat samples all stated to be of UK origin from four different major supermarket chains, PCR analysis of the initial “sweep” of ~ 10 to 20 (as per SOP) suspect *E. coli* colonies were positive for *mcr-3*. However, it was not possible to isolate individual *mcr-3* positive *E. coli* despite sub-culture of multiple different colonies from primary culture plates. Only lactose fermenters with characteristics typical of *E. coli* were investigated. As such it is possible that *mcr-3* could be present in other bacterial genera. This might include non-target lactose fermenters such as *Klebsiella* and *Citrobacter* [6] as well as non-lactose fermenters.

At the request of the FSA, further work was performed on the *mcr-1 E. coli* to resolve the plasmids, as reported in Appendix 3.

Table 15 – WGS results for *mcr-1 E. coli* from turkey crown meat sample T00512133 (sampled 9-12-20, UK origin).

Colony replicate (Predicted *E. coli* serotype)	**All AMR genes detected**	**Mutations in QRDRs‡**	**MLST† (% ID)**	**Plasmid types**	**Bacterial ID***
5 (O9/O9a:H10)	*blaTEM-1B, dfrA36,* ***mcr-1.1,*** *mdf(A), qnrS1, qnrB19, sul2.*	None	8778 (100)	Col(pHAD28), IncX1/4.	*E. coli*
7 (O9/O9a:H10)	*blaTEM-1B, dfrA36,* ***mcr-1.1,*** *mdf(A), qnrS1, qnrB19, sul2.*	None	8778 (100)	Col(pHAD28), IncX1/4.	*E. coli*
10 (O9/O9a:H10)	*blaTEM-1B, dfrA36,* ***mcr-1.1****,* *mdf(A), qnrS1, qnrB19, sul2.*	None	8778 (100)	Col(pHAD28), IncX1/4.	*E. coli*

***mcr* gene in bold**. † - MLST compared to DTU *Escherichia coli#1* - Achtman scheme.
* Bacterial ID was determined by use of oxidase and indole test and confirmed from WGS data by use of DTU KmerFinder
‡ - QRDR – quinolone resistance determining region.

Table 16 – WGS results for *mcr-1 E. coli* from turkey crown meat sample T00512101 (sampled 16-12-20, UK origin).

Colony replicate (Predicted *E. coli* serotype)	All AMR genes detected	Mutations in QRDRs‡	MLST† (% ID)	Plasmid type	Bacterial ID*
5 (O101:H25)	*aadA1/2, aph(3")-lb, aac(3)-lld, aph(6)-Id, catA1, cmlA1, blaTEM-1B, blaOXA-1 dfrA1/12/15/36, floR,* ***mcr-1.1****, mdf(A), qnrS1, sul1,2,3, tet(A).*	*gyrA* (p.S83L)	889 (100)	Col(MG828), Col156, IncFIA, IncFIB(AP001918), IncFII, IncHI2/A, IncI1-I(Alpha), IncI2(Delta), IncQ1, IncR, IncX4, IncY, p0111.	*E. coli*
9 (O101:H25)	*aadA1/2, aph(3")-la/Ib, aph(6)-Id, catA1, cmlA1, blaTEM-1B/106/126/135/220, blaOXA-1 dfrA1/12/15/36, floR,* ***mcr-1.1****, mdf(A), qnrS1, sul1,2,3, tet(A).*	*gyrA* (p.S83L)	58 (100)	Col(MG828), Col156, Col440I, IncFIA, IncFIB(AP001918), IncFIC(FII), IncFII, IncHI2/A, IncI1-I(Alpha), IncR, IncX1/4, p0111.	*E. coli*
10 (O9/O9a:H25)	*aadA1, aph(3")-Ib, aph(6)-Id, catA1, cmlA1, blaTEM-1B/106/126/135/220, blaOXA-1 dfrA1/12/36, floR,* ***mcr-1.1,*** *mdf(A), qnrS1, sul1,2,3, tet(A).*	*gyrA* (p.S83L)	58 (100)	Col(MG828), Col156, Col440I, IncFIA, IncFIB(AP001918), IncFIC(FII), IncI1-I(Alpha), IncR, IncX1/4, p0111.	*E. coli*

***mcr* gene in bold**. † - MLST compared to DTU *Escherichia coli#1* - Achtman scheme.

* Bacterial ID was determined by use of oxidase and indole test and confirmed from WGS data by use of DTU KmerFinder
‡ - QRDR – quinolone resistance determining region.

Table 17 – WGS results for *mcr-1 E. coli* from turkey breast meat sample T00512003 (sampled 4-01-21, German origin).

Colony replicate (Predicted *E. coli* serotype)	**All AMR genes detected**	**Mutations in QRDRs‡**	**MLST† (% ID)**	**Plasmid type**	**Bacterial ID***
5 (O101:H9/H21)	*aadA5, aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, catA1, blaTEM-1B, dfrA17,* ***mcr-1.1****, mdf(A), mph(A), sul1,2, tet(A), tet(B), tet(Y).*	*gyrA* (p.S83L), *gyrA* (p.D87N), *parC* (p.A56T)	744 (100)	IncFIB(AP001918), IncFII, IncFII(pSE11), IncI2, IncQ1, p0111.	*E. coli*
9 (O101:H9)	*aadA5, aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, catA1, blaTEM-1B, dfrA17,* ***mcr-1.1,*** *mdf(A), sul1,2, tet(A), tet(B).*	*gyrA* (p.S83L), *gyrA* (p.D87N), *parC* (p.A56T)	744 (100)	IncFIB(AP001918), IncFII(pSE11), IncI2, IncQ1.	*E. coli*
10 (O101:H9)	*aadA5, aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, catA1, blaTEM-1B, dfrA17,* ***mcr-1.1,*** *mdf(A), sul1,2, tet(B).*	*gyrA* (p.S83L), *gyrA* (p.D87N), *parC* (p.A56T)	744 (100)	IncFIB(AP001918), IncFII, IncFII(pSE11), IncI2, IncP6, IncQ1, p0111.	*E. coli*

***mcr* gene in bold**. † - MLST compared to DTU *Escherichia coli#1* - Achtman scheme.
* Bacterial ID was determined by use of oxidase and indole test and confirmed from WGS data by use of DTU KmerFinder
‡ - QRDR – quinolone resistance determining region.

Results specific for *Campylobacter*

Turkey samples positive for *Campylobacter*

The test method used in this study could detect *Campylobacter* in samples with 10 cfu/g or higher. *Campylobacter* was detected in 22 of the 210 samples tested (10.5%) (Table 18).

Table 18- Turkey meat sample types tested for *Campylobacter*.

Sample category	**Sample description**	**Number of samples tested**	**% *Campylobacter* positive**	**Lower 95% confidence interval for the % positive**	**Upper 95% confidence interval for the % positive**
Mixed other pieces (with skin)	Drumsticks	14	14.3	0	34.5
Mixed other pieces (skinless)	† See below	111	0.0	0.0	0.0
Breast (with skin)	Breast steak/joint	9	0.0	0.0	0.0
Breast (skinless)	Breast steak/joint/crown	19	5.3	0	16.0
Turkey leg (with skin)	Leg	14	64.3	36.6	92.0
Crown (with skin)	Crown/joint/butterfly	38	23.7	10.2	37.2
Whole bird (with skin)	Whole bird	5	20.0	0	69.7
All samples (with skin)	As above	80	26.3	16.7	35.9
All samples (skinless)	As above	130	0.8	0	2.3
All samples	As above	210	10.5	6.4	14.6

† Turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks.

The proportion of positive samples varied between the type of sample tested, 64.3% of turkey leg samples were positive for *Campylobacter*, and 23.7% and 20% of crown and whole bird samples were positive respectively. *Campylobacter* was not detected in any of the skinless mixed other piece samples (n=111) or in any of the breast samples with skin (n=9). The proportion of positive samples was significantly higher (Chi^2=34.24, , P-Value = 0.000) in samples with skin (26.3%) compared to samples without skin (0.8%). Up to five *Campylobacter* colonies from each positive sample were identified to species level. *C. jejuni* was identified in 20 samples (9.5% [95% confidence interval 5.6% to 13.5%]), *C. coli* was identified in seven samples (3.3% [95% confidence interval 0.9% to 5.8%]), and both species were identified in five samples (2.4% [95% confidence interval 0.3% to 4.4%]) (Table 19). Positive samples came from across the four nations and all of the positive samples were of UK origin. No meat samples that originated from outside the UK (Italy n=2, Germany n=1, Ireland n=1) were positive for *Campylobacter*.

Counts of *Campylobacter* in turkey samples

The enumeration method used in this study allowed for a calculation of *Campylobacter* levels in samples containing more than 45 cfu/g. Of the 22 positive samples, 10 were below this threshold, and were considered as positive samples that contain between 10 and 45 cfu/g (Table 19).

Contamination levels in chicken broiler carcasses of over 1,000 campylobacters per gram are classified as highly contaminated, and this level is often used as a significant threshold in assessing the relative risk of exposure to humans.[12] Considering this criteria, only one sample would be considered in the higher risk category for consumers. This was a UK-sourced turkey crown with 5.1×10^3 cfu/g of sample.

The variations in levels of *Campylobacter* present in the various sample types are presented in Figure 2. 6.2% (95% confidence interval 2.9% to 9.4%) of all samples tested contained *Campylobacter* at levels between 10 and 99 cfu/g. Higher levels (100-999cfu/g) were observed in 3.8% (95% confidence interval 1.2% to 6.4%) of samples and just one sample (0.5% [95% confidence interval 0.0% to 1.5%]) had levels of over 1000cfu/g. The level of contamination on turkey leg samples appears higher than other sample types, however the number of observations in this group is relatively small.

Further characterisation of *Campylobacter* recovered from turkey meat

All *C. jejuni* (n=50) recovered from the 20 *C. jejuni*-positive samples were further characterised by MIC and WGS. In addition, five *C. coli* isolates collected from three of the *C. coli*-positive samples were characterised. During further characterisation mixed cultures were suspected for a single *C. jejuni* isolate and a single *C. coli* isolate, and these were removed from the analysis. This left 49 *C. jejuni* and 4 *C. coli* collected from 20 samples for further analysis.

MICs against six antimicrobials for *Campylobacter* isolated from turkey meat

Isolates were resistant to up to three of the six antimicrobials tested (Table 20). The most common resistance was to ciprofloxacin (a fluoroquinolone antibiotic), found in 66% of the isolates (Table 21). Resistance to nalidixic acid was present in 60.4% of the isolates as was resistance to tetracycline. No isolates displayed resistance to erythromycin, gentamicin or streptomycin. A total of 22 isolates (41.5%) were resistant to three antimicrobials (ciprofloxacin, nalidixic acid and tetracycline), whilst 13 (24.6%) were resistant to two antimicrobials. No resistance to any antimicrobial was observed for 11 isolates (20.8%). No isolates with multiple drug resistance (MDR, which is defined as resistant to three different classes of antimicrobial), were identified by phenotyping in this study.

As the number of isolates characterised for each *Campylobacter*-positive meat sample is variable, it is more insightful to determine the proportion of positive samples with resistant phenotypes present. This data was compiled for samples containing *C. jejuni* (n=20) as all available isolates (n=49) for this species had been characterised by MIC (Table 20).

Table 19 -Turkey meat samples positive for *Campylobacter* with viable counts per gram (cfu/g) of meat sample tested.

Sample Number	Scheduled Sampling Date	Food Category	Retailer Code	Location	Country of Origin	Species present	cfu/gram meat	log cfu/g meat
T00462643	30/10/2020	Turkey Breast	F	Swindon	UK	*C.jejuni*	20*	1.30
T02797778	24/11/2020	Turkey Leg	H	Great Manchester South West	UK	*C.jejuni*	50	1.70
T00512201	23/11/2020	Turkey Leg	E	Haringey and Islington	UK	*C.jejuni*	30*	1.48
T02797790	23/11/2020	Turkey Leg	E	Redbridge & Waltham Forest	UK	*C.jejuni*	10*	1.00
T02672449	09/12/2020	Turkey Leg	E	West Northamptonshire	UK	*C.jejuni*	20*	1.30
T02797779	09/12/2020	Turkey Leg	E	Essex Thames Gateway	UK	Mixed	150	2.18
T00512111	10/12/2020	Turkey Leg	E	City of Edinburgh	UK	*C.jejuni*	10*	1.00
T00512114	10/12/2020	Turkey Crown	K	City of Edinburgh	UK	*C.jejuni*	20*	1.30
T00512116	11/12/2020	Turkey Leg	E	South Hampshire	UK	*C.jejuni*	30*	1.48
T00462772	11/12/2020	Turkey Whole Bird	B	Bristol, City of	UK	*C.jejuni*	270	2.43
T00462771	11/12/2020	Turkey Leg	E	Bath, N & NE Somerset	UK	*C.jejuni*	720	2.86
T00462810	14/12/2020	Turkey Crown	E	Mid Ulster	UK	Mixed	720	2.86
T00512074	14/12/2020	Turkey Crown	D	Brent	UK	Mixed	260	2.41
T00512057	14/12/2020	Turkey Crown	E	Camden & City of London	UK	Mixed	410	2.61
T00512068	14/12/2020	Turkey Crown	E	Birmingham	UK	*C.coli*	5100	3.71
T00512069	14/12/2020	Turkey Crown	E	Birmingham	UK	*C.coli*	500	2.70
T00512043	15/12/2020	Turkey Crown	L	Berkshire	UK	*C.jejuni*	30*	1.48
T00512103	16/12/2020	Turkey Crown	E	Inverclyde, East Renfrewshire	UK	Mixed	40*	1.60
T00512102	16/12/2020	Turkey Crown	E	Glasgow City	UK	*C.jejuni*	10*	1.00
T00512000	04/01/2021	TMOP	F	South West Wales	UK	*C.jejuni*	10*	1.00
T00511960	08/02/2021	Turkey Leg	H	Lewisham & Southwark	UK	*C.jejuni*	100	2.00
T00511961	08/02/2021	TMOP	D	Lewisham & Southwark	UK	*C.jejuni*	60	1.78

TMOP- turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks. Mixed- both *C. coli* and *C. jejuni* detected. * Count considered an estimate (between 10 and 45cfu/g)

Figure 2 -Distribution of *Campylobacter* in turkey meat samples

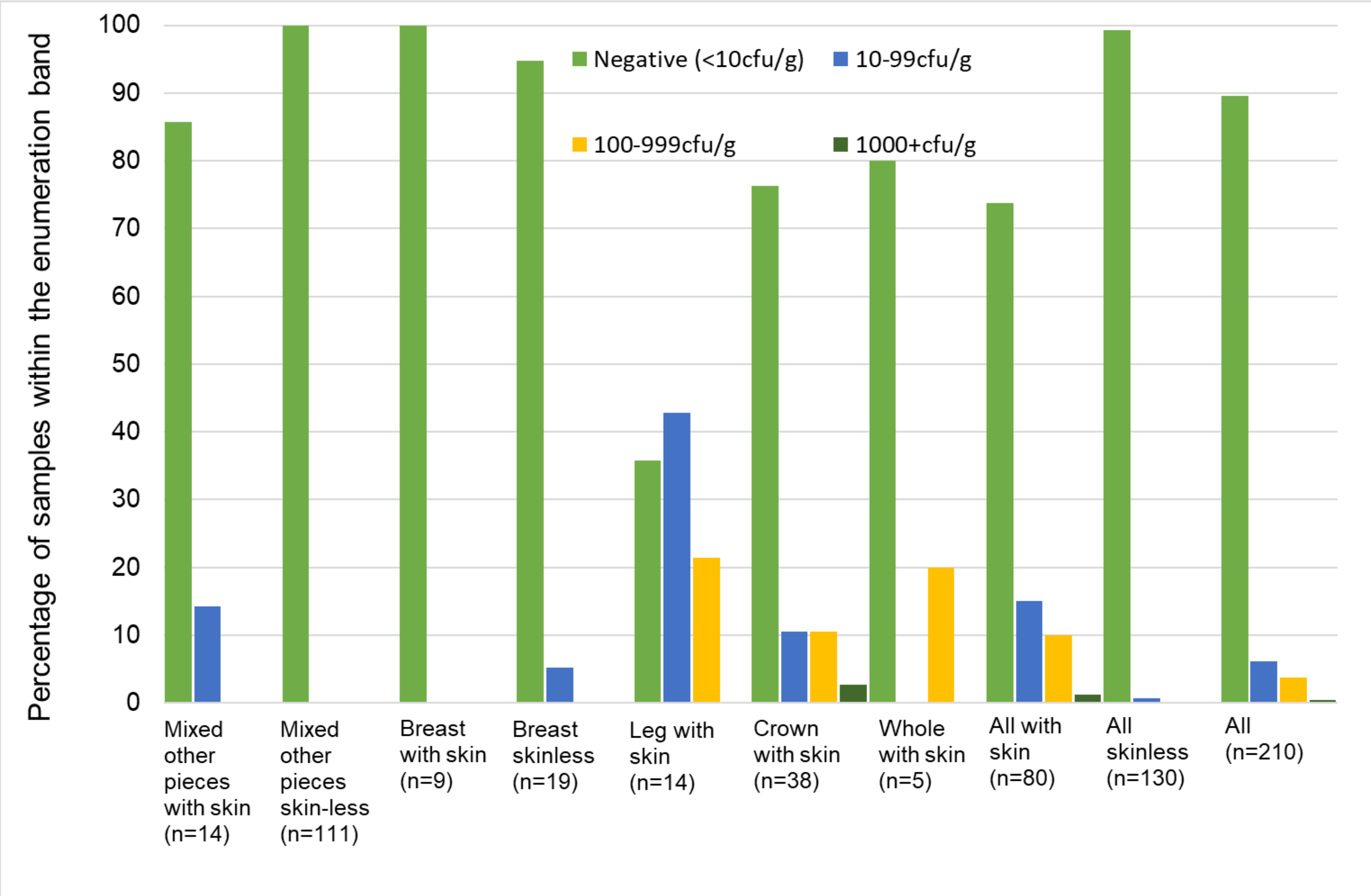


Table 20 - MIC results for six antimicrobials against *Campylobacter* isolated from turkey meat. - Resistant (R) or Sensitive (S) for different antimicrobials

Isolate Ref	Species	CIP	ERY	GEN	NAL	STR	TET
TU462643ACAMP20	*C. jejuni*	S	S	S	S	S	S
TU462643BCAMP20	*C. jejuni*	S	S	S	S	S	S
TU2797790ACAMP20	*C. jejuni*	R	S	S	R	S	R
TU512201ACAMP20	*C. jejuni*	R	S	S	R	S	R
TU512201BCAMP20	*C. jejuni*	R	S	S	R	S	R
TU512201CCAMP20	*C. jejuni*	R	S	S	R	S	R
TU2797778ACAMP20	*C. jejuni*	S	S	S	S	S	S
TU2797778BCAMP20	*C. jejuni*	S	S	S	S	S	S
TU2797778CCAMP20	*C. jejuni*	S	S	S	S	S	S
TU2797778DCAMP20	*C. jejuni*	S	S	S	S	S	S
TU462772ACAMP20	*C. jejuni*	R	S	S	R	S	S
TU462772BCAMP20	*C. jejuni*	R	S	S	R	S	S
TU462772CCAMP20	*C. jejuni*	R	S	S	R	S	S
TU462772DCAMP20	*C. jejuni*	R	S	S	R	S	S
TU462772ECAMP20	*C. jejuni*	R	S	S	R	S	S
TU462771ACAMP20	*C. jejuni*	R	S	S	R	S	R
TU462771BCAMP20	*C. jejuni*	R	S	S	R	S	R
TU462771CCAMP20	*C. jejuni*	R	S	S	R	S	R
TU462771DCAMP20	*C. jejuni*	R	S	S	R	S	R
TU462771ECAMP20	*C. jejuni*	R	S	S	R	S	R
TU512043ACAMP20	*C. jejuni*	R	S	S	S	S	R
TU512043BCAMP20	*C. jejuni*	R	S	S	S	S	R
TU512043DCAMP20	*C. jejuni*	R	S	S	S	S	R
TU512103ACAMP20	*C. jejuni*	R	S	S	R	S	R
TU512103BCAMP20	*C. jejuni*	R	S	S	R	S	R
TU512103DCAMP20	*C. jejuni*	R	S	S	R	S	R
TU512103CCAMP21	*C. coli*	R	S	S	R	S	S
TU512116ACAMP20	*C. jejuni*	R	S	S	R	S	R
TU512116BCAMP20	*C. jejuni*	R	S	S	R	S	R
TU512116CCAMP20	*C. jejuni*	R	S	S	R	S	R
TU462810CCAMP20	*C. coli*	R	S	S	R	S	S
TU462810DCAMP20	*C. jejuni*	R	S	S	R	S	R
TU462810ECAMP20	*C. jejuni*	R	S	S	R	S	R
TU512057CCAMP20	*C. jejuni*	R	S	S	R	S	R
TU512102CCAMP20	*C. jejuni*	R	S	S	R	S	R

Isolate Ref	Species	CIP	ERY	GEN	NAL	STR	TET
TU512111ACAMP20	*C. jejuni*	S	S	S	S	S	S
TU512114CCAMP20	*C. jejuni*	S	S	S	S	S	S
TU512114DCAMP20	*C. jejuni*	S	S	S	S	S	S
TU2672449ACAMP20	*C. jejuni*	R	S	S	R	S	S
TU2672449BCAMP20	*C. jejuni*	R	S	S	R	S	S
TU2797779BCAMP20	*C. jejuni*	R	S	S	R	S	S
TU2797779CCAMP20	*C. jejuni*	R	S	S	R	S	R
TU2797779ECAMP20	*C. jejuni*	R	S	S	R	S	R
TU512000ACAMP21	*C. jejuni*	S	S	S	S	S	S
TU512074ACAMP21	*C. coli*	R	S	S	R	S	R
TU512074ECAMP21	*C. coli*	S	S	S	S	S	R
TU512074CCAMP20	*C. jejuni*	S	S	S	S	S	R
TU511960ACAMP21	*C. jejuni*	S	S	S	S	S	R
TU511961ACAMP21	*C. jejuni*	S	S	S	S	S	R
TU511961BCAMP21	*C. jejuni*	S	S	S	S	S	R
TU511961CCAMP21	*C. jejuni*	S	S	S	S	S	S
TU511961DCAMP21	*C. jejuni*	S	S	S	S	S	R
TU511961ECAMP21	*C. jejuni*	S	S	S	S	S	R

R – Resistant; S – Sensitive.

Interpretative criteria were EUCAST ECOFFs according to Table 2 in Commission Implementing Decision 2013/652/EU, which were CIP– ciprofloxacin (R>0.5mg/l); ERY- erythromycin (R>4mg/l, or >8mg/l coli); GEN- gentamicin (R>2mg/l); NAL- nalidixic acid (R>16mg/l); STR- streptomycin (R>4mg/l); TET- tetracycline (R>1mg/l, or >2mg/l *C. coli*).

Table 21 - Summary of resistance phenotypes of *Campylobacter* isolated from turkey meat.

Antimicrobial	Number of isolates resistant (out of 53)	Percentage resistant (%)
Ciprofloxacin (CIP)	35	66.0
Erythromycin (ERY)	0	0.0
Gentamicin (GEN)	0	0.0
Nalidixic acid (NAL)	32	60.4
Streptomycin (STR)	0	0.0
Tetracycline (TET)	32	60.4
CIP+NAL+TET	22	41.5
CIP+NAL	10	18.9

Antimicrobial	Number of isolates resistant (out of 53)	Percentage resistant (%)
CIP+TET	3	7
TET only	7	13.2
Fully sensitive	11	20.8

Table 22 - Summary of resistance phenotypes of *C. jejuni* isolated from turkey meat samples.

Antimicrobial	Number of samples with phenotype (out of 20)	Percentage of samples with phenotype
Ciprofloxacin (CIP)	12	60.0
Erythromycin (ERY)	0	0.0
Gentamicin (GEN)	0	0.0
Nalidixic acid (NAL)	11	55.0
Streptomycin (STR)	0	0.0
Tetracycline (TET)	13	65
CIP+NAL+TET	10	50
CIP+NAL	2	10
CIP+TET	1	5
TET only	2	10
Fully sensitive	5	25

Resistance to ciprofloxacin and nalidixic acid was observed in 60% (95% confidence interval 38.5% to 81.5%) and 55% (95% confidence interval 33.2% to 55.0%) of the *C. jejuni*-positive samples respectively. Resistance to tetracycline was observed in 65% (95% confidence interval 44.1% to 85.9%) of positive samples. A total of 10 positive samples (50% contained *C. jejuni* resistance to three antimicrobials, ciprofloxacin, nalidixic acid and tetracycline. Whilst three samples contained resistance to two antimicrobials and two samples contained resistance to tetracycline only. One sample contained *C. jejuni* that was resistant to ciprofloxacin but not nalidixic acid.

Resistance in *C. coli* is not considered at the sample level as all available isolates were not retained for characterisation. All of the phenotyped *C. coli* isolates (n=4) were resistant to at least one antimicrobial, 75% were resistant to ciprofloxacin and nalidixic acid, 50% were resistant to tetracycline (Table 20). One isolate was resistant to all three antimicrobials, two isolates were resistant to ciprofloxacin and nalidixic acid and one isolate was resistant to tetracycline alone.

Application of WGS for MLST and to identify resistance genes in *Campylobacter*

The resistance genes detected and MLST outputs are shown in Table 23, profiles are displayed for 48 *C. jejuni* and four *C. coli* isolates. The sequencing output for one *C. jejuni* isolate was not sufficient for MLST or resistance genotyping. Variable numbers of isolates were characterised from each positive sample. Some samples with multiple isolates retained a single MLST type (ST), whilst other samples contained a variety of STs. The detection of STs and resistance genes in each individual meat sample is summarised in Table 24.

A total of 21 different STs were identified and 17 were assigned to recognised sequence types. At least 14 of the STs can be linked to clinical cases in humans via the PubMLST website.[2] In 14 turkey meat samples a single ST was detected, in four meat samples two different STs were detected and there were two samples that contained three different STs. Only five different STs were found in more than one sample, with ST21, ST354, ST441, and a non-assigned ST being found in two meat samples each. However, ST573 was most the widespread as it was detected in four different samples. In general, multiple isolates of the same ST had consistent AMR profiles (phenotypic and genotypic). The exceptions were ST21, that had two different AMR profiles and a ST2863 isolate which had additional resistance genes.

Each isolate was assigned to a clonal complex (CC) where possible,[2] isolates within a colonal complex all share common sequences for at least four loci of the MLST system. Eleven different complexes were identified which accounted for 40 isolates. The remaining 12 isolates were not assigned to any specific CC. Clonal complex 21 and CC573 were relatively widespread, each present in four different meat samples, whilst CC354 was present in three samples and CC828 was present in two samples.

The genotypic AMR profile can be compared against the phenotypic results (Table 23). In general, the genotype predictions for AMR to quinolones and tetracyclines were complementary to the phenotypic profile but there were some anomalies. The ResFinder pipeline failed to detect resistance genes in isolates that displayed phenotypic resistance. This was seen for ciprofloxacin in three different STs and for tetracycline in two different STs. Conversely resistance genes were detected in some isolates that were not phenotypically resistant, this was the case for a single ST (unassigned) with tetracycline and in another ST (ST2836) for nalidixic acid and streptomycin.

In one *C. jejuni* isolate (ST2836), resistance determinants for quaternary ammonium compounds (biocides used in disinfectants) and for the sulphonamide antimicrobial class were detected however, phenotypic resistance was not determined in this study. This isolate was of interest as phenotypic and genotypic results combined suggest the isolate may have an MDR profile..

The ResFinder pipeline determined that 51.9% of the isolates had the $bla_{OXA\text{-}61}$ gene, and it was detected in 14 of the 20 meat samples analysed (Table 23). This gene confers resistance to β-lactam antibiotics such as ampicillin.[13] In this study it was not possible to correlate the gene presence to β-lactam resistance as no β-lactams were included in the MIC panel.

Combining the phenotypic and genotypic results identified putative MDR isolates. A *C. jejuni* ST5136/CC464 and a *C. coli* ST1089/CC828 were phenotypically resistant to quinolones and tetracycline and genotypically shown to have β-lactam resistance genes. These isolates were isolated from UK grown turkey meat.

For quinolone resistance, the majority of the resistant isolates had a single mutation in *gyrA* gene in the quinolone resistance determinant region (p.T86I), but three isolates (ST441/CC unassigned) that were recovered from two samples had double mutations in *gyrA* (p.T86I and p.104S).

Table 23 – Characterisation of *Campylobacter jejuni* (n=48) and *coli* (n=4) using WGS to determine MLST, presence of resistance genes and mutations in *gyrA*, Maldi-ToF to identify and resistance phenotype from MICs.

Isolate Ref. No.	Meat Ref. No. and colony replicate	MLST* (% match if <100%)	AMR genes detected (% match if <100%)	*GyrA* **	MALDI ***	Resistance phenotype
S20-343	T462643-B	520	*blaOXA-61 (99.87%)*	N	J	None
S20-344	T462643-A	520	*blaOXA-61 (99.87%)*	N	J	None
S20-376	T2797790-A	2-17-2-64-23-7-23	None	A	J	C/N/T
S20-377	T512201-A	2-17-2-64-23-7-23	None	A	J	C/N/T
S20-378	T512201-B	2-17-2-64-23-7-23	None	A	J	C/N/T
S20-383	T512201-C	2-17-2-64-23-7-23	None	A	J	C/N/T
S20-379	T2797778-A	1044	*blaOXA-61 (99.74%)*	N	J	None
S20-380	T2797778-B	1044	*blaOXA-61 (99.74%)*	N	J	None
S20-381	T2797778-C	1044	*blaOXA-61 (99.74%)*	N	J	None
S20-382	T2797778-D	1044	*blaOXA-61 (99.74%)*	N	J	None
S20-441	T462772-A	21	*blaOXA-61 (99.87%)*	A	J	C/N
S20-442	T462772-B	21	*blaOXA-61 (99.87%)*	A	J	C/N
S20-443	T462772-C	21	*blaOXA-61 (99.87%)*	A	J	C/N
S20-444	T462772-D	21	*blaOXA-61 (99.87%)*	A	J	C/N
S20-446	T462771-A	354	*tet(O) (99.32%)*	A	J	C/N/T
S20-447	T462771-B	354	*tet(O) (99.32%)*	A	J	C/N/T
S20-448	T462771-C	354	*tet(O) (99.32%)*	A	J	C/N/T
S20-449	T462771-D	354	*tet(O) (99.32%)*	A	J	C/N/T
S20-450	T462771-E	354	*tet(O) (99.32%)*	A	J	C/N/T
S20-451	T512043-A	2863	*aadA1b, qacE, sul1,*	A	J	C/T
S20-452	T512043-B	2863	*tet(O) (99.74%)*	A	J	C/T
S20-453	T512043-D	2863	*tet(O) (99.74%)*	A	J	C/T
S20-454	T512103-A	573	*tet(O) (99.63%)*	A	J	C/N/T
S20-455	T512103-B	573	*tet(O) (99.63%)*	A	J	C/N/T

Isolate Ref. No.	Meat Ref. No. and colony replicate	MLST* (% match if <100%)	AMR genes detected (% match if <100%)	*GyrA* **	MALDI ***	Resistance phenotype
S20-456	T512103-D	573	*tet(O) (99.63%)*	A	J	C/N/T
S20-457	T512116-A	354	*tet(O) (99.32%)*	A	J	C/N/T
S20-458	T512116-B	354	*tet(O) (99.32%)*	A	J	C/N/T
S20-459	T512116-C	8-10-2-NHF-NHF-12-6	*tet(O) (99.32%)*	A	J	C/N/T
S20-460	T462810-C	4550	None	N	C	C/N
S20-461	T462810-D	573	*tet(O) (99.63%)*	A	J	C/N/T
S20-462	T462810-E	573	*tet(O) (99.63%)*	A	J	C/N/T
S20-463	T512057-C	573	*tet(O) (99.63%)*	A	J	C/N/T
S20-464	T512074-C	877	*tet(O) (99.89%)*	N	J	T
S20-465	T512102-C	573	*tet(O) (99.63%)*	A	J	C/N/T
S20-466	T512111-A	583	*blaOXA-61 (99.87%)*	N	J	None
S20-467	T512114-C	50	*blaOXA-61 (99.87%)*	N	J	None
S20-468	T512114-D	50	*blaOXA-61 (99.87%)*	N	J	None
S20-469	T2672449-A	441	*blaOXA-61 (99.74%)*	B	J	C/N
S20-470	T2672449-B	441	*blaOXA-61 (99.74%)*	B	J	C/N
S20-471	T2797779-B	441	*blaOXA-61 (99.74%)*	B	J	C/N
S20-472	T2797779-C	5136	*blaOXA-61 (99.87%),*	A	J	C/N/T
S20-473	T2797779-E	5136	*blaOXA-61 (99.87%),*	A	J	C/N/T
S21-008	T512000-A	3-392-5-17-11-11-8	*blaOXA-61 (99.87%)*	N	J	None
S21-009	T512074-A	1089	*blaOXA-61 (99.87%)*	N	C	C/N/T
S21-011	T512074-E	548 (98.1132%)-39-30-82-104-44-17	*tet(O) (99.42%)*	N	C	T
S21-012	T512103-C	9566	*blaOXA-61 (99.87%),*	N	C	C/N
S21-029	T511960-A	21	*blaOXA-61 (99.87%)*	N	J	T
S21-030	T511961-A	48	*blaOXA-61, tet(O)*	N	J	T
S21-031	T511961-B	51	*blaOXA-61 (99.87%),*	N	J	T
S21-032	T511961-C	8962	None	N	J	None
S21-033	T511961-D	51	*blaOXA-61 (99.87%),*	N	J	T
S21-034	T511961-E	51	*blaOXA-61 (99.87%),*	N	J	T

* MLST – Multi locus sequence type from WGS data. **C**- ciprofloxacin, **N**- nalidixic acid, **T**- tetracycline.
** Mutations in the quinolone resistance determining region; **A** - *gyrA* (p.T86I), **B** - *gyrA* (p.T86I) + *gyrA* (p.P104S), **N** – None.
*** Bacterial ID by MALDI-ToF; **C** – *Campylobacter coli,* **J** - *Campylobacter jejuni.*

Table 24 – The distribution of MLST types within *Campylobacter*-positive samples, assignment to clonal complexes and the antimicrobial resistance genes identified for each isolate within a MLST type.

Sample ID	Number of isolates per type	Sequence type	Clonal Complex	*bla*$_{OXA-61}$ detected	*gyrA** detected	*tet(O)* detected
462643	2	521	21	1	0	0
462771	5	354	354	0	A	1
462772	4	21	21	1	A	0
511960	1	21	21	1	0	1
512000	1	NA	NA	1	0	0
512043	3**	2863	354	0	A	1
512057	1	573	573	0	A	1
512102	1	573	573	0	A	1
512111	1	583	45	1	0	0
512114	2	50	21	1	0	0
512201	3	NA	NA	0	A	0
2672449	2	441	NA	1	B	0
2797778	4	1044	658	1	0	0
2797790	1	NA	NA	0	A	0
462810	2	573	573	0	A	1
462810	1	4450	1150	0	0	0
512103	3	573	573	0	A	1
512103	1	9566	828	1	0	1
512116	2	354	354	0	A	1
512116	1	NA	NA	0	A	1
2797779	2	5136	464	1	A	1
2797779	1	441	NA	1	B	0
511961	3	51	443	1	0	1
511961	1	48	48	1	0	1
511961	1	8962	1034	0	0	0
512074	1	877	NA	0	0	1
512074	1	1089	828	1	0	0
512074	1	NA	NA	0	0	1

NA - no sequence type or clonal complex assigned
* Mutations in the quinolone resistance determining region; **A** - *gyrA* (p.T86I), **B** - *gyrA* (p.T86I) + *gyrA* (p.P104S)
**Additional resistance genes detected in one isolate from this sample; *aadA1b, qacE, sul1.*

6. Discussion

ESBL- and/or AmpC-producing *E. coli*

Since 2013/14 APHA have been involved in studies to determine the presence of AmpC/ESBL-producing *E. coli* in retail chicken meat. This included a study in 2013/14 with Public Health England,[14] and in 2016, 2018 and 2020 EU/FSA studies.[12, 15] Results from these studies led to a publication in 2020 entitled "A decline in the occurrence of extended-spectrum β-lactamase-producing *Escherichia coli* in retail chicken meat in the UK between 2013 and 2018."[16] It was considered that "significant reductions in antimicrobials used in the UK poultry meat sector between 2012 and 2016 may be linked to significant reductions in AmpC/ESBL-phenotype *E. coli* in retail chicken between 2013/14 and 2018."[16]

Of interest, the EFSA report for 2017/18 EU AMR surveys also showed a reduction in the prevalence of presumptive ESBL- and/or AmpC-producing *E. coli* in broiler meat from several member states between 2016 and 2018.[17] Overall EFSA reported that the prevalence of presumptive ESBL- and/or AmpC-producing *E. coli* in meat from broilers in 2018 was 39.8%, which is markedly lower compared to 57.4% in 2016.[17] Between 2016 and 2020 the reduction seen in the UK for AmpC/ESBL-producing *E. coli* from retail chicken meat was from 45% to 13%.

Testing of lamb and turkey meat for ESBL- and/or AmpC-producing *E. coli* is not a mandatory part of EU surveys, and there is limited published data for the presence of ESBL- and/or AmpC-producing *E. coli* in lamb and turkey meat. However, testing turkey caecal contents for ESBL- and/or AmpC-producing *E. coli* has been part of EU monitoring as reported by EFSA. The prevalence of presumptive ESBL, AmpC or ESBL+AmpC-producing *E. coli* observed in fattening turkeys in 2016 / 2018 was 42.4% / 39.3%, so similar at both time points.[18]

There appear to be few studies in the literature that have tested for ESBL- and/or AmpC-producing producing *E. coli* in retail turkey meat.

A German study that tested 227 turkey meat samples in 2012-13 determined 40.1% to be positive for cefotaxime-resistant *E. coli* and this was the second highest prevalence (second to chicken meat) of the chicken, turkey, beef and pork meat samples tested.[19] Further analysis showed that 30.5% of turkey samples were positive for mainly $bla_{\text{CTX-M}}$ ESBL genes, but also for bla_{SHV} genes and for the AmpC gene bla_{CMY}.[19] The predominant CTX-M type in this German study for both chicken and turkey meat samples was CTX-M 1, but the CTX-M types from turkey meat were more diverse and also included CTX-M types 2, 3, 14, 15, 24, 32 and 55.[19] There also appears to be few published studies testing lamb caecal contents or meat samples for ESBL, AmpC or ESBL+AmpC-producing *E. coli.* In one study testing caecal samples in Switzerland, 5% of 40 lamb samples collected in 2009-10 were positive for ESBL *E. coli.*[20] A Spanish study reported AmpC/ESBL -resistant *E. coli* from 7% of lamb/sheep flocks surveyed in 2014 to 2016[21] and there is a UK report of ESBL-producing *E. coli* in two of 24 flocks in 2007/8.[22]

Overall, these reports are broadly consistent with the present findings of the presence of AmpC/ESBL type resistance in both meat types, and at a higher prevalence for turkey meat.

In view of the above, this study provides important new information on the presence of ESBL- and/or AmpC-producing and carbapenem-resistant *E. coli* in lamb and turkey retail meat samples in the UK.

In this study, after enrichment, two (0.95%) of the lamb samples and twenty-four (11.4%) of the turkey meat samples were positive on MCA-CTX agar. MICs showed that all of the relevant turkey isolates had an ESBL-phenotype, whilst one lamb isolate had an AmpC-phenotype and the other an ESBL-phenotype. Additionally, MICs against isolates from MCA-CTX showed that none of the isolates were resistant to the ‘last resort’ antibiotics ertapenem, imipenem or meropenem or to colistin.

In lamb meat, the prevalence of AmpC/ESBL phenotype *E. coli* was similar to that observed in the UK in 2015, 2017 and 2019 for beef and pork.[12] Similarly, the results for the turkey meat were aligned with the 2018 results for UK chicken meat.[12]

It was interesting to note that for turkey meat, the samples with the highest counts of background *E. coli* were the turkey mixed other pieces which included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks. In a study using high resolution molecular data the authors found “evidence for the cross-contamination of chicken broiler carcasses with ESBL-producing Enterobacteriaceae during scalding and de-feathering in the slaughterhouse.”[23] The authors suggested that the evidence “clearly shows the need not only for intervention measures on farm level, but also for effective interventions against cross contamination with ESBL-producing Enterobacteriaceae in the slaughterhouse.”[23] Whilst these high counts in the turkey mixed other pieces pre enrichment were not present on the agar containing cefotaxime*,* the results suggest that the extra mechanical processes to produce these pieces and the mixing of samples may lead to higher bacterial contamination.

Campylobacter **on turkey meat**

A study carried out in 2001/2002 across three cities on the island of Ireland[24] found *Campylobacter* in 37.5% of turkey retail samples tested whilst a 2009 survey in Northern Ireland[25] reported 56% of the samples were positive. This survey detected *Campylobacter* in 10.5% of turkey meat samples, which appears lower than previous studies.

These studies used enrichment methods that will enhance the detection of *Campylobacter* in samples, where numbers of *Campylobacter* may be low or stressed. This is likely to be the case with retail meat samples, original contamination levels should be low as they been processed in hygienic conditions and the period (approximately 1 week) between processing and retail will mean that the level of viable *Campylobacter* contamination on the retail meats will have decreased further. This is demonstrated in the quantitative results from this study where the majority (59.1%) of *Campylobacter*-positive turkey samples contained less than 100 cfu/g of *Campylobacter*. A similar finding was made in a survey of retail chicken meats in the UK in 2017[26] that used a similar sampling and test method as the current study. It is also worth noting that the proportion of *Campylobacter* positive chickens in the 2017 study was higher (25.1%) than the 10.5% reported for turkeys in this report. Previous studies have also reported lower prevelance of *Campylobacter* contamination on turkeys relative to chicken,[24, 25] an observation that may indicate that public health related risks associated with *Campylobacter* in turkey meat production are lower than in broiler chicken meat production.

In this survey, the *Campylobacter* prevalence reported is also influenced by predominance of samples tested without skin. The results clearly show that skinless samples are significantly less likely to be positive (0.8%) than samples with skin (26.3%). A recent study of skinless turkey (conventional flocks) meat at retail in Germany reported *Campylobacter* contamination in 19.4% of samples, however this was using enrichment culture.[27] Enrichment methods should be able to detect lower levels of viable *Campylobacter* contamination on a sample than the direct culture method used in this study.

There were only 5 samples of imported turkey meat tested in the current survey from a total of 210 turkey meat samples, suggesting that meat imports were unlikely to have a major influence on the prevalence reported here.

In this survey, *C. jejuni* was the predominant species recovered from 90.9% of *Campylobacter* positive samples, followed by *C. coli* which was detected in 31.8% of positive samples. This finding is similar to observations from broiler chickens at retail in the UK[28] and with a survey of retail turkey meat in Ireland in 2001/2.[24] However it contrasts to a study of retail samples in Poland (2009-13) that reported *C. coli* as the predominant species.[29]

E. coli resistant to carbapenems and *mcr* plasmid-mediated colistin resistance

None of the lamb or turkey samples were positive for carbapenem resistant *E. coli* and none of the lamb samples were positive for *mcr-1* plasmid -mediated colistin resistance *E. coli.*

Three (1.43%) retail turkey samples originating from the UK (n=2) and Germany (n=1) were positive for the *mcr-1* transferable colistin resistance gene post enrichment. This is the first detection of the *mcr* transferable colistin resistance gene in turkey meat on retail sale in the UK. Interestingly in 2020 *mcr-1 E. coli* were isolated for the first time from 0.95% of 315 chicken meat samples in the UK, although the origin of all of these samples was Poland.[15]

It was interesting to note that one sample positive for *mcr-1 E. coli* (T00512003) was from Germany, as previous studies have isolated *mcr-1 E. coli* from samples of turkey meat in Germany and other countries.[6]

In a recent study in the Netherlands, *mcr-1* was detected from 24.8% of 214 retail chicken meat samples, and the presence of Enterobacteriaceae carrying *mcr-1* was confirmed from 34 of the positives.[30] *E. coli* carrying the plasmid-mediated colistin resistance gene have also been reported from retail chicken in other countries such as South Korea[31] and Latin America.[32]

A German study which looked at over 10,600 *E. coli* isolates from the national monitoring on zoonotic agents from the years 2010–2015 for phenotypic colistin resistance found that the highest prevalence of *mcr-1* was detected in the turkey food chain (10.7%), followed by broilers (5.6%).[33] Interestingly, *mcr-1 E. coli* was recovered from samples such as broiler and turkey faeces and turkey meat originating from as early as 2010.[33] These originated from a total of 505 phenotypically colistin-resistant *E. coli* isolates that were part of the German monitoring program on antimicrobial resistance in zoonotic agents.[33] This predates the original detection of *mcr-1* in China in November 2015.[33]

In a more recent study of retail meat samples in Czechia (or Czech Republic) that originated from Czechia (n= 9), Poland (n=19), Hungary (n=8), Germany (n=6), Slovakia (n=4), France (n=4), Austria (n=2), Spain (n=1), Netherlands (n=1), Belgium (n=1), Great Britain (n=1), Brazil (n=8), and China (n=2), bacteria of the Enterobacteriaceae family carrying the *mcr-1* gene were detected in 21% (18/86) of the examined samples, especially in turkey meat and turkey liver (16/24 positive for *mcr-1* or 66.7%) originating from EU and non-EU countries.[6]

These different studies often have different methodology, for example, the above study used selective culture on Brilliance UTI Clarity agar, supplemented with 3.5 mg/L colistin.[6] Differences in methodology are likely to affect observed prevalence / detection rate.

Based on predicted serotype and ST, there was some commonality between *mcr-1* positive *E. coli* from UK retail chicken[15] and turkey meat, and from turkey meat previously determined to be positive for *mcr-1 E. coli* in Brazil, Czechia, Germany and Poland.[6] Also, with respect to plasmid type, there was some commonality between isolates from turkey meat in this study and isolates from poultry, turkey meat and humans in Switzerland.[34]

***Campylobacter*, antimicrobial resistance and MLST**

In the 2018 EU harmonised monitoring survey on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food, 31% of the *C. jejuni* isolated from UK turkey caecal samples collected at slaughter were resistant to ciprofloxacin and 45% were resistant to tetracycline.[35] In this study, we report higher levels of resistance in *C. jejuni* from retail turkey meat samples, as 60% and 65% of samples contained *Campylobacter* resistant to ciprofloxacin and tetracycline respectively.

The number of retail turkey meat samples that were contaminated with *Campylobacter* (25.1%) appeared higher than for retail chicken meat (10.5%) that was sampled in a similar survey in 2017.[26] For the majority of positive meat samples from either survey (turkey or chicken), the levels (quantity) of contamination were relatively low.

The resistance for *C. jejuni* isolated from German flocks in the EU harmonised survey in 2018 was 67% and 50% for ciprofloxacin and tetracycline respectively. A retail survey of German turkey meat over the same year reported 64% of *C. jejuni* isolates were resistant to fluoroquinolones and 39% were resistant to tetracycline.[27] The percentages of *C. jejuni* from UK retail turkey meat resistant to ciprofloxacin (60%) is higher in comparison to UK retail broiler meat (38%).[36] Prevalence of resistance to other antimicrobials tested (tetracycline, gentamicin, streptomycin) was similar for *C. jejuni* recovered from turkey meat or from chicken meat in the earlier survey. Resistance to erythromycin was not observed in *Campylobacter* from turkey meat but did occur in a low number of isolates from chicken meat. The occurrence of resistance in *C. jejuni* from turkey meat in the current study is also comparable to reports by some EU Member States (MS) in 2018.[18]

It is not clear why the prevalence of ciprofloxacin resistance in *C. jejuni* collected at retail (60%) is much higher than for *C. jejuni* collected at the slaughter stage in the UK (31%). However, it is important to note that the isolates are collected at different points in the production chain, which may influence the types of *Campylobacter* recovered and tested for AMR. There is also the potential for cross-contamination from other flocks during the slaughter process.

The use of WGS data with DTU pipelines “MLST” and “ResFinder 4.1” provided insights to complement the phenotypic characterisation of isolates. The ResFinder tool allowed confirmation of phenotypic results in the majority of isolates but the results were not 100% complementary for all isolates. Further work is needed to investigate the reasons behind the anomalies but similar level of discrepancies have been reported elsewhere.[37]

ResFinder added value in the identification of β-lactam resistance in *Campylobacter* isolates via the $bla_{OXA\text{-}61}$ gene and the gene was detected in 70% of *Campylobacter*-positive samples in this study. Investigations of *Campylobacter* in poultry flocks in

Italy have also reported the presence of this β-lactam resistance gene in most flocks.[38]

ResFinder also determined that a minority of quinolone resistant isolates had double mutations in the quinolone resistance determinant region of the *gyrA* gene, (p.T86I and p.104S), this genotypic profile has also been identified as a minority resistant variant in *C. jejuni* isolated from clinical cases in Denmark.[37]

Using the phenotypic MIC assay, no MDR isolates were identified in this study. However, when phenotypic data is supplemented with the genotypic predictions from ResFinder, putative MDR isolates from UK meat samples could be identified for further investigation. An example of this was the *C. jejuni* (ST5136) which was isolated from one (5%) of the *Campylobacter*-positive meat samples (UK produced turkey leg). This ST is considered an emerging lineage of *C. jejuni* that is strongly associated with MDR and with fluoroquinolone resistance.[39] In this survey, the ST5136 isolate was a putative MDR isolate (phenotypic resistance to quinolones and tetracyclines with genotypic resistant determinants for β-lactam antibiotics). The ST5136 isolate did not have resistance to aminoglycosides unlike other ST5136 isolates described.

In another UK turkey leg sample a putative MDR *C. coli* was detected (ST1089/CC828) with phenotypic resistance to quinolones and tetracyclines and genotypic resistance determinants for β-lactam antibiotics. Recent surveys of *Campylobacter* from poultry meat indicate that MDR is more common in *C. coli* than for *C. jejuni*[36, 40]. As relatively few *C. coli* were characterised in this study, it is not possible to establish this trend for UK retail turkey meats in 2020. Further work is needed to establish the prevalence and temporal dynamics of MDR *Campylobacters* in the UK turkey population. In addition, the ResFinder identified a *C. jejuni* isolate (ST2863) that had an exceptional MDR genotypic profile indicating resistance for disinfectant biocides (QAC), sulphonamides, streptomycin (not seen by phenotyping), quinolones and tetracyclines. The occurrence of genes associated with resistance to QAC biocides and sulphonamides suggests the presence of a class 1 intergron which is unusual for *Campylobacter*. Integrons are genetic elements that are linked with the dissemination of antimicrobial resistance, further examination of the WGS data for this isolate is recommended.

Diversity of *Campylobacter* populations present on turkey meat samples at retail was assessed using MLST, and up to three different STs were identified in a single sample. Across all the samples, a range of different STs were recovered. Some of the STs were widespread and detected on multiple turkey meat samples. The individual STs can be placed into groups of related isolates known as a clonal complex. Common clonal complexes can be associated with specific hosts, and the CCs observed in this study included those associated with poultry hosts (e.g., CC573, CC354, CC464) and other CC groups that are generalist in terms of host association (CC21 and CC828).[41] The majority of STs (14/21) recovered in this survey can be associated with infection in humans, inferring the potential risk pathway from turkey meat to public health impact.

The low relative prevalence of contaminated turkey meat compared to broiler chicken meat, the lower levels of turkey meat consumption, and the low prevalence of highly contaminated samples (<1000 cfu/g), suggest that turkey meat consumption is not the main *Campylobacter* exposure risk for humans in the UK.

Resistance to 'critically-important' and / or 'last resort' antibiotics – *Campylobacter*

Fluoroquinolones such as ciprofloxacin and macrolides like erythromycin are considered critically important antibiotics by the World Health Organisation.[42] They can form part of therapeutic treatment for *Campylobacter* infection in humans, although the occurrence of resistant strains can impact on the utility of these therapies.

In the UK, fluoroquinolones are licensed for use in meat producing poultry flocks. There has been a reduction (*ca.* 97%) in the usage of fluoroquinolone in turkey meat production in the UK since 2014, although this antibiotic is still in use (0..08mg per kg meat).[43] Despite the dramatic drops in usage, the levels of resistance to fluoroquinolones have not declined in a similar fashion and remains at 37% resistance for ciprofloxacin in *C. jejuni* isolated from turkey caecal samples collected at slaughter in 2020.[43] This has translated to even higher levels of ciprofloxacin resistance observed in the *Campylobacter* isolates from this study, as 60% of *C. jejuni*-positive samples harboured ciprofloxacin resistance.

Continued monitoring of resistance in *Campylobacter* is recommended in turkey production as an emerging lineage of *C. jejuni* (ST5136) which is strongly associated with MDR and fluoroquinolone resistance[39] was detected in this study. This sequence type has been linked to clinical cases in humans and therefore the use of MLST to characterise *C. jejuni* isolates from harmonised EU monitoring surveys could indicate if this ST and other variants of concern are an increasing resistance issue for turkey meat production.

This study did not identify any resistance to erythromycin in the turkey meat samples. In the UK, the recent EU harmonised monitoring of turkeys identified erythromycin resistance in less than 1% of *C. jejuni* isolates.[35] Therefore in our study of 55 isolates, detection of a resistant strain was unlikely and a more expansive sampling strategy would be required to detect these isolates.

Turkey meat samples initially positive for *mcr-3 E. coli*

The lack of confirmation of the *mcr-3* status of a further five meat samples from purified *E. coli* may be because other lactose fermenters such as *Klebsiella* and *Citrobacter*[6] or non-lactose fermenters carried *mcr-3*. The current protocol used to detect *mcr* focuses on *E. coli* in that lactose fermenters (a “sweep” of ~ 10 to 20 colonies) are as far as is possible selected for the PCR.

Another explanation may be that *mcr-3* positive *E. coli* were outnumbered by isolates that were chromosomally resistant to colistin. Also, previous studies have detected chromosome-mediated *mcr-3* variants in *Aeromonas veronii* from chicken meat.[44] As *Aeromonas* are non-lactose fermenters, if such isolates that were *mcr-3* positive were contaminating the *mcr-3* positive samples in this study, they would have only been detected in the initial “sweep” of multiple colonies, but not when lactose fermenters were purified and subsequently tested.

Traceback of *E. coli mcr-1* positive turkey samples

FSA traceback investigations into the three turkey products positive for *mcr*-1 have been unable to determine the farm origin as batch/lot numbers were not provided for these samples and therefore they cannot be easily traced. Two of the products were stated as UK origin and one was of German origin. All three products were carcase portions (turkey crown or turkey breast) indicating preparation of the carcase after slaughter. Investigations confirmed that, for the product of German origin, no further processing took place in the UK. Although unproven, it is therefore possible that the contamination of this particular product originated in Germany.

Traceback investigations into the five turkey products positive for *mcr*-3 on the initial PCR sweep found that three UK approved premises were involved, although no common links back to a farm origin have been identified. Investigations at the processing plants have confirmed that the processing lines are split by type of process and therefore products produced for different customers are processed on the same lines. It is therefore possible that cross-contamination could occur on these processing lines between turkey meat products sourced from different farms. None of the approved premises supply free range or organic meat products and they only receive UK meat, indicating that contamination is likely to have occurred in the UK.

7. Conclusions

For E. coli

- To the best of our knowledge this is the first time that retail lamb and turkey meat in the UK has been tested for AmpC/ESBL-phenotype *E. coli* and as such gives baseline results for this type of AMR in retail lamb and turkey meat. For lamb and turkey meat samples 0.95% and 11.4% respectively were positive for AmpC/ESBL-phenotype *E. coli*.

- The results for lamb meat are similar to those obtained previously in the UK for retail beef and pork in recent years, whilst the results for turkey are similar to results obtained previously for chicken meat.

- None of the retail lamb and turkey samples were positive for *E. coli* on the two carbapenemase agars. Additionally, none of the AmpC/ESBL-phenotype *E. coli* isolates from MCA-CTX agar were resistant to the 'last resort' carbapenem antibiotics ertapenem, imipenem or meropenem or to colistin.

- A total of three (1.4%) turkey samples originating from the UK (n=2) and Germany (n=1) were positive for the plasmid mediated *mcr-1* transferable colistin resistance gene after enrichment. This is the first time the authors are aware that *mcr-1 E. coli* have been detected from retail turkey meat in the UK. These isolates showed similarity (based on ST and plasmids) to *mcr-1 E. coli* isolated from poultry meat in Europe.

- The predominant CTX M type recovered from retail lamb and turkey meat was CTX-M 15. In humans CTX-M 15 is associated with the pandemic O25-ST131 CTX-M-15-producing clone.[45] Based on results from WGS data, none of the CTX-M 15 positive isolates were ST131, and as such not within the human pandemic clone. Previous UK studies have detect CTX-M 1, 14 (predominant), 15 and 55 *E. coli* from turkey faecal samples,[46] whilst a recent study in Portugal identified CTX-M 1, 55 and SHV 12 as being the predominant ESBL genes in *E. coli* from poultry meat.[47]

- Using the EU method with a detection limit of 3,000 cfu/g, none of the pre-enrichment retail lamb meat samples had counts of background *E. coli* on MCA or to presumptive ESBL/AmpC-producing *E. coli* on MCA-CTX. Turkey samples also did not give rise to counts of presumptive ESBL/AmpC-producing *E. coli* on MCA-CTX, but seven samples pre-enrichment gave rise to viable counts that ranged from 3,100 to 17,100 cfu/gram on MCA without cefotaxime. These samples were all “Turkey mixed other pieces” that all had the UK as their stated country of origin.

For Campylobacter

- The prevalence of *Campylobacter* in turkey meat is low (10.5%), with low numbers of viable counts in the great majority (95% <1000cfu/g) of positive samples.

- Sample type influences the prevalence of *Campylobacter* on turkey meat. Turkey samples that include skin are significantly more likely to be contaminated with detectable levels of *Campylobacter* than skinless turkey meat.

- Resistance to quinolones (ciprofloxacin, nalidixic acid) and tetracyclines was relatively high (*ca.*60%) in *C. jejuni* isolated from turkey meat samples at retail (2020). Continual monitoring is recommended to determine if levels of resistance are stable within the *Campylobacter* population at different points of the turkey meat production system, as the prevalence of resistance in *C. jejuni* recovered from UK turkey flocks at slaughter (caecal samples) indicated lower levels of resistance to ciprofloxacin (31%) and to tetracycline (45%) in 2018.

- All *Campylobacter* isolates were sensitive to erythromycin, gentamicin and streptomycin, however the expected prevalence of resistance to these antimicrobials is low. Expanded monitoring programmes with a greater focus on sampling meats with skin on would be required to assess their prevalence within the turkey production system.

- The application of WGS to isolates recovered from *ad hoc* surveys and on-going harmonised monitoring of the turkey production system provides an opportunity to scan for emerging variants (e.g., ST5136) of *Campylobacter* and to characterise their antimicrobial resistance profiles. This would assist with the AMR risk assessments for the consumption of turkey meat.

Appendix 1 - Details of all 210 lamb samples tested, sorted by date

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L02664396	19/10/2020	Lamb Cubed / Diced or Lamb Stewing	D	Nottingham	United Kingdom
L00462458	19/10/2020	Lamb Frying/Grilling Chops	I	Chorley and West Lancashire	United Kingdom
L00462460	19/10/2020	Lamb Leg Roasting Joint	L	East Lancashire	United Kingdom
L02797994	19/10/2020	Lamb Frying/Grilling Chops	L	Lancaster and Wyre	United Kingdom
L02797992	19/10/2020	Lamb Leg Roasting Joint	K	Blackburn with Darwen	United Kingdom
L02664389	19/10/2020	Lamb Leg Roasting Joint	D	South Nottinghamshire	United Kingdom
L02798003	19/10/2020	Lamb Leg Roasting Joint	D	South and West Derbyshire	United Kingdom
L02798005	19/10/2020	Lamb Leg Roasting Joint	H	East Derbyshire	United Kingdom
L00462459	19/10/2020	Lamb Shoulder Roasting Joint	I	Blackburn with Darwen	United Kingdom
L00462381	20/10/2020	Lamb Frying/Grilling Chops	L	Kent Thames Gateway	United Kingdom
L00462468	20/10/2020	Lamb Cubed / Diced or Lamb Stewing	E	Angus and Dundee City	United Kingdom
L00462412	20/10/2020	Lamb Leg Roasting Joint	C	Perth and Kinross and Stirling	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00462380	20/10/2020	Lamb Leg Roasting Joint	F	Medway	New Zealand
L00462382	20/10/2020	Lamb Frying/Grilling Steak	K	Kent Thames Gateway	United Kingdom
L00462377	20/10/2020	Lamb Shoulder Roasting Joint	I	Mid Kent	United Kingdom
L00462415	20/10/2020	Lamb Shoulder Roasting Joint	D	Clackmannanshire and Fife	United Kingdom
L00462411	20/10/2020	Lamb Frying/Grilling Chops	B	Perth and Kinross and Stirling	United Kingdom
L00462375	20/10/2020	Lamb Leg Roasting Joint	F	East Kent	New Zealand
L02798008	21/10/2020	Lamb Cubed / Diced or Lamb Stewing	A	Merton, Kingston upon Thames and Sutton	United Kingdom
L00462508	21/10/2020	Lamb Leg Roasting Joint	E	Croydon	United Kingdom
L02798013	21/10/2020	Lamb Leg Roasting Joint	L	Merton, Kingston upon Thames and Sutton	United Kingdom
L00462480	21/10/2020	Lamb Leg Roasting Joint	C	Sheffield	United Kingdom
L02798012	21/10/2020	Lamb Frying/Grilling Steak	D	Merton, Kingston upon Thames and Sutton	United Kingdom
L02797990	21/10/2020	Lamb Frying/Grilling Chops	H	North and North East Lincolnshire	United Kingdom
L00462485	21/10/2020	Lamb Frying/Grilling Steak	D	Barnsley, Doncaster and Rotherham	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00462483	21/10/2020	Lamb Frying/Grilling Chops	D	Barnsley, Doncaster and Rotherham	United Kingdom
L00462421	21/10/2020	Lamb Frying/Grilling Steak	D	Inverness, Nairn, Moray, Badenoch, Strathspey	United Kingdom
L00462420	21/10/2020	Lamb Frying/Grilling Chops	H	Aberdeen City and Aberdeenshire	United Kingdom
L00462487	21/10/2020	Lamb Leg Roasting Joint	H	Sheffield	United Kingdom
L00462504	21/10/2020	Lamb Leg Roasting Joint	D	Bromley	United Kingdom
L00462519	26/10/2020	Lamb Frying/Grilling Chops	F	Staffordshire CC	New Zealand
L00462516	26/10/2020	Lamb Leg Roasting Joint	E	Staffordshire CC	United Kingdom
L02797985	26/10/2020	Lamb Leg Roasting Joint	L	Staffordshire CC	United Kingdom
L00462531	26/10/2020	Lamb Leg Roasting Joint	D	Hounslow and Richmond upon Thames	New Zealand
L00462533	26/10/2020	Lamb Frying/Grilling Steak	E	Hounslow and Richmond upon Thames	United Kingdom
L00462535	26/10/2020	Lamb Leg Roasting Joint	F	Ealing	Ireland
L02797986	26/10/2020	Lamb Frying/Grilling Chops	D	Stoke-on-Trent	United Kingdom
L00462518	26/10/2020	Lamb Frying/Grilling Chops	H	Staffordshire CC	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L02797964	29/10/2020	Lamb Cubed / Diced or Lamb Stewing	L	Glasgow City	United Kingdom
L00462552	29/10/2020	Lamb Leg Roasting Joint	D	Glasgow City	United Kingdom
L00462649	29/10/2020	Lamb Frying/Grilling Steak	A	Cardiff and Vale of Glamorgan	United Kingdom
L00462647	29/10/2020	Lamb Leg Roasting Joint	D	Cardiff and Vale of Glamorgan	United Kingdom
L00462646	29/10/2020	Lamb Frying/Grilling Steak	D	Cardiff and Vale of Glamorgan	United Kingdom
L00462648	29/10/2020	Lamb Leg Roasting Joint	C	Cardiff and Vale of Glamorgan	United Kingdom
L02797946	29/10/2020	Lamb Frying/Grilling Chops	H	Monmouthshire and Newport	United Kingdom
L02797980	29/10/2020	Lamb Leg Roasting Joint	L	Gwent Valleys	United Kingdom
L00462449	29/10/2020	Lamb Leg Roasting Joint	H	Wirral	United Kingdom
L00462442	29/10/2020	Lamb Leg Roasting Joint	D	Sefton	New Zealand
L00462443	29/10/2020	Lamb Leg Roasting Joint	D	Liverpool	United Kingdom
L00462564	29/10/2020	Lamb Shoulder Roasting Joint	H	Liverpool	United Kingdom
L00462551	29/10/2020	Lamb Frying/Grilling Steak	H	Glasgow City	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00462644	30/10/2020	Lamb Leg Roasting Joint	L	Gloucestershire	United Kingdom
L00462537	30/10/2020	Lamb Cubed / Diced or Lamb Stewing	L	Gloucestershire	United Kingdom
L00462715	30/10/2020	Lamb Leg Roasting Joint	E	Swindon	United Kingdom
L00462697	30/10/2020	Lamb Leg Roasting Joint	H	Bexley and Greenwich	United Kingdom
L00462711	30/10/2020	Lamb Leg Roasting Joint	C	Wiltshire	United Kingdom
L00462696	30/10/2020	Lamb Frying/Grilling Chops	K	Bexley and Greenwich	United Kingdom
L00462716	30/10/2020	Lamb Leg Roasting Joint	H	Wiltshire	United Kingdom
L02797953	30/10/2020	Lamb Frying/Grilling Chops	L	Barking & Dagenham and Havering	United Kingdom
L02797932	17/11/2020	Lamb Frying/Grilling Chops	D	East Sussex CC	United Kingdom
L00462791	17/11/2020	Lamb Leg Roasting Joint	D	West Kent	New Zealand
L00462792	17/11/2020	Lamb Cubed / Diced or Lamb Stewing	E	West Kent	United Kingdom
L02797933	17/11/2020	Lamb Leg Roasting Joint	D	West Sussex (North East)	United Kingdom
L02797934	17/11/2020	Lamb Cubed / Diced or Lamb Stewing	D	West Sussex (North East)	New Zealand

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00563163	17/11/2020	Lamb Leg Roasting Joint	D	East Sussex CC	United Kingdom
L00462793	17/11/2020	Lamb Frying/Grilling Steak	I	East Sussex CC	United Kingdom
L00563178	17/11/2020	Lamb Leg Roasting Joint	B	Brighton and Hove	United Kingdom
L00512256	17/11/2020	Lamb Leg Roasting Joint	I	Tyneside	United Kingdom
L00512257	17/11/2020	Lamb Leg Roasting Joint	K	Northumberland	United Kingdom
L00512252	17/11/2020	Lamb Frying/Grilling Chops	J	Northumberland	United Kingdom
L00462794	17/11/2020	Lamb Frying/Grilling Steak	A	East Sussex CC	United Kingdom
L00462747	18/11/2020	Lamb Cubed / Diced or Lamb Stewing	D	York	United Kingdom
L00462744	18/11/2020	Lamb Leg Roasting Joint	K	East Riding of Yorkshire	United Kingdom
L00462806	18/11/2020	Lamb Leg Roasting Joint	D	South Lanarkshire	United Kingdom
L02797920	18/11/2020	Lamb Frying/Grilling Chops	L	South Ayrshire	United Kingdom
L02797918	18/11/2020	Lamb Leg Roasting Joint	E	East Ayrshire and North Ayrshire mainland	United Kingdom
L00462750	18/11/2020	Lamb Leg Roasting Joint	E	York	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00462796	18/11/2020	Lamb Leg Roasting Joint	D	Sandwell	New Zealand
L00462797	18/11/2020	Lamb Frying/Grilling Chops	L	Sandwell	United Kingdom
L02797928	18/11/2020	Lamb Frying/Grilling Steak	D	Shropshire CC	New Zealand
L02797929	18/11/2020	Lamb Cubed / Diced or Lamb Stewing	A	Warwickshire	United Kingdom
L00563400	18/11/2020	Lamb Leg Roasting Joint	D	Worcestershire	New Zealand
L02797919	18/11/2020	Lamb Leg Roasting Joint	H	East Ayrshire and North Ayrshire mainland	United Kingdom
L00462743	18/11/2020	Lamb Shoulder Roasting Joint	F	Kingston upon Hull, City of	United Kingdom
L02797966	19/11/2020	Lamb Cubed / Diced or Lamb Stewing	B	Oxfordshire	United Kingdom
L02797925	19/11/2020	Lamb Leg Roasting Joint	K	Devon CC	United Kingdom
L00462801	19/11/2020	Lamb Frying/Grilling Steak	D	Cornwall and Isles of Scilly	New Zealand
L00462546	19/11/2020	Lamb Leg Roasting Joint	D	Oxfordshire	United Kingdom
L00462545	19/11/2020	Lamb Leg Roasting Joint	E	Oxfordshire	United Kingdom
L00462547	19/11/2020	Lamb Frying/Grilling Chops	E	Oxfordshire	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L02797967	19/11/2020	Lamb Frying/Grilling Steak	H	Milton Keynes	United Kingdom
L00462550	19/11/2020	Lamb Leg Roasting Joint	F	Milton Keynes	New Zealand
L02797968	19/11/2020	Lamb Cubed / Diced or Lamb Stewing	D	Oxfordshire	United Kingdom
L02797923	19/11/2020	Lamb Leg Roasting Joint	D	Cornwall and Isles of Scilly	New Zealand
L00512235	23/11/2020	Lamb Cubed / Diced or Lamb Stewing	D	Norwich and East Norfolk	New Zealand
L00462572	23/11/2020	Lamb Frying/Grilling Chops	E	Cheshire East	United Kingdom
L00512199	23/11/2020	Lamb Leg Roasting Joint	I	Enfield	United Kingdom
L02797789	23/11/2020	Lamb Leg Roasting Joint	C	Enfield	United Kingdom
L02797788	23/11/2020	Lamb Leg Roasting Joint	D	Redbridge and Waltham Forest	New Zealand
L00512198	23/11/2020	Lamb Leg Roasting Joint	F	Redbridge and Waltham Forest	New Zealand
L00512200	23/11/2020	Lamb Leg Roasting Joint	E	Haringey and Islington	United Kingdom
L00512219	23/11/2020	Lamb Leg Roasting Joint	D	Norwich and East Norfolk	New Zealand
L00512216	23/11/2020	Lamb Cubed / Diced or Lamb Stewing	L	Norwich and East Norfolk	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00462615	23/11/2020	Lamb Frying/Grilling Chops	H	Cheshire East	United Kingdom
L00512237	23/11/2020	Lamb Shoulder Roasting Joint	E	Breckland and South Norfolk	United Kingdom
L00462570	23/11/2020	Lamb Frying/Grilling Steak	C	Cheshire West and Chester	United Kingdom
L00462571	23/11/2020	Lamb Leg Roasting Joint	F	Cheshire West and Chester	New Zealand
L00462616	23/11/2020	Lamb Frying/Grilling Chops	E	Warrington	United Kingdom
L02797776	23/11/2020	Lamb Frying/Grilling Chops	D	East Merseyside	United Kingdom
L00462617	23/11/2020	Lamb Shoulder Roasting Joint	F	Warrington	United Kingdom
L00462548	24/11/2020	Lamb Leg Roasting Joint	I	West Surrey	United Kingdom
L00462433	24/11/2020	Lamb Shoulder Roasting Joint	D	West Surrey	United Kingdom
L00560886	24/11/2020	Lamb Frying/Grilling Chops	E	West Surrey	United Kingdom
L00462434	24/11/2020	Lamb Leg Roasting Joint	E	West Surrey	United Kingdom
L00512191	24/11/2020	Lamb Leg Roasting Joint	D	Greater Manchester North West	New Zealand
L00512192	24/11/2020	Lamb Frying/Grilling Steak	D	Greater Manchester North West	New Zealand

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00512190	24/11/2020	Lamb Frying/Grilling Steak	E	Greater Manchester North West	United Kingdom
L00512193	24/11/2020	Lamb Leg Roasting Joint	H	Greater Manchester South West	United Kingdom
L00462435	24/11/2020	Lamb Frying/Grilling Steak	E	West Surrey	United Kingdom
L02797786	27/11/2020	Lamb Frying/Grilling Chops	F	Gwynedd	New Zealand
L00462370	27/11/2020	Lamb Frying/Grilling Steak	H	Buckinghamshire CC	United Kingdom
L00563189	27/11/2020	Lamb Cubed / Diced or Lamb Stewing	L	Flintshire and Wrexham	United Kingdom
L00563190	27/11/2020	Lamb Shoulder Roasting Joint	L	Gwynedd	United Kingdom
L00462689	27/11/2020	Lamb Shoulder Roasting Joint	D	East Surrey	United Kingdom
L00561079	27/11/2020	Lamb Leg Roasting Joint	F	East Surrey	New Zealand
L00462690	27/11/2020	Lamb Shoulder Roasting Joint	E	Buckinghamshire CC	United Kingdom
L02797759	27/11/2020	Lamb Leg Roasting Joint	D	Leicestershire CC and Rutland	New Zealand
L00462653	27/11/2020	Lamb Frying/Grilling Steak	E	Leicestershire CC and Rutland	United Kingdom
L02797798	27/11/2020	Lamb Leg Roasting Joint	E	Leicestershire CC and Rutland	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L02797760	27/11/2020	Lamb Cubed / Diced or Lamb Stewing	L	Leicestershire CC and Rutland	United Kingdom
L00462629	27/11/2020	Lamb Leg Roasting Joint	I	Leicester	United Kingdom
L00462652	27/11/2020	Lamb Leg Roasting Joint	L	Leicester	United Kingdom
L02797796	27/11/2020	Lamb Frying/Grilling Chops	L	South and West Derbyshire	United Kingdom
L00560931	27/11/2020	Lamb Shoulder Roasting Joint	L	Buckinghamshire CC	United Kingdom
L02797868	09/12/2020	Lamb Leg Roasting Joint	C	North Northamptonshire	United Kingdom
L00512152	09/12/2020	Lamb Frying/Grilling Steak	D	West Northamptonshire	United Kingdom
L02672450	09/12/2020	Lamb Frying/Grilling Steak	B	West Northamptonshire	United Kingdom
L02797867	09/12/2020	Lamb Frying/Grilling Chops	C	North Northamptonshire	United Kingdom
L00512178	10/12/2020	Lamb Leg Roasting Joint	F	Hackney and Newham	New Zealand
L00512119	11/12/2020	Lamb Frying/Grilling Steak	B	North Hampshire	United Kingdom
L00512120	11/12/2020	Lamb Frying/Grilling Chops	B	North Hampshire	United Kingdom
L00462774	11/12/2020	Lamb Leg Roasting Joint	I	Bath, N & NE Somerset, South Gloucestershire	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00512121	11/12/2020	Lamb Frying/Grilling Chops	C	South Hampshire	United Kingdom
L02797785	11/12/2020	Lamb Frying/Grilling Steak	D	Bristol, City of	United Kingdom
L00512071	14/12/2020	Lamb Leg Roasting Joint	C	Birmingham	United Kingdom
L00512066	14/12/2020	Lamb Leg Roasting Joint	L	Dudley	United Kingdom
L02664360	15/12/2020	Lamb Frying/Grilling Chops	F	Greater Manchester North East	Australia
L00462764	15/12/2020	Lamb Shoulder Roasting Joint	B	Cambridgeshire CC	United Kingdom
L00512039	15/12/2020	Lamb Leg Roasting Joint	I	Berkshire	United Kingdom
L00512048	16/12/2020	Lamb Leg Roasting Joint	I	Calderdale and Kirklees	United Kingdom
L00512052	16/12/2020	Lamb Cubed / Diced or Lamb Stewing	F	Wandsworth	United Kingdom
L02797767	16/12/2020	Lamb Frying/Grilling Chops	H	Manchester	United Kingdom
L00512002	04/01/2021	Lamb Leg Roasting Joint	D	South West Wales	United Kingdom
L00512005	04/01/2021	Lamb Shoulder Roasting Joint	H	Bridgend and Neath Port Talbot	United Kingdom
L00512004	04/01/2021	Lamb Frying/Grilling Chops	H	Bridgend and Neath Port Talbot	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00512035	04/01/2021	Lamb Leg Roasting Joint	E	Devon CC	United Kingdom
L02797924	04/01/2021	Lamb Leg Roasting Joint	H	Devon CC	United Kingdom
L00512038	04/01/2021	Lamb Shoulder Roasting Joint	I	Plymouth	United Kingdom
L02797784	05/01/2021	Lamb Frying/Grilling Steak	A	Warwickshire	United Kingdom
L00512021	05/01/2021	Lamb Leg Roasting Joint	E	Solihull	United Kingdom
L00512022	05/01/2021	Lamb Shoulder Roasting Joint	E	Coventry	United Kingdom
L00512040	05/01/2021	Lamb Cubed / Diced or Lamb Stewing	D	Solihull	United Kingdom
L00512041	05/01/2021	Lamb Leg Roasting Joint	H	Warwickshire	United Kingdom
L02797830	05/01/2021	Lamb Shoulder Roasting Joint	A	Warwickshire	United Kingdom
L00511998	06/01/2021	Lamb Leg Roasting Joint	E	Portsmouth	New Zealand
L00512029	06/01/2021	Lamb Shoulder Roasting Joint	E	Dorset CC	United Kingdom
L00512030	06/01/2021	Lamb Leg Roasting Joint	E	Dorset CC	United Kingdom
L00511982	06/01/2021	Lamb Frying/Grilling Chops	B	Dorset CC	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00511999	06/01/2021	Lamb Leg Roasting Joint	C	Portsmouth	United Kingdom
L00511996	06/01/2021	Lamb Leg Roasting Joint	D	West Sussex (South West)	United Kingdom
L00511995	06/01/2021	Lamb Shoulder Roasting Joint	D	West Sussex (South West)	United Kingdom
L00511997	06/01/2021	Lamb Cubed / Diced or Lamb Stewing	E	West Sussex (South West)	United Kingdom
L00511985	06/01/2021	Lamb Leg Roasting Joint	H	Somerset	United Kingdom
L00511970	18/01/2021	Lamb Shoulder Roasting Joint	F	Southampton	United Kingdom
L00512027	18/01/2021	Lamb Shoulder Roasting Joint	H	Durham CC	United Kingdom
L00511968	18/01/2021	Lamb Leg Roasting Joint	B	Isle of Wight	United Kingdom
L00511969	18/01/2021	Lamb Leg Roasting Joint	D	Southampton	United Kingdom
L00511976	18/01/2021	Lamb Leg Roasting Joint	E	Sunderland	United Kingdom
L00511977	18/01/2021	Lamb Frying/Grilling Steak	I	Sunderland	New Zealand
L00512025	18/01/2021	Lamb Frying/Grilling Steak	F	Durham CC	Australia
L00512063	18/01/2021	Lamb Leg Roasting Joint	L	Hertfordshire	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00512062	18/01/2021	Lamb Leg Roasting Joint	F	Hertfordshire	New Zealand
L02797851	18/01/2021	Lamb Frying/Grilling Chops	F	Hertfordshire	New Zealand
L00462766	18/01/2021	Lamb Leg Roasting Joint	F	Hertfordshire	New Zealand
L00511981	18/01/2021	Lamb Frying/Grilling Chops	L	Hartlepool and Stockton-on-Tees	United Kingdom
L00511978	18/01/2021	Lamb Leg Roasting Joint	E	Hartlepool and Stockton-on-Tees	New Zealand
L00511967	18/01/2021	Lamb Shoulder Roasting Joint	F	Isle of Wight	United Kingdom
L00512028	18/01/2021	Lamb Frying/Grilling Chops	I	Durham CC	United Kingdom
L00512091	21/01/2021	Lamb Frying/Grilling Chops	A	Tyneside	United Kingdom
L00540768	21/01/2021	Lamb Frying/Grilling Steak	G	Causeway Coast and Glens	United Kingdom
L00540767	21/01/2021	Lamb Leg Roasting Joint	F	Causeway Coast and Glens	New Zealand
L00364558	21/01/2021	Lamb Leg Roasting Joint	E	Newry, Mourne and Down	United Kingdom
L00364559	21/01/2021	Lamb Frying/Grilling Steak	E	Newry, Mourne and Down	United Kingdom
L00512090	21/01/2021	Lamb Cubed / Diced or Lamb Stewing	C	West Cumbria	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L02797935	21/01/2021	Lamb Leg Roasting Joint	F	Lincolnshire	New Zealand
L02797858	21/01/2021	Lamb Leg Roasting Joint	I	North Nottinghamshire	United Kingdom
L02797860	21/01/2021	Lamb Frying/Grilling Steak	F	North Nottinghamshire	Australia
L00462818	21/01/2021	Lamb Frying/Grilling Chops	L	Tyneside	United Kingdom
L00462819	21/01/2021	Lamb Cubed / Diced or Lamb Stewing	L	Tyneside	United Kingdom
L02797804	21/01/2021	Lamb Frying/Grilling Steak	H	East Cumbria	United Kingdom
L00512231	21/01/2021	Lamb Frying/Grilling Chops	D	Tyneside	United Kingdom
L00462685	22/01/2021	Lamb Cubed / Diced or Lamb Stewing	E	Luton	United Kingdom
L00511993	22/01/2021	Lamb Frying/Grilling Steak	I	Bedford	New Zealand
L00462686	22/01/2021	Lamb Cubed / Diced or Lamb Stewing	E	Luton	United Kingdom
L00511994	22/01/2021	Lamb Cubed / Diced or Lamb Stewing	D	Central Bedfordshire	United Kingdom
L00511945	08/02/2021	Lamb Frying/Grilling Chops	A	Essex Haven Gateway	United Kingdom
L00511946	08/02/2021	Lamb Shoulder Roasting Joint	C	Suffolk	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category	Retailer code	Location Name	Country of Origin
L00511941	08/02/2021	Lamb Cubed / Diced or Lamb Stewing	L	Suffolk	Ireland
L00511965	08/02/2021	Lamb Cubed / Diced or Lamb Stewing	L	Lewisham and Southwark	United Kingdom
L00511962	08/02/2021	Lamb Shoulder Roasting Joint	E	Lambeth	New Zealand

Appendix 2 - Details of all 210 turkey samples tested, sorted by date

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T02798004	19/10/2020	Turkey Mixed Other Pieces	C	East Derbyshire	United Kingdom
T02664390	19/10/2020	Turkey Mixed Other Pieces	I	South and West Derbyshire	United Kingdom
T02664388	19/10/2020	Turkey Mixed Other Pieces	E	Nottingham	United Kingdom
T02664387	19/10/2020	Turkey Breast	F	South Nottinghamshire	United Kingdom
T02797993	19/10/2020	Turkey Mixed Other Pieces	F	Lancaster and Wyre	United Kingdom
T02797995	19/10/2020	Turkey Mixed Other Pieces	D	Mid Lancashire	United Kingdom
T02797996	19/10/2020	Turkey Mixed Other Pieces	I	East Lancashire	United Kingdom
T00462376	20/10/2020	Turkey Mixed Other Pieces	D	Mid Kent	United Kingdom
T00462378	20/10/2020	Turkey Mixed Other Pieces	F	East Kent	United Kingdom
T00462379	20/10/2020	Turkey Mixed Other Pieces	D	Medway	United Kingdom
T00462413	20/10/2020	Turkey Mixed Other Pieces	D	Perth and Kinross and Stirling	United Kingdom
T00462414	20/10/2020	Turkey Mixed Other Pieces	F	Clackmannanshire and Fife	United Kingdom
T02797991	21/10/2020	Turkey Mixed Other Pieces	H	North and North East Lincolnshire	United Kingdom
T00462422	21/10/2020	Turkey Mixed Other Pieces	I	Inverness, Nairn, Moray, Badenoch, Strathspey	United Kingdom
T00462419	21/10/2020	Turkey Mixed Other Pieces	C	Aberdeen City and Aberdeenshire	United Kingdom
T02797988	21/10/2020	Turkey Mixed Other Pieces	H	Sheffield	United Kingdom
T02797989	21/10/2020	Turkey Breast	F	Barnsley, Doncaster and Rotherham	United Kingdom
T00462507	21/10/2020	Turkey Mixed Other Pieces	E	Merton, Kingston upon Thames and Sutton	United Kingdom
T00462506	21/10/2020	Turkey Mixed Other Pieces	E	Croydon	United Kingdom
T00462505	21/10/2020	Turkey Mixed Other Pieces	F	Merton, Kingston upon Thames and Sutton	United Kingdom
T00462481	21/10/2020	Turkey Mixed Other Pieces	E	Barnsley, Doncaster and Rotherham	United Kingdom
T00462534	26/10/2020	Turkey Mixed Other Pieces	B	Hounslow and Richmond upon Thames	United Kingdom
T02797987	26/10/2020	Turkey Mixed Other Pieces	K	Stoke-on-Trent	United Kingdom
T02797984	26/10/2020	Turkey Mixed Other Pieces	F	Staffordshire CC	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T00462536	26/10/2020	Turkey Mixed Other Pieces	D	Ealing	United Kingdom
T00462517	26/10/2020	Turkey Leg	H	Staffordshire CC	United Kingdom
T00462532	26/10/2020	Turkey Mixed Other Pieces	D	Hounslow and Richmond upon Thames	United Kingdom
T00462553	29/10/2020	Turkey Breast	F	Glasgow City	United Kingdom
T00462444	29/10/2020	Turkey Mixed Other Pieces	C	Liverpool	United Kingdom
T00462561	29/10/2020	Turkey Mixed Other Pieces	H	Liverpool	United Kingdom
T00462441	29/10/2020	Turkey Mixed Other Pieces	C	Sefton	United Kingdom
T00462450	29/10/2020	Turkey Mixed Other Pieces	E	Wirral	United Kingdom
T00462650	29/10/2020	Turkey Mixed Other Pieces	C	Gwent Valleys	United Kingdom
T02797981	29/10/2020	Turkey Mixed Other Pieces	C	Monmouthshire and Newport	United Kingdom
T00462643	30/10/2020	Turkey Breast	F	Swindon	United Kingdom
T00462538	30/10/2020	Turkey Mixed Other Pieces	C	Gloucestershire	United Kingdom
T02797954	30/10/2020	Turkey Mixed Other Pieces	I	Bexley and Greenwich	United Kingdom
T02797955	30/10/2020	Turkey Mixed Other Pieces	C	Bexley and Greenwich	United Kingdom
T00462695	30/10/2020	Turkey Mixed Other Pieces	E	Barking & Dagenham and Havering	United Kingdom
T00462642	30/10/2020	Turkey Mixed Other Pieces	E	Gloucestershire	United Kingdom
T02797855	17/11/2020	Turkey Mixed Other Pieces	F	Tyneside	United Kingdom
T02797930	17/11/2020	Turkey Mixed Other Pieces	C	Brighton and Hove	United Kingdom
T02797927	18/11/2020	Turkey Mixed Other Pieces	B	Shropshire CC	United Kingdom
T00462751	18/11/2020	Turkey Mixed Other Pieces	I	North Yorkshire CC	United Kingdom
T00462748	18/11/2020	Turkey Mixed Other Pieces	F	North Yorkshire CC	United Kingdom
T00462749	18/11/2020	Turkey Mixed Other Pieces	D	York	United Kingdom
T00462745	18/11/2020	Turkey Mixed Other Pieces	F	East Riding of Yorkshire	United Kingdom
T00462752	18/11/2020	Turkey Mixed Other Pieces	F	Kingston upon Hull, City of	United Kingdom
T00462798	18/11/2020	Turkey Breast	L	Walsall	Italy
T00462805	18/11/2020	Turkey Crown joint	H	South Lanarkshire	United Kingdom
T00462804	18/11/2020	Turkey Mixed Other Pieces	C	South Ayrshire	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T02797926	18/11/2020	Turkey Breast	H	Walsall	United Kingdom
T00462746	18/11/2020	Turkey Mixed Other Pieces	D	North Yorkshire CC	United Kingdom
T00462549	19/11/2020	Turkey Breast	F	Milton Keynes	United Kingdom
T02797761	19/11/2020	Turkey Mixed Other Pieces	I	Cornwall and Isles of Scilly	United Kingdom
T00462802	19/11/2020	Turkey Crown joint	H	Cornwall and Isles of Scilly	United Kingdom
T00512197	23/11/2020	Turkey Mixed Other Pieces	D	North and West Norfolk	United Kingdom
T02797777	23/11/2020	Turkey Breast	F	Warrington	United Kingdom
T02797790	23/11/2020	Turkey Leg	E	Redbridge and Waltham Forest	United Kingdom
T02797787	23/11/2020	Turkey Mixed Other Pieces	I	Redbridge and Waltham Forest	United Kingdom
T00512196	23/11/2020	Turkey Breast	H	Breckland and South Norfolk	United Kingdom
T00512195	23/11/2020	Turkey Mixed Other Pieces	C	North and West Norfolk	United Kingdom
T00462760	23/11/2020	Turkey Crown joint	H	Norwich and East Norfolk	United Kingdom
T00512201	23/11/2020	Turkey Leg	E	Haringey and Islington	United Kingdom
T00540737	24/11/2020	Turkey Mixed Other Pieces	F	West Surrey	United Kingdom
T02797778	24/11/2020	Turkey Leg	H	Greater Manchester South West	United Kingdom
T02797775	24/11/2020	Turkey Mixed Other Pieces	F	Greater Manchester North West	United Kingdom
T00512172	27/11/2020	Turkey Breast	F	Flintshire and Wrexham	United Kingdom
T00462369	27/11/2020	Turkey Breast	F	Buckinghamshire CC	United Kingdom
T02797797	27/11/2020	Turkey Mixed Other Pieces	C	South and West Derbyshire	United Kingdom
T00512153	09/12/2020	Turkey Crown joint	C	North Northamptonshire	United Kingdom
T00512247	09/12/2020	Turkey Crown joint	C	Leeds	United Kingdom
T00512099	09/12/2020	Turkey Mixed Other Pieces	D	Leeds	United Kingdom
T00512100	09/12/2020	Turkey Mixed Other Pieces	H	Leeds	United Kingdom
T00462809	09/12/2020	Turkey Mixed Other Pieces	H	Leeds	United Kingdom
T02672449	09/12/2020	Turkey Leg	E	West Northamptonshire	United Kingdom
T00512151	09/12/2020	Turkey Crown joint	C	North Northamptonshire	United Kingdom
T00512098	09/12/2020	Turkey Breast	L	Leeds	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T00512097	09/12/2020	Turkey Breast	L	Leeds	United Kingdom
T00512126	09/12/2020	Turkey Breast	F	Essex Haven Gateway	United Kingdom
T02797779	09/12/2020	Turkey Leg	E	Essex Thames Gateway	United Kingdom
T00512217	09/12/2020	Turkey Mixed Other Pieces	F	Essex Thames Gateway	United Kingdom
T02797794	09/12/2020	Turkey Mixed Other Pieces	B	Essex Haven Gateway	United Kingdom
T02797782	09/12/2020	Turkey Mixed Other Pieces	I	West Essex	United Kingdom
T00462755	09/12/2020	Turkey Crown joint	E	West Essex	United Kingdom
T02797792	09/12/2020	Turkey Breast	L	Heart of Essex	United Kingdom
T02797780	09/12/2020	Turkey Mixed Other Pieces	D	Heart of Essex	United Kingdom
T02797781	09/12/2020	Turkey Mixed Other Pieces	D	Essex Haven Gateway	United Kingdom
T00512248	09/12/2020	Turkey Mixed Other Pieces	D	Bradford	United Kingdom
T02797756	09/12/2020	Turkey Mixed Other Pieces	D	West Northamptonshire	United Kingdom
T00512133	09/12/2020	Turkey Crown joint	L	Leeds	United Kingdom
T00512110	10/12/2020	Turkey Mixed Other Pieces	F	East Lothian and Midlothian	United Kingdom
T00512109	10/12/2020	Turkey Crown joint	H	East Lothian and Midlothian	United Kingdom
T00512113	10/12/2020	Turkey Mixed Other Pieces	I	City of Edinburgh	United Kingdom
T00512114	10/12/2020	Turkey Crown joint	K	City of Edinburgh	United Kingdom
T00512111	10/12/2020	Turkey Leg	E	City of Edinburgh	United Kingdom
T00512107	10/12/2020	Turkey Breast	K	West Lothian	United Kingdom
T00512177	10/12/2020	Turkey Leg	E	Tower Hamlets	United Kingdom
T00512108	10/12/2020	Turkey Crown joint	K	West Lothian	United Kingdom
T00512144	10/12/2020	Turkey Crown joint	C	Lewisham and Southwark	United Kingdom
T00512176	10/12/2020	Turkey Mixed Other Pieces	D	Hackney and Newham	United Kingdom
T00512189	10/12/2020	Turkey Whole Bird	L	Hackney and Newham	United Kingdom
T00512179	10/12/2020	Turkey Mixed Other Pieces	F	Hackney and Newham	United Kingdom
T00512187	10/12/2020	Turkey Mixed Other Pieces	E	Tower Hamlets	United Kingdom
T00512188	10/12/2020	Turkey Leg	E	Tower Hamlets	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T00512123	11/12/2020	Turkey Mixed Other Pieces	D	Central Hampshire	United Kingdom
T00462769	11/12/2020	Turkey Crown joint	E	Bath, N & NE Somerset, South Gloucestershire	United Kingdom
T00462771	11/12/2020	Turkey Leg	E	Bath, N & NE Somerset, South Gloucestershire	United Kingdom
T00462770	11/12/2020	Turkey Whole Bird	L	Bath, N & NE Somerset, South Gloucestershire	United Kingdom
T00512122	11/12/2020	Turkey Mixed Other Pieces	D	Central Hampshire	United Kingdom
T00512117	11/12/2020	Turkey Mixed Other Pieces	H	Central Hampshire	United Kingdom
T00512125	11/12/2020	Turkey Mixed Other Pieces	D	South Hampshire	United Kingdom
T00512115	11/12/2020	Turkey Crown joint	E	South Hampshire	United Kingdom
T00512116	11/12/2020	Turkey Leg	E	South Hampshire	United Kingdom
T00512124	11/12/2020	Turkey Mixed Other Pieces	D	Central Hampshire	United Kingdom
T00462772	11/12/2020	Turkey Whole Bird	B	Bristol, City of	United Kingdom
T02797914	14/12/2020	Turkey Mixed Other Pieces	D	Belfast	United Kingdom
T00512070	14/12/2020	Turkey Mixed Other Pieces	C	Birmingham	United Kingdom
T00512072	14/12/2020	Turkey Mixed Other Pieces	I	Birmingham	United Kingdom
T00512073	14/12/2020	Turkey Mixed Other Pieces	E	Birmingham	United Kingdom
T00512068	14/12/2020	Turkey Crown joint	E	Birmingham	United Kingdom
T00512069	14/12/2020	Turkey Crown joint	E	Birmingham	United Kingdom
T00462808	14/12/2020	Turkey Crown joint	E	Belfast	United Kingdom
T00462810	14/12/2020	Turkey Crown joint	E	Mid Ulster	United Kingdom
T00462807	14/12/2020	Turkey Breast	I	Mid Ulster	Ireland
T00512076	14/12/2020	Turkey Mixed Other Pieces	D	Harrow and Hillingdon	United Kingdom
T00512059	14/12/2020	Turkey Crown joint	L	Camden and City of London	United Kingdom
T00512075	14/12/2020	Turkey Crown joint	D	Harrow and Hillingdon	United Kingdom
T00512057	14/12/2020	Turkey Crown joint	E	Camden and City of London	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T00512058	14/12/2020	Turkey Crown joint	E	Westminster	United Kingdom
T00512056	14/12/2020	Turkey Mixed Other Pieces	D	Westminster	United Kingdom
T00512078	14/12/2020	Turkey Mixed Other Pieces	H	Barnet	United Kingdom
T00512077	14/12/2020	Turkey Crown joint	H	Barnet	United Kingdom
T00512074	14/12/2020	Turkey Crown joint	D	Brent	United Kingdom
T00512079	14/12/2020	Turkey Mixed Other Pieces	K	Harrow and Hillingdon	United Kingdom
T00512067	14/12/2020	Turkey Crown joint	E	Dudley	United Kingdom
T02797793	15/12/2020	Turkey Mixed Other Pieces	D	Cambridgeshire CC	United Kingdom
T00462447	15/12/2020	Turkey Crown joint	E	Mid Lancashire	United Kingdom
T00462773	15/12/2020	Turkey Mixed Other Pieces	E	Berkshire	United Kingdom
T00512042	15/12/2020	Turkey Mixed Other Pieces	I	Berkshire	United Kingdom
T00512044	15/12/2020	Turkey Mixed Other Pieces	I	Berkshire	United Kingdom
T00512043	15/12/2020	Turkey Crown joint	L	Berkshire	United Kingdom
T02797899	15/12/2020	Turkey Mixed Other Pieces	C	Berkshire	United Kingdom
T00462448	15/12/2020	Turkey Whole Bird	L	Chorley and West Lancashire	United Kingdom
T00462446	15/12/2020	Turkey Mixed Other Pieces	C	Greater Manchester North East	United Kingdom
T00512130	15/12/2020	Turkey Crown joint	D	Cambridgeshire CC	United Kingdom
T00512129	15/12/2020	Turkey Crown joint	K	Cambridgeshire CC	United Kingdom
T00512128	15/12/2020	Turkey Crown joint	E	Peterborough	United Kingdom
T00512218	15/12/2020	Turkey Mixed Other Pieces	C	Peterborough	United Kingdom
T02664627	15/12/2020	Turkey Mixed Other Pieces	C	Greater Manchester North East	United Kingdom
T00512053	16/12/2020	Turkey Mixed Other Pieces	I	Wandsworth	United Kingdom
T00512049	16/12/2020	Turkey Crown joint	H	Wakefield	United Kingdom
T00512105	16/12/2020	Turkey Mixed Other Pieces	D	Falkirk	United Kingdom
T00462464	16/12/2020	Turkey Mixed Other Pieces	F	City of Edinburgh	United Kingdom
T00462463	16/12/2020	Turkey Breast	H	Falkirk	United Kingdom
T00512102	16/12/2020	Turkey Crown joint	E	Glasgow City	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T00512104	16/12/2020	Turkey Crown joint	L	North Lanarkshire	Italy
T00512103	16/12/2020	Turkey Crown joint	E	Inverclyde, East Renfrewshire, Renfrewshire	United Kingdom
T00512101	16/12/2020	Turkey Crown joint	L	Inverclyde, East Renfrewshire, Renfrewshire	United Kingdom
T02797766	16/12/2020	Turkey Whole Bird	K	Manchester	United Kingdom
T00512054	16/12/2020	Turkey Crown joint	E	Kensington & Chelsea, Hammersmith & Fulham	United Kingdom
T00512055	16/12/2020	Turkey Breast	F	Wandsworth	United Kingdom
T00512222	16/12/2020	Turkey Crown joint	H	Greater Manchester North East	United Kingdom
T00512174	16/12/2020	Turkey Breast	F	Greater Manchester South East	United Kingdom
T00512051	16/12/2020	Turkey Breast	F	Sheffield	United Kingdom
T00512046	16/12/2020	Turkey Mixed Other Pieces	C	Calderdale and Kirklees	United Kingdom
T00512045	16/12/2020	Turkey Breast	F	Calderdale and Kirklees	United Kingdom
T00512047	16/12/2020	Turkey Mixed Other Pieces	I	Calderdale and Kirklees	United Kingdom
T00512050	16/12/2020	Turkey Mixed Other Pieces	C	Wakefield	United Kingdom
T00512175	16/12/2020	Turkey Crown joint	H	Greater Manchester South East	United Kingdom
T00512003	04/01/2021	Turkey Breast	L	South West Wales	Germany
T00512034	04/01/2021	Turkey Mixed Other Pieces	H	Devon CC	United Kingdom
T00512032	04/01/2021	Turkey Mixed Other Pieces	D	Devon CC	United Kingdom
T00462803	04/01/2021	Turkey Leg	H	Plymouth	United Kingdom
T00512037	04/01/2021	Turkey Breast	L	Torbay	United Kingdom
T00512000	04/01/2021	Turkey Mixed Other Pieces	F	South West Wales	United Kingdom
T00512036	04/01/2021	Turkey Mixed Other Pieces	I	Torbay	United Kingdom
T00512001	04/01/2021	Turkey Breast	F	South West Wales	United Kingdom
T00512033	04/01/2021	Turkey Mixed Other Pieces	D	Devon CC	United Kingdom
T02797829	05/01/2021	Turkey Mixed Other Pieces	D	Warwickshire	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T02797753	05/01/2021	Turkey Mixed Other Pieces	D	Coventry	United Kingdom
T00511990	06/01/2021	Turkey Mixed Other Pieces	D	West Sussex (South West)	United Kingdom
T00512031	06/01/2021	Turkey Mixed Other Pieces	C	Bournemouth and Poole	United Kingdom
T00511984	06/01/2021	Turkey Mixed Other Pieces	I	Somerset	United Kingdom
T00511983	06/01/2021	Turkey Mixed Other Pieces	H	Dorset CC	United Kingdom
T00512173	06/01/2021	Turkey Mixed Other Pieces	I	Bournemouth and Poole	United Kingdom
T00462765	18/01/2021	Turkey Breast	H	Hertfordshire	United Kingdom
T00462767	18/01/2021	Turkey Leg	H	Hertfordshire	United Kingdom
T00512020	18/01/2021	Turkey Mixed Other Pieces	B	Hertfordshire	United Kingdom
T00512026	18/01/2021	Turkey Mixed Other Pieces	C	Durham CC	United Kingdom
T02797852	18/01/2021	Turkey Mixed Other Pieces	C	Hertfordshire	United Kingdom
T02797937	21/01/2021	Turkey Mixed Other Pieces	I	Lincolnshire	United Kingdom
T02797857	21/01/2021	Turkey Breast	F	Lincolnshire	United Kingdom
T00512181	21/01/2021	Turkey Mixed Other Pieces	H	Lincolnshire	United Kingdom
T02797936	21/01/2021	Turkey Breast	H	Lincolnshire	United Kingdom
T02797938	21/01/2021	Turkey Mixed Other Pieces	D	Lincolnshire	United Kingdom
T00512092	21/01/2021	Turkey Mixed Other Pieces	C	West Cumbria	United Kingdom
T00512232	21/01/2021	Turkey Mixed Other Pieces	D	East Cumbria	United Kingdom
T02797808	21/01/2021	Turkey Mixed Other Pieces	C	North Nottinghamshire	United Kingdom
T02797859	21/01/2021	Turkey Mixed Other Pieces	I	North Nottinghamshire	United Kingdom
T00511991	22/01/2021	Turkey Mixed Other Pieces	D	Central Bedfordshire	United Kingdom
T00511992	22/01/2021	Turkey Mixed Other Pieces	D	Bedford	United Kingdom
T00511947	08/02/2021	Turkey Mixed Other Pieces	E	Harrow and Hillingdon	United Kingdom
T00511960	08/02/2021	Turkey Leg	H	Lewisham and Southwark	United Kingdom
T00511961	08/02/2021	Turkey Mixed Other Pieces	D	Lewisham and Southwark	United Kingdom
T00511964	08/02/2021	Turkey Mixed Other Pieces	B	Lambeth	United Kingdom
T00511948	08/02/2021	Turkey Mixed Other Pieces	K	Brent	United Kingdom

Sample Number	Scheduled Sampling Date	Food Category †	Retailer code	Location Name	Country of Origin
T00511963	08/02/2021	Turkey Mixed Other Pieces	K	Lewisham and Southwark	United Kingdom
T00511943	08/02/2021	Turkey Mixed Other Pieces	E	Suffolk	United Kingdom
T00511944	08/02/2021	Turkey Mixed Other Pieces	D	Suffolk	United Kingdom

† Turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks.

Appendix 3 – Further molecular characterisation of mcr-1 plasmids

Background

This report contains results for the detection of and WGS analysis for *mcr-1 E. coli* from three turkey meat samples. At the request of the FSA, long read sequencing was performed on *mcr-1 E. coli* from turkey meat to resolve the plasmid type and the additional results are included here.

In order that results from turkey meat can be considered in the wider context of *mcr-1 E. coli* from poultry, the analysis also includes results for *mcr-1 E. coli* from 2020 chicken meat samples that were also tested for the FSA.

Results and discussion

Long read sequencing was performed on selected isolates, and hybrid assemblies produced from long- and short-read data was used to resolve the *mcr* bearing plasmid genome for further characterisation.

In the UK, presence of *mcr-1* harbouring *E. coli* has previously only been reported from pig caeca or faeces, where the *mcr-1* gene was present in plasmids of the following Inc-types: X4, pO111, I2 and HI2.[48] In addition, the *mcr* gene was also detected in chicken meat samples collected during monitoring of AMR in 2020[15] and in retail beef in 2017.[12] Therefore, as part of this work we compared the genomes of plasmids from *E. coli* poultry isolates from 2020 (turkey and chicken), as well as that from pigs in the UK, to determine whether the 2020 poultry isolates harboured the same *mcr* plasmids as each other and that present in pig isolates; or were these genes harboured on new plasmid variants. The results showed the *mcr-1* gene in *E. coli* from samples T_521133 and T_512101 were on an Inc-X4 plasmid. Comparison of the resolved *mcr-1* Inc-X4 genome indicated the plasmid to be highly conserved within all *E. coli* isolated from the two turkey meat samples (Fig 1). It also showed high sequence identity with the *mcr-1* IncX-4 bearing plasmid isolates from chicken meat (C_2672451-A and C_2798073-B) and pig faeces (RB5). In contrast, the *mcr-1* harbouring *E. coli* in sample T_512003 was carried on an IncI2 plasmid. The resolved plasmid genomes indicated that this plasmid was highly conserved in both *E. coli* from turkey sample T_512003, and an IncI2 plasmid reported from *mcr-1* harbouring *Salmonella* (S3) and *E. coli* (data not shown) previously isolated from UK pigs (Fig. 2). It was notable that despite all *mcr* bearing *E. coli* being multidrug resistance, the *mcr* plasmid did not harbour any other resistance gene, as reported previously.[48, 49]

Conclusions

This is, to our knowledge, the first reported occurrence of *mcr E. coli* from retail turkey meat in the UK. Molecular characterisation, using WGS, of *E. coli* isolated from *mcr-1* PCR positive sweeps, indicated that the *mcr-1* carrying plasmid was present on two different plasmid types in *E. coli* from three turkey meat samples, although the same *mcr*-plasmid type was present in isolates from the same sample. Also, the *mcr-1* plasmids showed a high percentage identity to *mcr-1* harboured on IncX4 and IncI2 plasmids previously reported from UK pigs; which are highly similar to ones reported globally.[50] With respect to plasmid type there was some commonality between isolates from turkey meat in this study and isolates from poultry, turkey meat and humans in Switzerland.[34]

The presence of *mcr* in retail meat, although novel for the UK, has been reported from elsewhere. In a recent study from the Netherlands, *mcr-1* was detected from 24.8% of 214 retail chicken meat samples, and the presence of *Enterobacteriaceae* carrying *mcr1* was confirmed in 34 of the positives.[30] *E. coli* carrying the plasmid mediated colistin resistance gene have also been reported from retail chicken in other countries such as South Korea [31] and Latin America.[32] A German study which looked at over 10,600 *E. coli* isolates from the national monitoring on zoonotic agents from the years 2010–2015 for phenotypic colistin resistance found that the highest prevalence of *mcr-1* was detected in the turkey food chain (10.7%), followed by broilers (5.6%).[33] In a more recent study of retail meat, bacteria of the Enterobacteriaceae family carrying the *mcr-1* gene were detected in 21% (18/86) of the examined samples, especially in turkey meat and liver (16/24 positive for *mcr-1* or 66.7%) originating from EU and non-EU countries.[6]

Based on the predicted serotype and ST, there was some commonality between *mcr-1* positive *E. coli* from UK retail chicken and turkey meat, and from turkey meat previously determined to be positive for *mcr-1 E. coli* in Brazil, Czechia, Germany and Poland.[6] A phylogenetic analysis in future will help determine how genetically closely related these isolates may be.

Figure 1 - Comparison of the resolved genome of a *mcr-1* Inc-X4 plasmid from an *E. coli* isolated from turkey meat with other *mcr-1* Inc-X4 *E. coli*, including those from other turkey meat samples, chicken meat samples, as well as a pig isolate from the UK.

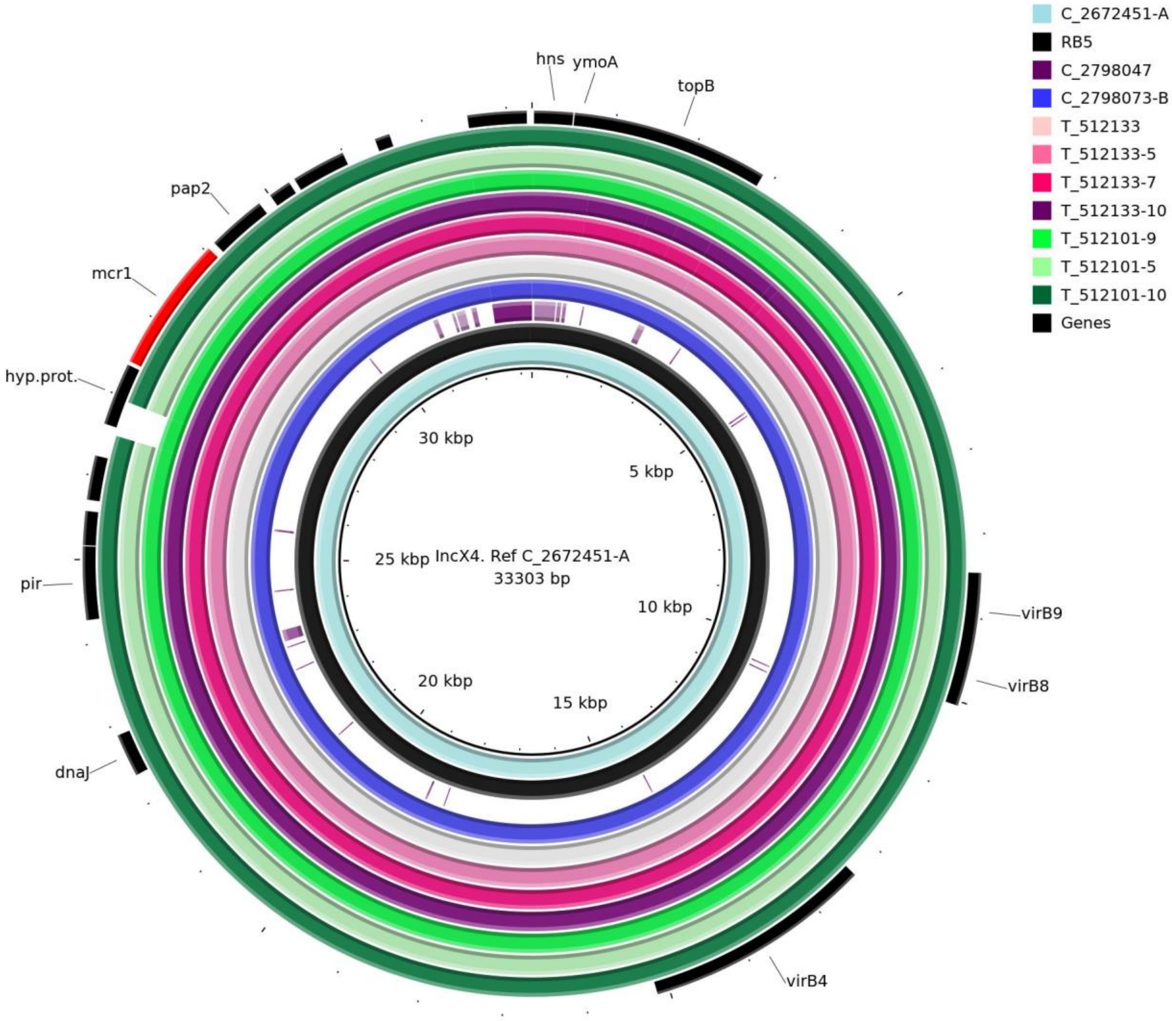
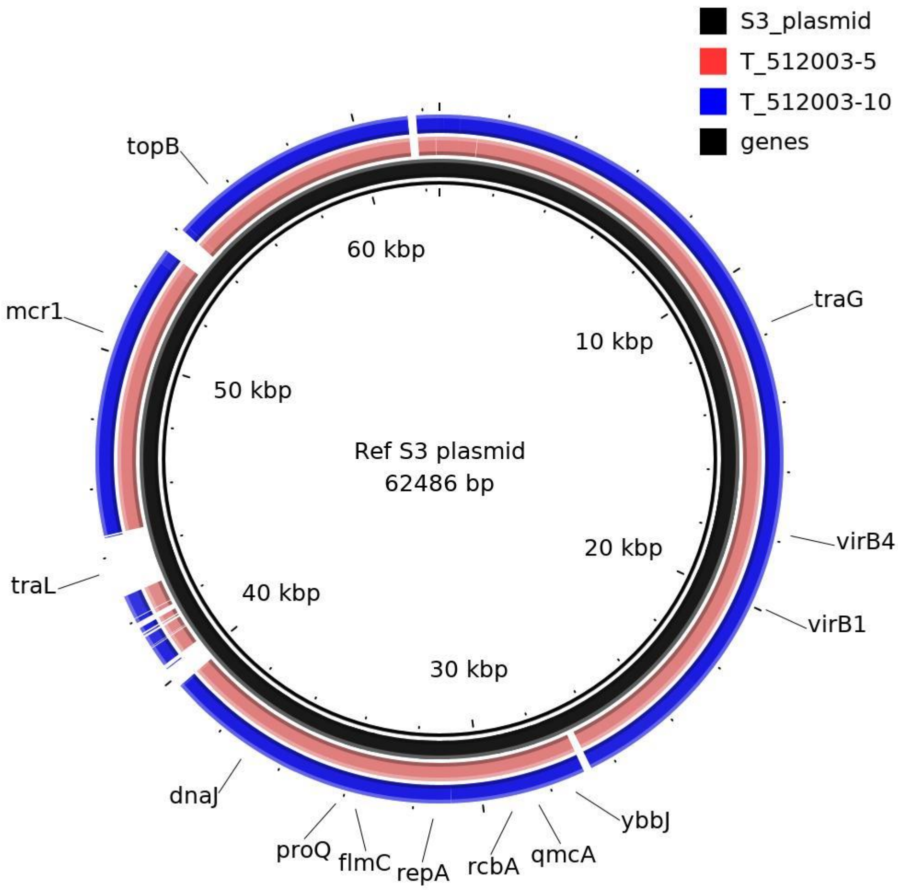


Figure 2 - Comparison of the resolved genome of a *mcr-1* Inc-2 plasmid from an *E. coli* isolated from turkey meat with other *mcr-1* Inc-I2 *E. coli*, including another from the same sample, as well as a pig isolate from the UK.



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