FINAL REPORT

FS430917: Surveillance of AMR in *E. coli*, *Campylobacter* and *Salmonella* on raw fresh chicken and turkey meat on retail sale in the UK in 2022.

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1. Lay Summary

It should be noted that epidemiological cut-off values (ECOFFs) have been used in this survey to determine 'resistance', but ECOFFs do not necessarily indicate clinical resistance. The Advisory Committee on Microbiological Safety of Food (ACMSF) Working Group on Antimicrobial Resistance is currently considering how best to integrate ECOFFs, clinical breakpoint and genomic data in order to most accurately reflect the AMR status of bacterial isolates found in our AMR-food surveys. The Group will report in 2024 and consequently the findings of this report may be updated in light of the revised guidance in due course.

Antimicrobial resistance (AMR) in microorganisms is a growing problem. While it is a natural process, the extensive use of antimicrobials in humans and animals has been a significant driving force in its development. The development of multi-drug resistant (MDR) bacteria has been described as the 'silent pandemic' with infections in humans and animals caused by MDR organisms associated with substantial morbidity and mortality.

Escherichia coli (*E. coli*) is a normal inhabitant of the mammalian gut (termed a commensal) and most isolates do not cause observable clinical disease in healthy animals or humans. Commensal bacteria can be reservoirs of AMR genes and are therefore useful 'indicators' of AMR. They are ubiquitous in animals and allow monitoring of the presence of AMR typically circulating in food-producing animals. *E. coli* that produce ESBL or AmpC enzymes which confer resistance to the 3rd and 4th generation cephalosporins are called 'extended spectrum beta-lactamase-producing' (ESBL) *E. coli*. Surveillance of antimicrobial-resistant bacteria, especially ESBL-producing organisms in humans, environments and food- producing animals is crucial to monitor and understand the threat posed to public and animal health. *Campylobacter* spp., by contrast, is frequently present in the gut of healthy poultry, but certain species (*Campylobacter jejuni* and *Campylobacter coli*) typically cause food-poisoning in people. *Salmonella* spp. are carried by wild-animal vectors, especially birds. The most common serovar in both humans and food-producing animals, *Salmonella* Enteritidis, is controlled in poultry in the UK by vaccination and careful management of farms and poultry processing units. Prevalence in fresh UK poultry meat is very low (approx. 5% of samples).

The APHA have been carrying out surveys to monitor AMR *E. coli* in retail meat since 2015. In this survey, 306 raw chicken and 302 raw turkey meats were purchased from supermarkets and butchers' shops across the UK and tested for the presence and levels

of *E. coli* (including ESBL-producing *E. coli), Campylobacter jejuni*, *Campylobacter coli* and *Salmonella* spp. The AMR of these bacterial species was assessed using panels of antimicrobials.

The results show that ESBL- producing *E. coli* are present in chicken and turkey retail meat. Twelve percent of chicken and 12% of turkey samples were shown to contain these bacteria. For chicken samples we have observed the levels of ESBL-producing *E. coli* have dropped dramatically since it was first surveyed it in 2016, with 45% of chicken samples containing these bacteria. For turkey meat we do not know if the drop has been the same, as this meat was not surveyed over the same period, and only tested once before, in 2020-21, and the prevalence of ESBLs was the same at 12%. A small decrease in ESBL-producing *E. coli* prevalence has been observed in chicken over the same period (13% in 2020 to 12% in 2022). It is thought that the 81% (data from VARSS report 2021 [1]) reduction in use of antimicrobials used in the poultry industry in the UK since 2014 (especially between 2014 to 2017 [2]) may have caused the initial drop, but overall usage since then has stabilised (both in turkey and chickens) and may explain the steadying of ESBL prevalence seen.
Samples from both meat types were also shown to contain *E. coli* that harbour *a mcr* gene, a transferable gene responsible for resistance to the 'last resort' antibiotic colistin. *E. coli* positive for *mcr-1* were found in 1.3% of chicken and 1.3% of turkey meat samples tested although these were predominantly due to isolates from imported meat (seven of the eight).

Salmonella spp. were detected in 1.9% (n=6/306) of chicken samples and 0.7% (n=2/302) of turkey samples, but the salmonella organisms were not ESBL producers.

The prevalence of *Campylobacter* spp. was lower in turkey samples (5%; *n*=15/302) but more common in chicken samples (47.5%; *n*=145/306). *Campylobacter jejuni* (*C. jejuni*) was the most common species of *Campylobacter* detected in both types of meat, *Campylobacter coli* (*C. coli*) was less frequently detected. Approximately 10% of whole chickens sampled were contaminated with the highest levels of *Campylobacter* spp. as defined by hygiene regulations [3]. Meat containing skin was more likely to be contaminated with *Campylobacter* spp. than skinless meat - 15% of chicken samples without skin were contaminated *vs* 50% of chicken samples with skin. In turkey meat, 10.4% of samples with skin were contaminated whilst 1.6% without skin were contaminated.

Ciprofloxacin and tetracycline resistance was detected in the majority of *Campylobacter* spp. from turkey and chicken samples. It is encouraging to report no resistance to chloramphenicol, erythromycin and gentamicin in all *Campylobacter* spp. isolates. Although the frequency and intensity of *Campylobacter* spp. contamination on turkey meat was less than for chicken meat, the species, genomic types and the AMR profiles of turkey meat and chicken meat isolates were generally similar.

Whole genome sequencing (WGS) was conducted on a sub-set of antimicrobial-resistant *E. coli*, *Campylobacter* spp. and *Salmonella* spp. isolates in order to identify by genotypic methods specific resistance genes that may be present in these organisms and has shown that many harbour a range of AMR genes. Elements (plasmids) which assist in the horizontal transfer of such genes were also identified. Genes known to confer resistance to carbapenem, one of 'the last resort' antimicrobials used to treat human infections, were not found.

The results of this survey show the importance of continued monitoring for commensal *E. coli, Campylobacter* spp. and *Salmonella* spp. (when present) in retail meats, as well as provide new baseline data on AMR and genes conferring AMR in such organisms in chicken and turkey meat. These studies continue to deepen and extend our knowledge and understanding of AMR for the UK 5-year (2019-2024) AMR National Action Plan, closing data gaps and improving understanding of the hazards and risks from AMR, especially from foods that we regularly consume. It is especially important since UK's exit from the EU, that we continue to monitor AMR in retail meats so we can align our results to those of EU surveys and allow comparison of data with countries in the EU.

2. Executive summary

It should be noted that epidemiological cut-off values (ECOFFs) have been used in this survey to determine 'resistance', but ECOFFs do not necessarily indicate clinical resistance. The Advisory Committee on Microbiological Safety of Food (ACMSF) Working Group on Antimicrobial Resistance is currently considering how best to integrate ECOFFs, clinical breakpoint and genomic data in order to most accurately reflect the AMR status of bacterial isolates found in our AMR-food surveys. The Group will report in 2024 and consequently the findings of this report may be updated in light of the revised guidance in due course.

Surveys to monitor the presence of antimicrobial-resistant bacteria in foods of animal origin is a requirement of the European Directive 2003/99/EC and the commission

implementing decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance (AMR) in zoonotic and commensal bacteria. Since the UK's exit from the EU it is important to continue this monitoring for the UK AMR National Action Plan to ensure that there are no data gaps and comparison of UK data with the EU data can continue. Surveys have been conducted at APHA to monitor AMR in *E. coli* in retail meat since 2016. Surveys of AMR in *E. coli* in retail chicken have been performed using the same methodology in 2016, 2018, 2020 and in this 2022 study. For turkey meat, surveys have been conducted in 2020 and 2022. This is the first year in which the FSA have requested that we also monitor for the presence of *C. jejuni*, *C. coli* and *Salmonella* spp., and determine the AMR of these bacterial species.

Samples of raw fresh chicken (*n*=306) and turkey (*n*=302) were collected from retail premises across England, Scotland, Wales and Northern Ireland between January and December 2022. The prevalence of *E. coli* that produce ESBL- and/or AmpC-enzymes in chicken and turkey meat samples was shown to be 12% for both meat types. As detection was only observed from enriched samples rather than direct sampling, the actual bacterial numbers on the meat were below the level of detection before enrichment. The prevalence of ESBLs in *E. coli* from chicken meat was similar to the previous 2020 survey of 13%. For turkey meat there was no change in the prevalence from the 2020-21 survey. In the UK retail chicken survey undertaken in 2016, 45% of samples were positive for ESBL-producing *E. coli* but when the survey was repeated in 2018 the prevalence had declined to 14%. The reduction in ESBL/AmpC-producing *E. coli* in chicken during this time might be linked to the ban on the use of cephalosporins in poultry flocks by the British Poultry Council in 2012.

Carbapenem-resistant *E. coli* have not been detected within this or any previous surveys. There was a low prevalence in chicken (1.3%) and in turkey samples (1.3%) of *E. coli* carrying a *mcr* plasmid-mediated colistin resistance gene although these were predominantly due to isolates from imported meat (seven of the eight). Colistin *mcr*-positive samples were also identified in 2020 in chicken retail meat (0.95%) and in turkey meat in 2020-21 (1.4%).

The *Salmonella* spp. prevalence was extremely low with less than 2% (*n*=6/306) of chicken samples and less than 1% (*n*=2/302) of turkey samples positive for this bacterial species, and none were shown to be ESBL-producers. A *Salmonella* serovar Paratyphi variant Java was isolated from a chicken breast, where the origin of the meat was the Netherlands.

Other isolates were serovars Agona (*n*=4), Infantis (*n*=1), London (*n*=1) and Mbandaka (*n*=1), and with the exception of one of the Agona isolates, which was from a turkey breast from the UK, all were from chicken of UK origin.

The prevalence of *Campylobacter* spp. was low in turkey meat (5%, *n*=15/302) in comparison to chicken (47.5%, *n*=145/305). *C. jejuni* was the most common contaminant on both types of meat. High levels of contamination (>1000 cfu/g) were observed in 5.6% of chicken meat samples in total with 10.4% of whole chickens highly contaminated. Meat with skin was more likely to be contaminated with *Campylobacter* spp. than meat without skin with 21.8% of skinless chicken contaminated versus 60.3% of chicken samples containing skin. In turkey meat, 10.4% of samples with skin were contaminated whilst 1.6% without skin were contaminated.

Ciprofloxacin resistance was common for *C. jejuni* isolates from both chicken (64.5%, *n*=141) and turkey samples (47.3%, *n*=19) as was resistance to tetracycline in isolates from chicken (73.8% *n*=141) and turkey samples (57.9%, *n*=19). Resistance to chloramphenicol, erythromycin and gentamicin was not detected in any chicken and turkey samples. Although the threshold used to define a campylobacter organism as resistant to ertapenem is provisional, 17% of *C. jejuni* from chicken and 10.5% of *C. jejuni* from turkey were classified as resistant (MIC >0.5 mg/l). The detection of *Campylobacter* spp. resistant to carbapenems is unexpected since these antimicrobials are not used in food-producing animals. Therefore, the interpretation of MIC data for *Campylobacter* spp. in relation to ertapenem resistance may need to be reviewed.

Whole genome sequencing (WGS) was conducted on a sub-set of AMR *E. coli*, *Campylobacter* spp. and *Salmonella* spp. isolates in order to identify specific resistance genes in these organisms and the underlying mechanisms of AMR. The analyses has shown that many isolates harbour several AMR genes. Elements (plasmids) which assist in the horizontal transfer of such genes were also identified. We have been able to correlate the AMR phenotype with the AMR genotype in most isolates examined. It has also been used to identify the MLST sequence types of the ESBL-producing *E. coli* and *Campylobacter* spp., which can then be compared with types of *Campylobacter* that are isolated from clinical cases and indicate potential transmission pathways for AMR in people. Genes known to confer resistance to carbapenem, one of ‘the last resort’ antimicrobials used to treat human infections, were not found.

In summary, these studies indicate that both fresh retail chicken and turkey meat do contain *E. coli* that are resistant to 3rd-generation cephalosporins, but the prevalence of such organisms is low. Both these meat types contain *E. coli* that harbour the transferable colistin resistance *mcr*-1 gene. This is the second time that *mcr-1* has been detected in *E. coli* in both chicken and turkey meat on retail sale in the UK. albeit at low levels (1%), the majority of which was imported into the UK.[4, 5]

Just under 2% of chicken and 1% of turkey samples were positive for *Salmonella* spp. The prevalence of *Campylobacter* spp. contamination is considerably higher (47.5%; 145/305) in chicken meat samples relative to turkey meat (5.0%; 15/302), and contamination is more frequent and of higher concentrations on samples that contain skin. *C. jejuni* was the predominant *Campylobacter* species on both chicken and turkey meat. As in previous studies, resistance to ciprofloxacin and tetracycline was common in *C. jejuni* isolates from chicken meat, with 65.4% and 73.8% resistant respectively. In *C. jejuni* from turkey meat, a lower proportion of resistant isolates were detected - 47.3% were resistant to ciprofloxacin and 57.9% to tetracycline. It is notable in that no resistance to chloramphenicol, gentamicin, and erythromycin was detected in any of the campylobacters characterised.

AMR was observed in two of the eight *Salmonella* spp. isolates. Multiple resistance (MDR) was observed in a *S. Agona* isolate from a turkey breast, which exhibited resistance to ampicillin, gentamicin and tetracycline. Another *S.* Agona isolate from a chicken leg was resistant to ampicillin. Both these *S.* Agona organisms were isolated from meat that originated in the UK.

The results show the importance of continued monitoring for *E. coli* and *Campylobacter* spp. in retail meats, as well as provide new baseline data on AMR *Salmonella* in chicken and turkey meat. These studies continue to deepen and expand our knowledge and understanding of AMR for the UK 5-year National Action plan 2019-2024, closing data gaps and improving understanding of the hazards and risks from AMR in common foods that we consume. It is especially important that, since UK's exit from the EU, that we continue to monitor AMR in retail meats so we can align our results to those of EU surveys and allow comparison of data with countries in the EU 27.

3. Introduction

3.1 Background

Antimicrobial resistance (AMR) in microorganisms is a growing problem. Many human and animal pathogens are becoming multidrug-resistant (MDR) and this is making it more difficult to treat some infections with the antibiotics that are available. The development of new antimicrobials has slowed down in recent years. While it is a natural process, the extensive use of antimicrobials in humans and animals has been a significant driving force in its development. Antimicrobials are used in the livestock industry to prevent and control bacterial disease. The use of subtherapeutic levels of antibiotics in animal feed (as growth promotors) since the 1950's is associated with the expansion of the pool of AMR bacteria[6]. In 2006, the use of these was banned in the EU (EU Regulation 1831/2003), and in the UK.

Escherichia coli (*E. coli*) is a normal inhabitant of the mammalian gut (termed a commensal) and most isolates do not cause observable clinical disease in healthy animals or humans. Commensal bacteria can be reservoirs of AMR genes. Horizontal gene transfer among bacteria allows them to exchange their genetic material including AMR genes. *E. coli* isolates are therefore useful 'indicators' of AMR. As they are ubiquitous in animals they allow monitoring of the presence of AMR typically circulating in food-producing animals.

If the bacteria are resistant to three or more different classes of antimicrobials, they are considered as 'multidrug resistant' (MDR). MDR bacteria may pose a health risk because fewer therapeutic agents are active against them, should therapy with antibiotics be considered necessary. This is a particular concern if the MDR includes resistance to certain classes of antibiotics (such as the carbapenems) which are used to treat severe bacterial infections when other treatment options are ineffective.

Mechanisms by which bacteria can develop resistance to antimicrobials include; the production of enzymes which break-down the drug; inactivation of the drug by modification; mutation of the drug target site or by transport of the drug out of the bacterial cell. Resistance to cephalosporins occurs by the production of beta-lactamase enzymes. Additionally, *E. coli* can possess resistance to carbapenems, one of the 'last resort' antibiotics. *E. coli* that are resistant to the 3rd-and 4th-generation cephalosporins are termed 'extended spectrum beta-lactamase-producing' *E. coli* and are referred to as ESBL-producing *E. coli*. Surveillance of AMR in bacteria in humans, environments and

food- producing animals is crucial to monitor and understand the threat posed to public and animal health.

Surveys to monitor the presence of antimicrobial-resistant bacteria in foods of animal origin was a UK requirement of the European Directive 2003/99/EC and the commission implementing decision 2013/652/EU on the monitoring and reporting of AMR in zoonotic and commensal bacteria. After the UK exit from the EU, monitoring of resistance in the UK was continued to mirror the EU specification to allow for comparison with past years surveillance. This work is conducted as part of the Food Standards Agency 5 year National Action Plan (NAP) on AMR, which runs from 2019 until 2024. APHA has surveyed fresh raw meat since 2015, alternating between testing 300 beef and 300 pork samples in one year (2015, 2017, 2019 and 2021 and then 300 turkey and 300 chicken retail meats the alternate years (2016, 2018, 2020 and including this 2022 survey). In this 2022 survey, the levels of *E. coli*, *Campylobacter* spp. and *Salmonella* spp.in fresh raw chicken and turkey on retail sale was determined, thereby giving important insight into foodborne disease and contamination.

Campylobacter jejuni (C. jejuni) is the most frequent contaminant of poultry products and is also the most common cause of bacterial gastroenteritis in people in the UK and for many developed countries [7-9]. The antimicrobial susceptibility of *C. jejuni* has been monitored through harmonised programmes that aligned with EU directives, which has demonstrated that *C. jejuni* present in the poultry production system is typically resistant to tetracycline and the fluoroquinolone antibiotic ciprofloxacin. Fluoroquinolones are recognised as 'high priority-critically important antibiotics' (HP-CIA) by the WHO [10] and therefore the increased incidence of resistant *Campylobacter* spp. isolates is a concern that has prompted changes in AMR prevalence and antimicrobial use (AMU) and a continuation of monitoring. This survey continues to monitor for resistance to ciprofloxacin, tetracycline, erythromycin and gentamicin, but will, for the first time, investigate the susceptibility of *Campylobacter* spp. to chloramphenicol and ertapenem [11].

3.2 Aims and Objectives

The purpose of this study was to monitor the presence of AMR bacteria in fresh raw chicken and turkey retail meat, and to compare to the levels seen in previous AMR monitoring surveys. As surveys on retail meat have been conducted since 2016 and the same methodology has been used it allows trends in the prevalence and levels of antimicrobial-resistant bacteria in foods over time. The methods used are based on current

EU protocols used for the EU harmonised AMR monitoring conducted in food producing animals in Member States. It includes the method for detection of ESBL and/or AmpC beta lactamase-producing *E. coli*, *Campylobacter* spp. and *Salmonella* spp., followed by determination of resistance using MIC testing. At the request of the FSA total counts of *E. coli* were performed on the meats prior to enrichment to determine the levels of the bacteria on the meat samples. Screening was also performed for *E. coli* that harbour a *mcr* gene which is responsible for resistance to colistin. The prevalence and numbers of *Salmonella* spp, *Campylobacter jejuni* and *Campylobacter coli* in the chicken and turkey were also determined. This is the first survey in which we have included antimicrobial-resistant *Salmonella* testing of retail meats. The resultant data will provide a baseline data for future surveys.

4. Methodology

4.1 Sampling

Samples of raw fresh chicken and turkey were collected from retail premises across England, Scotland, Wales and Northern Ireland between January and December 2022. As with previous years, the sampling plan mirrored previous surveys based on the EU specification as this will allow comparison of results between years. This was a 'proportionate stratified sampling' was used in 80 locations (i.e. NUTS-3 area) where the proportion taken was according to population size of the area and in proportion to the market share. Samples were taken from all parts of the UK except for the smallest locations, and this covered at least 80% of the total population. The plan included the 11 largest supermarkets in the UK and butchers' shops. The aim was to collect 300 fresh chicken and 300 fresh turkey samples that had not been previously frozen over the 12-month sampling period. These meats were the same cut categories for both meat types and same as previous AMR studies and included whole birds, breasts, legs, portions and wings (for chicken only). Basted, cook in the bag or meats that contained any other ingredients (such as breaded or herbs etc) were not sampled, neither were minced, frozen or cooked meat. The product categories were well defined to ensure consistency between surveyors.

4.2 Isolation and enumeration of *E. coli*

For detection of antimicrobial-resistant *E. coli* the methodology followed the DTU protocol for the isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* (EU methods

published online). Briefly, this involved preparing a 1 in 10 meat homogenate (of 25g meat) in buffered peptone water, which was then incubated for 18-22 hours at 37ºC. The homogenate was plated out on 3 different media types following EU survey requirements. These are MacConkey agar containing 1 mg/L cefotaxime (McC-CTX), chromID® CARBA (CARBA) and chromID® OXA-48 (OXA-48). In addition, samples were also plated to two non-EU stipulated screening agars at the request of the FSA (UK only tests). These were CHROMagar™ ESBL (CA-ESBL), for the specific detection of ESBL-producing *E. coli* and also onto MacConkey agar containing 2 mg/L colistin (McC-COL), for the detection of colistin-resistant *E. coli*. All agar plates were incubated for 18-22 hours at 37 ± 1ºC (or 44 ± 0.5 °C for MacConkey based agar plates) before checking for presumptive *E. coli.*

Three single presumptive *E. coli* colonies from each of these agars were picked and plated onto the same stated agars to ensure purity. One isolate was then used to confirm they were *E. coli* by indole/oxidase testing and/or by identification by MALDI-ToF, followed by storing in cryogenic material at -80ºC for further tests. Overall, this method of post-enrichment in BPW has the theoretical potential to detect one *E. coli* of interest per 25 grams of meat.

For total *E. coli* counts, the method involved plating 100 µL of the meat homogenate (prior to incubation) on to MacConkey agar with and without 1 mg/L cefotaxime. These two agars are used to enumerate the number of presumptive total counts of *E. coli* and the total number of presumptive ESBL- and/or AmpC-producing *E. coli* in 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 considered not necessary to further dilute the initial BPW homogenate for counts beyond the initial tenfold dilution.

4.3 Isolation and enumeration of *Salmonella* spp. and *Campylobacter* spp

For the detection and enumeration of *Campylobacter* spp. and *Salmonella* spp., the same meat homogenates as prepared for *E. coli* were used. To allow for detection and enumeration of *Campylobacter* spp., a procedure based on ISO10272-1,2:17 [12, 13] 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. Additional mCCDA and Butzler agar plates were 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 sub-cultured 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. If sub-cultures failed confirmation, the enumeration count was adjusted. The minimum detectable level of *Campylobacter* was 10 colony forming units (CFU) per gram of sample. It was possible to quantify the number of *Campylobacter* spp. organisms in a sample when levels were greater than 45 cfu/g and less than 15,100 cfu/g.

Confirmed *Campylobacter* spp. 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 the detection of *Salmonella* spp. the method BS EN ISO 6579:1:2017 [14] was followed, where the same enriched meat homogenate that had been incubated at 37±1ºC for 18± 2h was plated onto MSRV agar. Agar plates with grey-white turbid zone of growth from the inoculated drop were plated to Rambach and XLD media before confirmation by slide agglutination with poly H and poly O antisera. In addition, *Salmonella* brilliance agar (Thermoscientific™) was used with cefotaxime (1mg/L) added to detect ESBL- and/or AmpC-producing *Salmonella* spp. Enumeration of *Salmonella* spp. isolates was conducted using the miniaturized most probable number technique (MPN) according to BS EN ISO 6579:2:2012 [15]. This was used due to the large number of samples to be tested in the survey as it is less resourceful than the full MPN method. For characterisation of *Salmonella* spp. isolates, serotyping was conducted at the APHA *Salmonella* reference laboratory (Weybridge).

4.4 Determination of Minimum Inhibitory Concentration (MIC) for *E. coli*, *Salmonella* spp. and *Campylobacter* spp.

All isolates were screened against panels of antimicrobials to determine their susceptibility (according to ECOFFs) to determine the minimum inhibitory concentration (MIC). This was conducted using the microbroth dilution technique on the Thermofisher™ Sensititre instrument according to EN ISO 20776-1:2019 [16]. Commercially- prepared plates containing a two-fold dilution series of antimicrobial compounds were used as in accordance with Decision 2020/1729/EU [17]. These were EUVSEC2 and EUVSEC3 plates (Thermofisher™) for *E. coli* (from CTX-containing agar) and *Salmonella* spp., and the EUCAMP plate for *Campylobacter* spp. MIC for *E. coli, Salmonella* spp. and *Campylobacter* spp. isolates were interpreted using the ‘epidemiological cut-off values’

(ECOFFs) specified in the 2020/1729 EU decision, and if not available then current EUCAST ECOFFs published were considered.

To determine if an isolate was a ESBL- or AmpC producer, then the following criteria were used:

***E. coli* with an ESBL-phenotype:** Isolates resistant to one or both of cefotaxime and ceftazidime that 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 possess an ESBL phenotype.

***E. coli* with an AmpC phenotype:** 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 possess an AmpC phenotype.

***E. coli* with an AmpC+ESBL-phenotype:** Isolates resistant to cefotaxime or ceftazidime that also had an MIC of greater than 8mg/L against cefoxitin that 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 possess an AmpC+ESBL-phenotype.

4.5 PCR for plasmid-mediated *mcr-1-5* genes in *E. coli*

PCR was used to identify *E. coli* which carry *mcr1-5* genes, the plasmid encoded genes which confer resistance to colistin (which is a 'last-resort' antibiotic for treatment of bacterial infections in humans). The PCR method used was the EU method (PCR method for *mcr1-5*) . Any pink to red colonies that grew on McC-COL agar were tested. To make detection more sensitive, a 'sweep' of ~ 10 to 20 colonies was taken to prepare the crude DNA boilate for the PCR. This boilate was used in the PCR, and if the initial 'sweep' was positive by PCR for any of *mcr-1-5* genes, then multiple individual suspect *E. coli* colonies (up to 10 as available) were further examined by PCR.

4.6 Whole genome sequencing on selected *E. coli*, *Salmonella* spp. and *Campylobacter* spp. isolates to identify the MLST types and AMR genes they harbour.

Whole genome sequencing was conducted on 112 isolates covering all bacterial species by Illumina and the resulting WGS, FASTQ files were assembled using "SPAdes - St

Petersburg aligner" [18] and analysed using DTU pipelines "MLST" [19], APHA Seqfinder [20] and "ResFinder 4.1." [21].

For *E. coli,* WGS was used to determine the presence of the genes that are responsible for production of ESBLs (*bla*$_{CTX-M}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$) and to identify any genes that may encode for the production of carbapenemase enzymes. We looked at whether mutation in the promoter region of the *amp*C gene was present that allows over expression of AmpC beta-lactamase enzyme in *E. coli*. For the analysis of *E. coli* ESBL genes we examined isolates from CA-ESBL media only (*n*=69) and not from CTX media. This was to ensure continuity with previous surveys. Also, as both media isolate ESBL-producing *E. coli* this could have resulted in isolation of same ESBL-producing isolate from a sample and thus affected the calculation of the prevalence of ESBL types. All *mcr* gene-harbouring colistin-resistant *E. coli* were examined by WGS (n=15). For *Campylobacter* spp., isolates selected for sequencing were based on their AMR profile with focus on ciprofloxacin- and erythromycin-resistant isolates. For *Salmonella* spp. all 8 isolates were examined.

5. *E. coli* Results

5.1 Samples tested in the survey.

A total of 306 fresh chicken and 302 fresh turkey meats were collected from January to December 2022. The numbers of samples for each type of meat cut are shown in Table 1, and the country of origin of the samples are shown in Table 2. The origin of samples according to the different retailers (using anonymised codes) and country are shown in Table 3. In eight supermarkets, all the chicken and turkey meats were of UK origin. A few of the turkey meats from two supermarkets were non-UK origin, but 50% of meat from butchers' shops were non-UK origin meat. Fifty percent of chicken meat from one supermarket and 27% from butchers' shops came from EU countries. Meat from butchers' shops had the greatest variety of origin (see Table 3). Although this survey was of fresh retail meat, five turkey samples had been previously frozen due to a Defra derogation (which allowed retailers to sell frozen whole turkeys to maintain stock levels for the Christmas period, depleted due to an avian influenza outbreak).

Table 1 Numbers of chicken and turkey samples per meat cut type (number in brackets). *2 whole turkeys and 3 crown joints previously frozen

Chicken (306)	Turkey (302)

Breasts (whole, diced and sliced) (111)	Breast (whole, diced and sliced) (16)
Leg (67)	Leg (56)
Pieces (2)	Mixed/other pieces (191)
Whole (116)	Whole (13)*
Wings (10)	Crown joint (26)*

Table 2 Number of chicken and turkey samples per country of origin

Country of Origin	Number of chicken samples	Number of turkey samples
UK	299	293
Germany	-	1
Ireland	-	3
Italy	-	1
Lithuania	-	1
Netherlands	4	-
Poland	3	3
Total per meat	306	302

Table 3 Number and origin of chicken and turkey samples according to retailer code and country. Key to countries: Irl - Republic of Ireland, G - Germany, It - Italy, L - Lithuania, N - Netherlands, P - Poland. Retailer code C is butchers' shops

Retailer code	No Chicken samples	UK-produced chicken samples (%)	Non-UK produced chicken samples (%)	No Turkey samples	UK-produced turkey samples (%)	non-UK produced turkey samples (%)
A	40	100	-	40	100	-
B	45	100	-	41	100	-
C	15	73	27 (N)	12	50	50 (G, It, L, P)
D	6	50	50 (P)	0	0	-
E	15	100	-	1	100	-
F	11	100	-	7	86	14 (Irl)
G	7	100	-	13	100	-
H	32	100	-	30	100	-
I	26	100	-	44	100	-
J	2	100	-	0	0	-
K	41	100	-	53	100	-
L	66	100	-	61	97	3 (Irl)
Total	306	-	-	302	-	-

5.2 *E. coli* counts results (pre-enrichment)

Pre-enrichment counts of total *E. coli* and *E. coli* that grew on MacConkey agar containing cefotaxime (CTX) are shown in Table 4. Cefotaxime is a cephalosporin antimicrobial which is selective for isolating ESBL- and AmpC-beta lactamase producing *E. coli*. For *E. coli* only 2.6% of chicken and 4.6% of turkey samples yielded growth of total *E. coli* (above the limit of detection). No pre-enrichment samples contained *E. coli* that were resistant to cefotaxime.

Table 4 Counts results for total E. coli on non-selective agar and for E. coli that grew on MacConkey agar containing cefotaxime (CTX) pre-enrichment.

Meat	No tested	Number of samples with total *E. coli* above the detection limit (%)	Counts (cfu/g) min	Counts (cfu/g) max	Number of samples with *E. coli* on McC-CTX agar	Counts on McC-CTX agar
Chicken	306	8 (2.6 %)	4.3×10^3	1.7×10^4	0	0
Turkey	302	14 (4.6 %)	2.3×10^3	2.7×10^4	0	0

5.3 Microbiological testing for *E. coli* on antimicrobial selective agars (post enrichment).

Thirty-six chicken samples were positive for *E. coli* (12%, 95% confidence interval CI [9-15%]) and 35 turkey samples (12%, 95% CI [8-16%]) were positive for *E. coli* on cefotaxime-containing agar (see Table 5). Using a second ESBL selective agar (CHROMagar™ ESBL) that inhibits AmpC *E. coli*, 44 chicken samples (14%, 95% CI [10-18%]) and 25 turkey samples (8%, 95% CI [5-11%]) were positive after pre-enrichment. No carbapenem-resistant *E. coli* were isolated on the carbapenem-selective agar plates from chicken or turkey after enrichment.

Two hundred and sixteen pre-enriched chicken (71%) and 155 pre-enriched turkey (51%) samples yielded presumptive *E. coli* colonies on colistin-supplemented agar plates (see Table 5). When tested in the *mcr1-5* gene PCR only four (1.3% 95%CI [0.03-2.6%]) chicken samples and four (1.3%, 95% CI [0.05-2.6%]) turkey samples were shown to contain *E. coli* with *mcr* genes, and were positive for *mcr-1*-by PCR. The large numbers of colistin resistant colonies on colistin-supplemented agar plates are due to the presence of non-target lactose fermenters such as *Klebsiella* spp. and *Citrobacter* spp. which have intrinsic resistance (resistance that is naturally present in a microorganism) to colistin (such as efflux pumps) but do not possess *mcr* genes. The origin of the samples which possessed *E. coli* harbouring the *mcr1* gene were all non-UK origin except for one chicken breast sample. It is interesting to note that all but one of the *mcr-1*-positive meats came from butchers' shops (retailer code C). Two turkey and two chicken meats were of Polish origin; one turkey breast was from Lithuania and two chicken breasts were from the

Netherlands (Table 6). The five turkey samples that had been previously frozen were negative for total *E. coli* both pre- and post-enrichment (except for one colony for the count plate of T0086488, which is below the detection limit for a reliable count).

Table 5 Numbers and percentage of samples positive for E. coli on antimicrobial selective agars

Meat	Number Samples	McC + CTX	CHROM-agar™ESBL	chromID® CARBA	chromID® OXA-48	McC + Colistin
Chicken	306	36 (12%)	44 (14%)	0	0	216 (71%)
Turkey	302	35 (12%)	25 (8%)	0	0	155 (51%)

Table 6 Meat samples positive for E. coli with mcr-1 gene

Sample ID	Meat Category	Retailer Code	Country of Origin	Growth on colistin	*mcr* gene type by PCR
C02898536	Chicken Breasts	C	United Kingdom	Yes	*mcr*-1
C00864920	Chicken Breasts	C	Netherlands	Yes	*mcr*-1
C03038322	Chicken Breasts	C	Netherlands	Yes	*mcr*-1
C03038643	Chicken Breasts	D	Poland	Yes	*mcr*-1
T00826165	Turkey Breast	C	Poland	Yes	*mcr*-1
T00823416	Turkey Breast	C	Lithuania	Yes	*mcr*-1
T00865017	Turkey Breast	C	Poland	Yes	*mcr*-1
T03135215	Turkey Crown Joint	C	Poland	Yes	*mcr*-1

5.4 AMR phenotypes of *E. coli*

Microbroth dilution testing using a panel of 20 antimicrobials allowed determination of whether *E. coli* produced beta-lactamase enzymes, of which there are three main AMR phenotypes, AmpC-, ESBL- or carbapenem beta-lactamase-producers. All *E. coli* isolates from CTX containing media were tested to determine AMR phenotype, and the prevalence of these in the meats tested can be seen in Table 7. No samples were found to contain *E. coli* that were carbapenemase-producers. Only one AmpC *E. coli* was isolated from a chicken sample, whilst 10 AmpC-producing *E. coli* were isolated from the turkey samples (one of these was an *E. coli* producing both ESBL and AmpC-beta lactamase enzymes).

Table 7 Total number (and percentage) of samples positive for ESBL- and or AmpC-producing E. coli (from CTX containing media) positive for E. coli on carbapenem isolation media, and positive for E. coli harbouring mcr-1

Meat	All ESBL & AmpC	ESBL (including ESBL+AmpC)	AmpC (including ESBL+AmpC)	Carbapenem resistant	*mcr-1* gene (by PCR)
Chicken (n=306)	36 (11.8)	34 (11.1)	2 (0.7)	0	4 (1.3)
Turkey (n=302)	35 (11.6)	25 (8.6)	10 (3.3)	0	4 (1.3)

A comparison of the percentage of samples with ESBL- and/or AmpC-producing *E. coli* from previous surveys is shown in Table 8 and Table 9 for chicken and turkey meat samples respectively.

For 2022, 11.8% of the 306 retail chicken samples were positive for presumptive ESBL- and/or AmpC-producing *E. coli,* which is a drop from 13.0 % seen in chicken samples in 2020, and a significant reduction from the 45% detected in 2016. This reduction in 2022 was observed for samples positive on both CTX- containing media and for ESBL producers isolated on CA-ESBL media. Statistical analysis was conducted for each media type and using chi square statistic the p values are 7.1E-5 and 2.28E-5 respectively, which confirms that the drop in the occurrence of ESBL/ampC *E. coli* between the years for both meat types was statistically significant.

Table 8 Comparison of the percentage of chicken samples positive for ESBL-, AmpC-, and carbapenemase-producing E. coli, and E. coli harbouring the mcr-1 gene between the different chicken surveys.

Year	All ESBL & AmpC isolates on CTX (%)	ESBL only (including ESBL+ AmpC) on CTX (%)	AmpC only (including ESBL+ AmpC) on CTX (%)	ESBL on CA-ESBL (%)	Carba-penem resistant (%)	Colistin *mcr-1* gene by PCR (%)
2016	45.1	29.7	16.3	30.4	0	0
2018	13.6	8.4	6.1	10.0	0	0
2020	13.0	12.4	1.3	17.1	0	0.95
2022	11.8	11.1	0.7	14.4	0	1.3

Table 9 Comparison of the percentage of turkey samples positive for ESBL-, AmpC-, and carbapenemase-producing E. coli, and E. coli harbouring the mcr-1 gene between the 2 different turkey surveys

Year	All ESBL & AmpC isolates on CTX (%)	ESBL only on CTX (%)	AmpC only on CTX (%)	ESBL only on CA-ESBL (%)	Carba-penem resistant (%)	Colistin *mcr*-1 gene by PCR (%)
2020/21	11.4	11.4	0	11.9	0	1.4
2022	11.6	8.6	3.3	8.3	0	1.3

In the 302 retail turkey meat samples tested, the prevalence of ESBL and/or AmpC-producing *E. coli* was 11.6% which is the same prevalence seen in 2020-2021. This is the figure when both the *E. coli* ESBL and AmpCs are looked at together. However, when the percentage of samples that were positive for ESBL and AmpC-producing *E. coli* were assessed separately, the percentage of samples with ESBLs has dropped from 11.4% to 8.6% (and statistically significant for both media types, p values 0.039 and 0.0004), and the percentage of AmpC producers has increased from 0% to 3.3%. This increase in prevalence in turkey in 2022 is statistically significant using a chi square statistic with a p-value of 0.0077.

The trend in the prevalence of AmpC-producing bacteria in chicken samples is different to that in turkey samples, in that year on year there has been a reduction in the number of AmpC-producing isolates present in chicken, with a reduction from 1.3% in 2020 to 0.7% 2022, but for AmpC-producers in turkeys, this is the first report of such organisms.

We also looked at the effect of whether chicken and turkey meat both with and without skin had an impact in terms of ESBL- and/or AmpC-producers being detected. Results indicated that skinless chicken was more likely to be positive for ESBL- and/or AmpC-producing isolates [on both CTX and CA-ESBL media (95% CI 2.62E-7 and 2.65E-5)]. In contrast for turkey meat there was no statistical significance as to whether a sample was positive for ESBL- and/or AmpC-producing organismsbetween skin on or skin off for both media types (95% CI 0.117 and 0.107). This result has been observed in all of our previous chicken surveys except in 2020, when skin on was more likely to result in ESBL producers being present.

5.5 Resistances to antimicrobials in ESBL- and AmpC-producing *E. coli* (as determined by MIC testing).

The resistance of the ESBL- and/or AmpC-producing *E. coli* to the panels of antimicrobials that had been isolated on CTX-containing media are shown in Table 10 and Table 11 for chicken and turkey samples respectively. For interpretation on whether an isolate was sensitive or resistant to antibiotic ECOFF values were used. These are shown in Table 12. ECOFFs are published by EUCAST and defined in Decision 2020/1729/EU [17]. If an ECOFF was not available under this decision, then the ECOFF established by EFSA at that time was used. We also interpreted all MIC results using the current EUCAST ECOFFs to see if this affected the interpretation of the MIC result.

As expected for *E. coli* isolates from agar containing 1 mg/L of cefotaxime, all isolates were resistant to the ampicillin, and to cefotaxime (see Table 10 and Table 11). All chicken and turkey isolates were also resistant to the cephalosporin, ceftazidime and most were resistant to cefepime (except for AmpC-producing isolates). The majority of isolates were sensitive to cefoxitin except for all the turkey AmpC-producing isolates (a recognised resistance in AmpC-producing *Enterobacteriaceae*). For quinolones about half of the turkey and chicken isolates were resistant to ciprofloxacin. Some resistance (3 turkey and 12 chicken isolates) was seen to the quinolone nalidixic acid. One turkey isolate was resistant to the aminoglycoside amikacin (and two chicken isolates showed resistance to the macrolide azithromycin. No resistance was observed in any of the isolates to gentamicin, tigecycline or temocillin or to the ‘last resort’ antimicrobials colistin, ertapenem, imipenem or meropenem.

If the current EUCAST ECOFFs were used to determine the resistances of the isolates, then the only interpretation of MIC results that would change would be for two turkey isolates that were classed as resistant to cefoxitin would become sensitive (a doubling of ECOFF resistance from R>8 to R>16).

Table 10 MIC results and AMR phenotype for E. coli isolated on McC-CTX media from chicken meat. EUCAST ECOFFs used as stipulated by EU decision 2020/1729/EU. The antimicrobial resistances of the *E. coli* ESBL- and/or AmpC-producing isolates from chicken meat against the 20 different antimicrobials tested, and the total number of antimicrobial classes that to which each isolate exhibited resistance at ECOFF levels. The isolates were resistant to between 2 and 7 different classes of antimicrobials. The total number (and percentage) of isolates resistant against each antimicrobial is shown at the bottom of the columns. The highest (i.e more than 50% of isolates) were resistant to ampicillin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, chloramphenicol, sulfamethoxazole, trimethoprim and tetracycline. There would be no changes to the resistances if current EUCAST ECOFFs were used (as accessed on 27/03/23). See glossary for abbreviations to the antimicrobials.

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
CH-03038329	AmpC	R	S	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	R	R	6
CH-00861911	AmpC	R	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	4
CH-00823144	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	7
CH-00825390	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	7
CH-00864774	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	7
CH-03038339	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	7
CH-03047774	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	R	R	R	R	7
CH-03135103	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	7

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
CH-03135216	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	R	7
CH-00861806	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	6
CH-00864761	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	R	R	R	6
CH-00864779	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	R	6
CH-00864796	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	6
CH-00865049	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	6
CH-03038363	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	6
CH-03038584	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	6
CH-03038724	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	6
CH-03047413	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	6
CH-03135198	ESBL	R	S	R	R	R	S	S	R	S	S	S	S	S	S	S	S	R	R	R	S	6
CH-03135511	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	R	S	S	R	S	R	6
CH-00864702	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	5
CH-00864769	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	R	S	S	S	S	R	5
CH-00864798	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
CH-00864836	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
CH-00864856	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
CH-00864920	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	5
CH-00872268	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	5
CH-03038580	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	R	R	S	5
CH-00864986	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	4
CH-03038412	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	R	4
CH-03038680	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	4
CH-03135312	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	R	4
CH-03135391	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	4
CH-00861602	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3
CH-03047767	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3
CH-03038357	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	2
Total	–	100	0	97	100	100	6	53	33	0	0	0	0	0	0	6	0	58	75	67	75	–
Percentage	–	36	0	35	36	36	2	19	12	0	0	0	0	0	0	2	0	21	27	24	27	–

Table 11 MIC results and AMR phenotype for E. coli isolated on McC-CTX media from turkey meat. EUCAST ECOFFs used as stipulated by EU decision 2020/1729/EU. The antimicrobial resistances of *the E. coli* ESBL- and/or AmpC-producing isolates from turkey meat against the 20 different antimicrobials tested. The total number of antimicrobial classes that each isolate is resistant to is shown in the last column, which is between 2 and 6. The total number (and percentage) of isolates resistant against each antimicrobial is shown at the bottom of the columns. The highest (i.e more than 50% of isolates) were resistant to ampicillin, cefepime, cefotaxime, ceftazidime, and tetracycline. There would be two isolates that would be classed as sensitive to cefoxitin if current EUCAST ECOFFs were used (as accessed on 27/03/23) (not shown).
R represents resistance to the respective antimicrobial(s) and S represents sensitivity. See glossary for abbreviations to the antimicrobials

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
TU-00511951	AmpC	R	S	S	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	4
TU-00864838	AmpC	R	S	S	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	4
TU-00864877	AmpC	R	S	S	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	4
TU-00825407	AmpC	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	3
TU-00861612	AmpC	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	3
TU-00861613	AmpC	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	3

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
TU-00865053	AmpC	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	3
TU-02898677	AmpC	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	3
TU-03135043	AmpC	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	3
TU-00343300	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-00803067	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-00803068	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-00825266	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-00864694	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	S	R	S	R	5
TU-00864861	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R	5
TU-00864987	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
TU-02797658	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R	5
TU-02898486	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-02898492	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-03038594	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-03038723	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-03047415	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R	5
TU-03047812	ESBL	R	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	S	R	S	R	5
TU-00861818	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	4
TU-00864716	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	4
TU-00864722	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	4

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
TU-00864848	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	4
TU-03047800	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	4
TU-00825268	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	3
TU-02898617	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3
TU-03038492	ESBL	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3
TU-00825265	ESBL	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	2
TU-00825279	ESBL	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	2
TU-02996023	ESBL	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	2
TU-03009365	ESBL+AmpC	R	S	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	R	S	R	6
Total	–	35	0	24	35	35	10	15	3	0	0	0	0	0	1	0	0	13	15	0	27	–
Percentage	–	100	0	69	100	100	29	43	9	0	0	0	0	0	3	0	0	37	43	0	77	–

Table 12 EUCAST Interpretative thresholds of AMR (ECOFFS mg/L) applied in this report under EU decision 2020/1729/EU (including the current E. coli EUCAST ECOFFS as accessed on 27/03/23).

*** No confirmed ECOFF was available for ertapenem for *Campylobacter* spp.. Resistance threshold of >0.5mg/l used, and in the EU summary report on AMR in zoonotic and indicator bacteria for 2021 (REF). A tentative ECOFF (>0.125mg/l) is now published by EUCAST (05/04/2023) for *C. jejuni* and ERT. There is no published ECOFF for *C. coli*.**

( ) EUCAST ECOFF is tentative (TECOFF)

^ cut off as stipulated in EFSA Technical Report [22]

§EUCAST ECOFF for *Salmonella* spp. is 1ml/mL

Antibiotic	***E. coli***	**Current *E. coli* EUCAST ECOFFs**	***Salmonella* spp**	***Campylobacter jejuni and C. coli***
Amikacin	> 8	> 8	> (4)	NT
Ampicillin	> 8	> 8	>8	NT
Azithromycin	> 16	> (16)	> 16^	NT
Cefepime	> 0.125	> 0.125	>0.125^	NT
Cefotaxime	> 0.25	> 0.25	> 0.5	NT
Cefoxitin	> 8	> 16	>8^	NT
Ceftazidime	> 0.5	> 1	> 2	NT
Chloramphenicol	> 16	> 16	> 16	> 16
Ciprofloxacin	> 0.06	> 0.064	> 0.064	> 0.5
Colistin	> 2	> 2	> 2^	NT
Ertapenem	> 0.06	> (0.03)	>0.06^	0.5 (0.125)*
Erythromycin	NT	NT	NT	>4 [>8, *C. coli*]
Gentamicin	> 2	> 2	> 2§	> 2
Imipenem	> 0.5	> 0.5	>1^	NT
Meropenem	> 0.125	> 0.06	> 0.125	NT
Nalidixic acid	> 8	> 8	>8	NT
Sulfamethoxazole	> 64	> 64	> 256^	NT
Temocillin	> 16	> 16	>16^	NT
Tetracycline	> 8	> 8	> 8	> 1 [>2 *C. coli*]
Tigecycline	> 0.5	> 0.5	> 0.5 ^	NT

Antibiotic	*E. coli*	Current *E. coli* EUCAST ECOFFs	*Salmonella* spp	*Campylobacter jejuni and C. coli*
Trimethoprim	> 2	> 2	>2	NT

The total number of antimicrobial classes that the ESBL/AmpC *E. coli* isolates are resistant to are shown in the Table 13 and Table 14. Seven *E. coli* isolates from chicken possessed resistance up to seven different classes of antimicrobials, but on average they were resistant to 5 classes. *E. coli* turkey isolates were resistant to less antimicrobial classes. One turkey isolate was resistant to 6 antimicrobial classes, but the average for all turkey isolates was to 4 classes. A summary of the percentage of ESBL- and/or AmpC-producing isolates from broilers and turkeys resistant to each antimicrobial is shown in Table 13.

If we compare the levels of AMR of the ESBL and/or AmpC- positive chicken isolates to 2020 and 2018 surveys, ciprofloxacin resistance has reduced in incidence in chicken isolates compared to 2020, from 64% isolates to 50%; the level seen in 2018 was 50% so no overall change (Table 14). Nalidixic acid has dropped from 46% in 2018 to 35% in 2022. Combined resistance to third-generation cephalosporins and fluoroquinolones combined is still high, with 53% of chicken isolates and 45% of turkey isolates possessing this AMR phenotype. Co-resistance has decreased since 2020 which was 66% in chicken and 79% in turkey. There does not appear to be any changes in the percentage in AMR of AmpC producing chicken isolates, which have stayed the same between 2020 and 2022.

Table 13 Summary of percentage of ESBL- and/or AmpC-producing E. coli isolates from chicken and turkey meats with resistances to panel of antimicrobials (from CTX containing media only).

Antimicrobial	Antimicrobial class	Chicken isolates 2022	Chicken isolates 2020	Turkey isolates 2022	Turkey isolates 2020-2021
Ampicillin	Beta-Lactam	100	100	100	100
Temocillin	Beta-Lactam	0	0	0	0
Amikacin	aminoglycoside	0	NT	3	3
Gentamicin	aminoglycoside	0	2.4	0	0
Azithromycin	macrolide	6	2.4	0	0
Cefepime	cephalosporin (4^{th} gen)	97	93	69	100

Antimicrobial	Antimicrobial class	Chicken isolates 2022	Chicken isolates 2020	Turkey isolates 2022	Turkey isolates 2020-2021
Cefotaxime	cephalosporin (3rd gen)	100	100	100	100
Cefoxitin	cephalosporin (2nd gen)	6	10	29	0
Ceftazidime	cephalosporin (3rd gen)	100	100	100	100
Trimethoprim	trimethoprim	67	61	0	17
Colistin	polymyxin	0	0	0	0
Ertapenem	carbapenem	0	2.4	0	0
Imipenem	carbapenem	0	0	0	0
Meropenem	carbapenem	0	0	0	0
Ciprofloxacin	quinolone	53	66	43	79
Nalidixic acid	quinolone	33	61	9	58
Sulfamethoxazole	sulfonamide	75	88	43	16
Tetracycline	tetracycline	75	90	77	62
Tigecycline	glycylcycline	0	0	0	0
Chloramphenicol	amphenicols	58	63	37	8.3

For ESBL- and/or AmpC- producing isolates from turkey the percentage of isolates with resistance has stayed the same for the majority of antimicrobials since the previous turkey survey (ampicillin, amikacin, ceftazidime). There has been a drop in the occurrence of resistance to cefepime, trimethoprim and both the quinolones (i.e., for ciprofloxacin, from 79 to 43% and nalidixic acid from 58% to 9%). For temocillin, gentamicin, azithromycin, colistin and all the carbapenems they remained I sensitive to these antimicrobials. The percentage of isolates where resistance has increased since the previous survey was to the 2nd-generation cephalosporin cefoxitin, and the older antibiotics sulphamethoxaxole, tetracycline and chloramphenicol. The most significant increase is the increase in resistance to cefoxitin, from zero to 29% of isolates. This increase also can be attributed to the isolation of AmpC-producing *E. coli* in turkeys in 2022, which were all resistant to this antimicrobial. In the previous (2020/2021) turkey survey, no AmpC producers were isolated.

Table 14 Comparison of resistance phenotypes for E. coli from chicken meat isolated on CTX containing media between 2018, 2020 and 2022. Total number of resistant isolates for each AMR phenotype (with percentage number of isolates in

brackets). 0 = all isolates sensitive to antimicrobial.* ESBL-only producers and ESBL+AmpC-producers

Antimicrobial	ESBL* 2018 (n=26)	ESBL* 2020 (n=39)	ESBL* 2022 (n=34)	AmpC 2018 (n=16)	AmpC 2020 (n=2)	AmpC 2022 (n=2)
Ampicillin	26 (100%)	39 (100%)	34 (100%)	16 (100%)	2 (100%)	2 (100%)
Azithromycin	0	1 (2.6%)	2 (5.9%)	0	0	0
Cefepime	26 (100%)	37 (95%)	34 (100%)	13 (81%)	1 (50%)	1 (50%)
Cefotaxime	26 (100%)	39 (100%)	34 (100%)	16 (100%)	2 (100%)	2 (100%)
Cefoxitin	3 (11%)	2 (5%)	0	16 (100%)	2 (100%)	2 (100%)
Ceftazidime	26	39 (100%)	34 (100%)	16 (100%)	2 (100%)	2 (100%)
Chloramphenicol	2 (7%)	26 (66%)	21 (61%)	0	0	0
Ciprofloxacin	13 (50%)	25 (64%)	17 (50%)	4 (25%)	2 (100%)	2 (100%)
Colistin	0	0	0	0	0	0
Ertapenem	0	1 (3%)	0	0	0	0
Gentamicin	0	1 (3%)	0	3 (19%)	0	0
Imipenem	0	0	0	0	0	0
Meropenem	0	0	0	0	0	0
Nalidixic Acid	12 (46%)	24 (62%)	12 (35%)	4 (25%)	1 (50%)	1 (50%)
Sulfamethoxazole	25 (96%)	35 (90%)	26 (76%)	6 (38%)	1 (50%)	1 (50%)
Temocillin	0	0	0	0	0	0
Tetracycline	22 (85%)	35 (90%)	25 (73%)	6 (38%)	2 (100%)	2 (100%)
Tigecycline	0	0	0	0	0	0
Trimethoprim	7 (27%)	24 (62%)	23 (68%)	1(6%)	0	1 (50%)

5.6 Antimicrobial resistance of *mcr-1* positive *E. coli* isolates

All 15 *E. coli* isolates which possessed the *mcr-1* gene (from 4 chicken and 4 turkey samples) were found to be MDR and resistant up to seven different classes of antimicrobials (Table 15). There was no resistance to any of the three carbapenem antimicrobials tested against, and only one isolate demonstrated resistance to the cephalosporin cefepime. This is the second retail chicken survey and the second turkey meat survey in which *mcr-1* gene-harbouring *E. coli* have been identified. In 2020, three chicken samples of Polish origin were found to contain *E. coli* with the *mcr-1* gene (0.95% of samples), whilst in the 2020/2021 turkey survey, two UK samples and one sample from Germany were positive for *mcr-1 E. coli* (1.4% of samples). Unfortunately, the *mcr*-1 *E. coli* isolates from previous years were not MIC tested but were analysed by WGS. This

analysis allows them to be compared to 2022 survey isolates and is described later in section 6.9.

Table 15 MIC results of E. coli isolates harbouring mcr-1. The table shows the antimicrobial resistances of the E. coli isolates from turkey and chicken harbouring mcr-1. The isolates were resistant to many antimicrobials, especially to the quinolone antimicrobials ciprofloxacin and nalidixic acid, plus ampicillin, trimethoprim, sulfamethoxazole and tetracycline. The total number of antimicrobial classes that the isolates were resistant to varied between 4 and 7. Interpretative criteria were EUCAST ECOFFS as stipulated by EU decision 2020/1729/EU (see Table 14). There would be no changes if current EUCAST ECOFFs are used (accessed 27/03/23). R represents resistance to the respective antimicrobial(s) and S represents sensitivity. See glossary for abbreviations to the antimicrobials

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
CH-02898536-COL-a-22	NOT ESBL	R	S	S	S	S	S	R	R	R	S	S	S	R	S	S	S	R	R	R	R	7
CH-02898536-COL-b-22	NOT ESBL	R	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	R	R	R	6
CH-00864920(a)-COL-22	NOT ESBL	R	S	S	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	R	4
CH-00864920(b)-COL-22	NOT ESBL	R	S	S	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	R	4
CH-03038322(a)-COL-22	NOT ESBL	R	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	R	R	R	6

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
CH-03038322(b)-COL-22	NOT ESBL	R	S	S	S	S	S	R	R	R	S	S	S	R	S	S	S	S	R	R	R	6
CH-03038643-a-COL-22	NOT ESBL	R	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	R	R	R	6
TU-03135215-COL3-22	NOT ESBL	R	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	R	4
TU-03135215-COL5-22	NOT ESBL	R	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	R	4
TU-00865017(a)-COL-22	NOT ESBL	R	S	R	S	S	S	R	R	R	S	S	S	S	S	S	S	R	R	S	R	6
TU-00865017(b)-COL-22	NOT ESBL	R	S	S	S	S	S	R	S	R	S	S	S	S	S	S	S	R	R	R	R	7
TU-00826165(a)-COL-22	NOT ESBL	R	S	S	S	S	S	R	S	R	S	S	S	S	S	S	S	S	R	S	R	5
TU-00826165(b)-COL-22	NOT ESBL	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	R	3

Isolate ID	AMR Phenotype	AMP	TMC	FEP	CTX	CAZ	FOX	CIP	NAL	CST	ERT	IPM	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antimicrobial classes resistant to
TU-00823416(a)-COL-22	NOT ESBL	R	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	R	S	R	5
TU-00823416(b)-COL-22	NOT ESBL	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	R	R	R	5

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5.7 Whole genome sequencing (WGS) to determine underlying resistance to cephalosporins in *E. coli.*

WGS was conducted of all 69 *E. coli* isolates obtained from CA-ESBL media to determine the underlying mechanism for 3rd-generation cephalosporin resistance and the MLST sequence type, the results of which are shown in Table 16, Table 17 and Table 18. Most isolates contained one ESBL gene, which included *bla* CTX-M-15, *bla* CTX-M-55, *bla* SHV-12 and *bla* TEM-52c. Determining the underlying mechanism of the resistance to 3rd-generation cephalosporins was not possible from the WGS data for five of the chicken isolates and one of the turkey isolates as no known genes were present in the sequences. Table 19 shows the percentage of chicken and turkey isolates that possessed each ESBL gene type, and the ESBL types that have been observed in previous retail surveys. The most common genes in chicken isolates were *bla* CTX-M-55 (34%) and *bla* SHV-12 (32%), and in turkey isolates the most common gene was *bla*SHV-12 (48%) (Table 19). SHV is a beta lactamase enzyme found in Enterobacteriaceae, of which there are a large number of allelic variants including extended spectrum β-lactamases and non-ESBL variants [23]. Comparing the results of the ESBL gene types from previous surveys, there has also been an observed change in the types of CTX-M genes present in *E. coli* from CA-ESBL media over the years. In 2018 the predominant *bla*$_{CTX-M}$ type was *bla*$_{CTX-M\ 1}$ (with 84% of isolates), whilst for 2020 isolates the *bla*CTX-M types were more varied with *bla*CTX-M 55 (34%) being the most common. In 2022 *bla*SHV-12 was almost equal to the percentage of isolates with *bla*CTX-M 55. The presence of *bla*SHV-12 has not been seen in isolates from these retail surveys before.

For the ESBL isolates from turkey meat, the two main *bla*CTX-M types present in 2020-2021 CTX-M-15 and CTX-M-55 are still present in 2022, but *bla*SHV-12 variant is now predominant.

Table 16, 17 and 18 show that a ESBL gene was determined in the majority of isolates by WGS. The common genes identified in chicken were the same as the common ESBL genes identified in turkey isolates, which were *bla* CTX-M-15, *bla* CTX-M-55 and *bla* SHV-12. For the AmpC isolates, the AmpC promotor mutation was determined in half of the isolates.

Table 16 Presence of ESBL genes in E. coli isolated on CA-ESBL agar and isolates from CTX media with AmpC phenotype. Chicken ESBL-producing isolates phenotype. '-' absence of known ESBL gene according to APHA Seqfinder

Chicken *E. coli* isolate reference	Media	MLST	CTX-M-15	CTX-M-55	SHV-12	TEM-190	TEM-52c	CMY-2	AmpC promoter mutation
CH-02898536-ESBL-22	CA-ESBL	224	-	-	1	-	-	-	-
CH-03135511-ESBL-22	CA-ESBL	10	-	1	-	-	-	-	-
CH-03135312-ESBL-22	CA-ESBL	1163	1	-	-	-	-	-	-
CH-00872268-ESBL-22	CA-ESBL	117	-	-	-	-	-	-	-
CH-03038720-ESBL-22	CA-ESBL	752	-	-	-	-	-	-	-
CH-03038724-ESBL-22	CA-ESBL	10	-	-	-	-	1	-	-
CH-03135198-ESBL-22	CA-ESBL	155	-	-	-	-	-	-	-
CH-00861602-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
CH-00861940-ESBL-22	CA-ESBL	12633	-	1	-	-	-	-	-
CH-02898897-ESBL-22	CA-ESBL	10	-	-	1	-	-	-	-
CH-00865049-ESBL-22	CA-ESBL	2165	-	-	1	-	-	-	-
CH-03038412-ESBL-22	CA-ESBL	1163	1	-	-	-	-	-	-
CH-00803065-ESBL-22	CA-ESBL	349	-	1	-	-	-	-	-
CH-03038592-ESBL-22	CA-ESBL	12633	-	1	-	-	-	-	-
CH-03038584-ESBL-22	CA-ESBL	10	-	-	-	-	1	-	-
CH-03135391-ESBL-22	CA-ESBL	117	-	-	1	-	-	-	-
CH-00861782-ESBL-22	CA-ESBL	752	-	1	-	-	-	-	-
CH-03135216-ESBL-22	CA-ESBL	744	-	1	-	1	-	-	-
CH-00511950-ESBL-22	CA-ESBL	1163	1	-	-	-	-	-	-
CH-00865059-ESBL-22	CA-ESBL	752	-	1	-	-	-	-	-
CH-03038357-ESBL-22	CA-ESBL	1286	-	1	-	-	-	-	-
CH-03038397-ESBL-22	CA-ESBL	10	-	-	1	-	-	-	-
CH-00864920-ESBL-22	CA-ESBL	12069	-	-	1	-	-	-	-

Chicken *E. coli* isolate reference	Media	MLST	CTX-M-15	CTX-M-55	SHV-12	TEM-190	TEM-52c	CMY-2	AmpC promoter mutation
CH-00864914-ESBL-22	CA-ESBL	1163	1	-	-	-	-	-	-
CH-00865080-ESBL-22	CA-ESBL	2165	-	-	1	-	-	-	-
CH-03038363-ESBL-22	CA-ESBL	1137	-	-	1	-	-	-	-
CH-00861806-ESBL-22	CA-ESBL	101	-	1	-	-	-	-	-
CH-00861858-ESBL-22	CA-ESBL	752	-	-	-	-	-	-	-
CH-00864980-ESBL-22	CA-ESBL	12633	-	1	-	-	-	-	-
CH-00864769-ESBL-22	CA-ESBL	1196	1	-	-	-	-	-	-
CH-00864986-ESBL-22	CA-ESBL	57	-	-	1	-	-	-	-
CH-00864836-ESBL-22	CA-ESBL	3232	-	-	1	-	-	-	-
CH-03038339-ESBL-22	CA-ESBL	752	-	1	-	-	-	-	-
CH-03038580-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
CH-03047767-ESBL-22	CA-ESBL	117	-	-	1	-	-	-	-
CH-03047774-ESBL-22	CA-ESBL	12034	-	1	-	-	-	-	-
CH-00864774-ESBL-22	CA-ESBL	752	-	-	-	-	-	-	-
CH-00864702-ESBL-22	CA-ESBL	23	-	-	1	-	-	-	-
CH-00864856-ESBL-22	CA-ESBL	2165	-	-	1	-	-	-	-
CH-03047413-ESBL-22	CA-ESBL	2165	-	-	1	-	-	-	-
CH-03135103-ESBL-22	CA-ESBL	6448	-	1	-	-	-	-	-
CH-00823144-ESBL-22	CA-ESBL	6448	-	1	-	-	-	-	-
CH-00825390-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
CH-00825435-ESBL-22	CA-ESBL	12633	-	1	-	-	-	-	-
Total chicken isolates	–	–	8	15	14	1	2	0	0
Percentage of chicken isolates	–	–	18	34	32	2	5	0	0

Table 17 Presence of ESBL genes in E. coli isolated on CA-ESBL agar and isolates from CTX media with AmpC phenotype. Turkey eSBL-producing isolates. '-' absence of known ESBL gene according to APHA Seqfinder

Turkey *E. coli* isolate reference	Media	MLST	CTX-M-15	CTX-M-55	SHV-12	TEM-190	TEM-52c	CMY-2	AmpC promoter mutation
TU-03038723-ESBL-22	CA-ESBL	457	-	1	-	-	-	-	-
TU-00803068-ESBL-22	CA-ESBL	93	-	1	-	-	-	-	-
TU-02898617-ESBL-22	CA-ESBL	117	-	-	1	-	-	-	-
TU-00343300-ESBL-22	CA-ESBL	93	-	-	-	-	-	-	-
TU-00864801-ESBL-22	CA-ESBL	752	-	1	-	-	-	-	-
TU-00864987-ESBL-22	CA-ESBL	69	-	1	-	-	-	-	-
TU-00864848-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
TU-03038492-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
TU-03047800-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
TU-00864861-ESBL-22	CA-ESBL	515	-	-	1	-	-	-	-
TU-02797658-ESBL-22	CA-ESBL	515	-	-	1	-	-	-	-
TU-00864694-ESBL-22	CA-ESBL	515	-	-	1	-	-	-	-
TU-03047812-ESBL-22	CA-ESBL	515	-	-	1	-	-	-	-
TU-00462790-ESBL-22	CA-ESBL	515	-	-	1	-	-	-	-
TU-00862014-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
TU-00864716-ESBL-22	CA-ESBL	58	1	-	-	-	-	-	-
TU-00864855-ESBL-22	CA-ESBL	3580	1	-	-	-	-	-	-
TU-03047415-ESBL-22	CA-ESBL	69	-	1	-	-	-	-	-
TU-03047426-ESBL-22	CA-ESBL	515	-	-	1	-	-	-	-
TU-00825408-ESBL-22	CA-ESBL	515	-	-	1	-	-	-	-
TU-00825265-ESBL-23	CA-ESBL	155	-	-	1	-	-	-	-
TU-00825266-ESBL-23	CA-ESBL	69	-	1		-	-	-	-
TU-00825268-ESBL-23	CA-ESBL	515	-	-	1	-	-	-	-
TU-00825279-ESBL-23	CA-ESBL	515	-	-	1	-	-	-	-

Turkey *E. coli* isolate reference	**Media**	**MLST**	**CTX-M-15**	**CTX-M-55**	**SHV-12**	**TEM-190**	**TEM-52c**	**CMY-2**	**AmpC promoter mutation**
TU-02996023-ESBL-23	CA-ESBL	2690	-	-	1	-	-	-	-
Total turkey isolates	–	–	6	6	12	0	0	0	0
Percentage of turkey isolates	–	–	24	24	48	0	0	0	0

Table 18 Presence of ESBL genes in E. coli isolated on CA-ESBL agar and isolates from CTX media with AmpC phenotype. Turkey and chicken isolates with AmpC phenotype. '-' absence of known ESBL gene according to APHA Seqfinder

AmpC phenotype *E. coli* isolate reference	Media	MLST	CTX-M-15	CTX-M-55	SHV-12	TEM-190	TEM-52c	CMY-2	AmpC promoter mutation
CH-00861911-CTX-22	McC-CTX	1196	-	-	-	-	-	1	-
TU-00861612-CTX-22	McC-CTX	88	-	-	-	-	-	-	1
TU-00861613-CTX-22	McC-CTX	88	-	-	-	-	-	-	1
TU-00865053-CTX-22	McC-CTX	88	-	-	-	-	-	-	1
TU-02898677-CTX-22	McC-CTX	88	-	-	-	-	-	-	1
TU-03135043-CTX-22	McC-CTX	88	-	-	-	-	-	-	1
TU-00511951-CTX-22	McC-CTX	88	-	-	-	-	-	1	-
TU-00864838-CTX-22	McC-CTX	88	-	-	-	-	-	1	-
TU-03009365-CTX-22	McC-CTX	515	-	-	1	-	-	-	-
CH-03038329-CTX-22	McC-CTX	155	-	-	-	-	-	1	-
TU-00825407-CTX-22	McC-CTX	1706	-	-	-	-	-	-	1
TU-00864877-CTX-22	McC-CTX	88	-	-	-	-	-	1	-
Total AmpC producing isolates	–	–	0	0	1	0	0	5	6
Percentage of AmpC isolates	–	–	0	0	8	0	0	42	50

Table 19 Summary of E. coli MLST sequence types and the presence of ESBL genes or genetic determinants of AmpC AMR phenotype

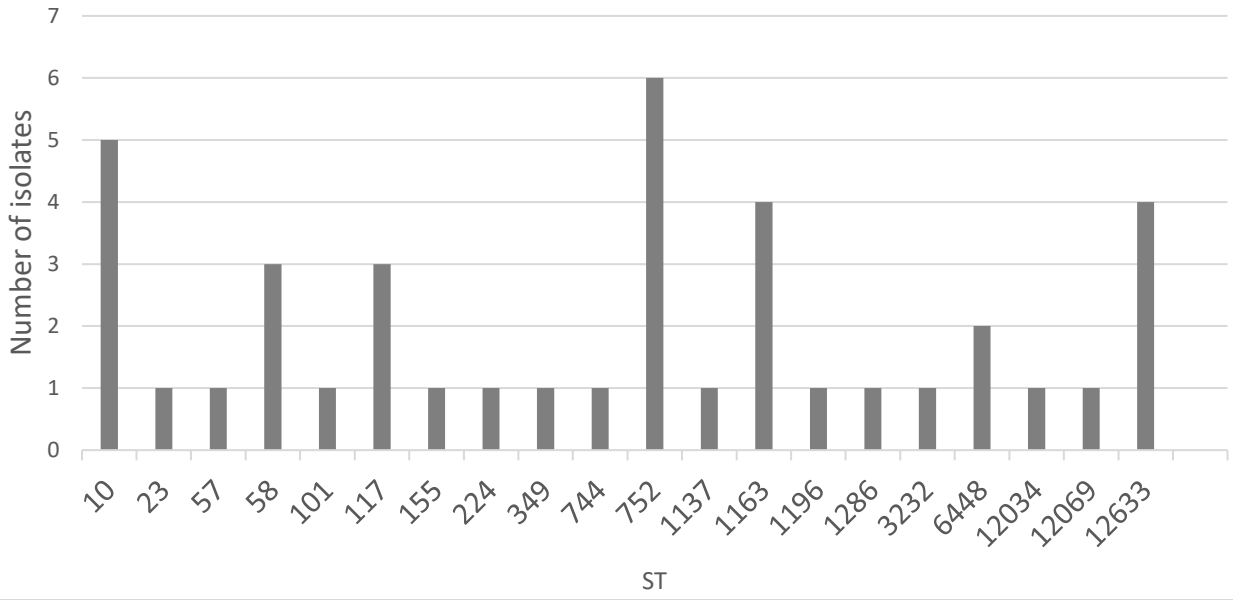
Gene	2018 Chicken	2020 Chicken	2022 Chicken	2020 Turkey	2022 Turkey
CTX-M-1	26 (84%)	10 (19%)	-	-	-
CTX-M-9	-	1 (2%)	-	-	-
CTX-M-14	-	1 (2%)	-	-	-
CTX-M-15	-	-	8 (18%)	13 (52%)	6 (24%)
CTX-M-27	-	3 (6%)	-	-	-
CTX-M-55	1 (3%)	26 (48%)	15 (34%)	6 (24%)	6 (24%)
CTX-M-65	-	-	-	1 (4%)	-
SHV-134	3 (10%)	2 (4%)	-	2 (8%)	-
SHV-12	-	-	14 (32%)	-	12 (48%)
TEM-190	-	-	1 (2%)	-	-
TEM-52c	-	-	2 (5%)	-	-
ND	4	1	-	1	-
No ESBL gene	-	6	5	1	1
Total isolates	31	54	44	25	25

5.8 MLST results of *E. coli* ESBLs

Table 16, Table 17, Table 18 and Figure 1 show the MLST types of the ESBL-producing chicken and turkey isolates (from CA-ESBL media) and the AmpC isolates from McC-CTX media. The chicken and turkey isolates were of diverse STs. Chicken isolates had 20 different STs (see Figure 1a) and turkey isolates have 10 STs (see Figure 1b). Only four STs were common between chicken and turkey isolates, and these were ST58, ST117, ST155 and ST752. The AmpC isolates fell into 5 different STs (see Figure 1c), with ST88 being the most common with 8 out of 12 isolates.

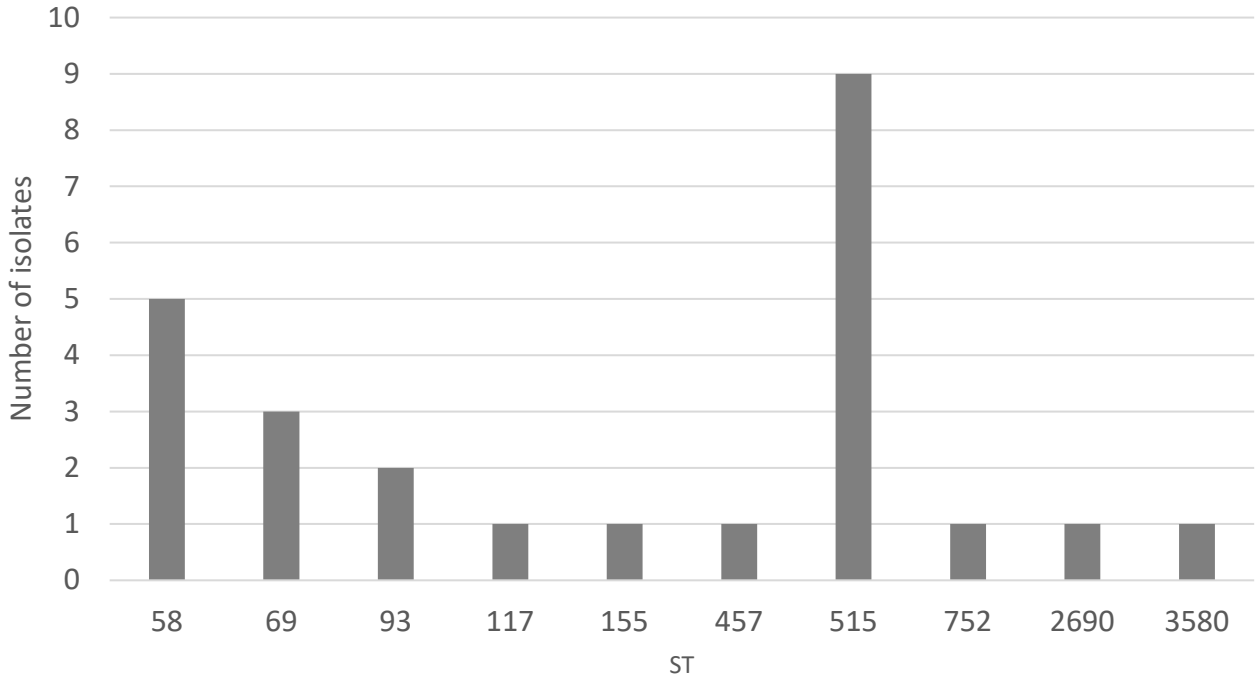
a) ST types for Chicken isolates (from CA-ESBL agar).

Chicken isolates belonged to 20 different ST types, with ST752 being the most common (six isolates), followed by five ST10 isolates.



b) ST types for Turkey isolates (from CA-ESBL agar).

ST 515 was the most common type of *E. coli* ESBL from turkey meat (9 isolates) followed by ST58 (5 isolates).



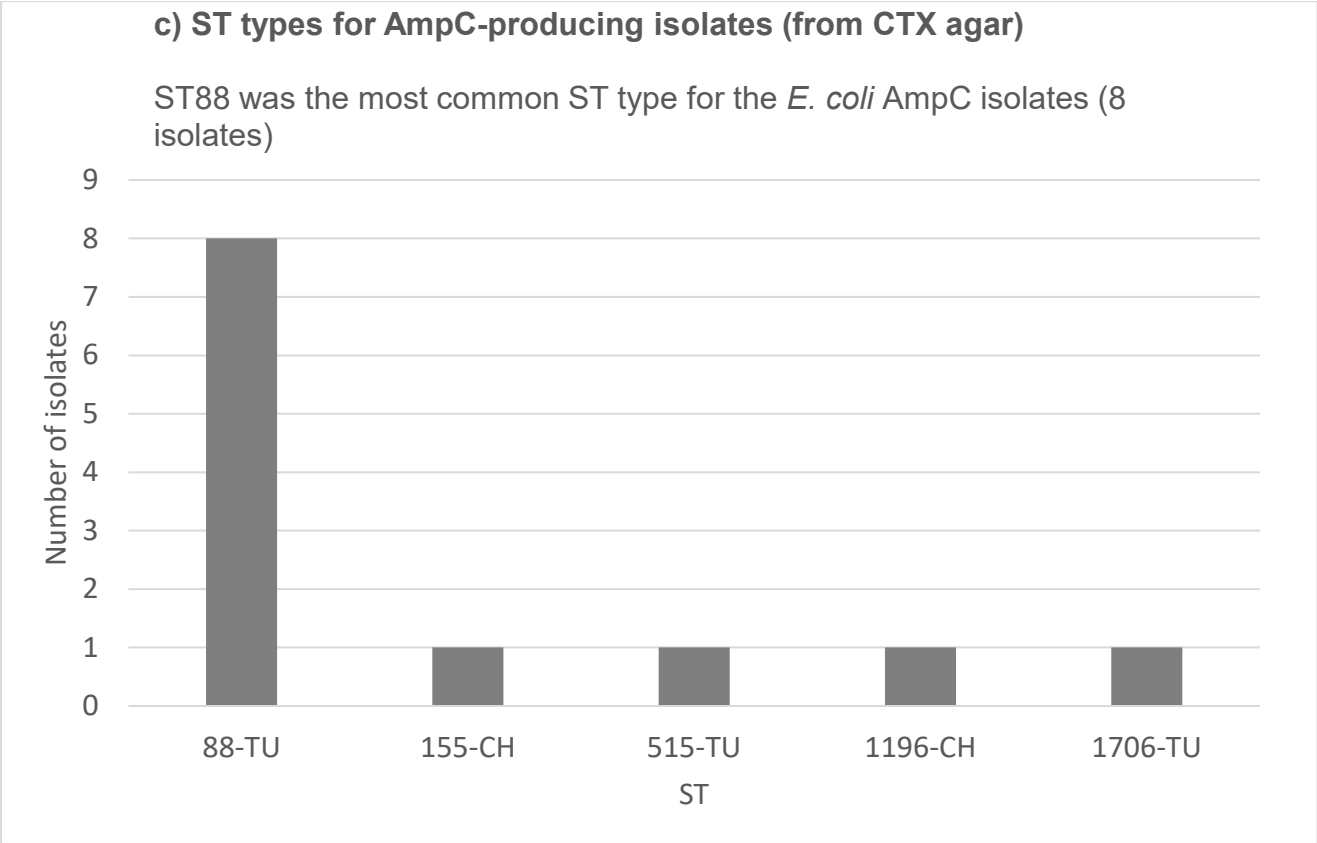


Figure 1 Number of isolates for each ST type (as determined by MLST) for E. coli ESBL producers from chicken (a), turkey (b) and AmpC E. coli isolates (c).

5.9 Results of WGS analysis of *mcr-1* -positive *E. coli*.

WGS analysis confirmed the presence of *mcr-1* genes in all the *mcr-1* PCR positive *E. coli* isolates (see Table 20). The analysis revealed many other genes responsible for encoding AMR in these isolates, both on the chromosome and plasmids. The genes present closely matched the AMR phenotype for the isolates. Many genes that were present were to the older antimicrobials used such as streptomycin (*aph*3 or *aph*6), sulfamethoxazole (*sul*2, *sul*3), tetracycline (*tet*A) and trimethoprim (*dfr*A1). There was also a very good correlation with resistance to quinolones in the *mcr-1*-positive isolates with the presence of a *qnr*B5 or *qnr*S1 gene or having the chromosomal mutation in the *parC* gene. None of the isolates harboured the mutation in *gyr*A or *gyr*B, which is a common mechanism of quinolone resistance in *E. coli*. For one isolate (CH-03038322(b)-COL-22) which exhibited resistance to the quinolone antimicrobials, no known resistance genes/known mutations for conferring quinolone resistance were evident. There were no genes encoding resistance to carbapenem present in any of the isolates. Comparing the sequence types (STs) and genes present in the '*mcr-1*- harbouring' *E. coli* isolates from the 2022 and 2020 surveys

the STs types and the combinations of AMR genes present were different. This suggests that the *mcr-1* isolates in the two surveys represent different clones.

The WGS analysis also showed that the recent isolates do not harbour AMR genes that have not been seen previously in *mcr-1*- positive isolates from the previous surveys (Table 21).

Table 20 Whole genome sequencing analysis results of E. coli isolates from chicken and turkey meat (2022 survey) harbouring a mcr-1 gene. Note that chicken isolates have an isolate ref beginning with 'CH' and turkey isolates with 'TU. Key to genes: *aac3* (Gentamicin*), aadA5* (Spectinomycin, Streptomycin), *ant3* (Streptomycin), *aph3* (Neomycin, Kanamycin*), aph3-1b_strA/aph6-1d_strB* (Streptomycin), HERA-3 (beta-lactams), TEM1, TEM135 (Ampicillin), *catA, catB3* (Chloramphenicol), *cmiA1* (Chloramphenicol, Florfenicol), *cmI* (Chloramphenicol*), mcr-1* (Colistin), InuF (Lincomycin*), mphB* (Erythromycin, Spiramycin, Telithromycin), *qnrB5, qnrS1* (Ciprofloxacin, Nalidixic Acid), *sat2A* (streptothricin), *sul2, sul3* (Sulfamethoxazole), *tetA, tetAB (*Tetracycline*), dfrA* (Trimethoprim)

2022 Isolate ref	MLST	AMR genes	Plasmid type	Country of origin *
CH-02898536-COL-a-22	457	*ant(3")-Ia, aph(3")-Ia, aac(3)-IId, bla$_{TEM-1B}$, cmlA1, floR, mcr-1.1,* InuF*, parC, sul2, sul3, dfrA12, tetA*	Col-156, IncFIB, IncFIC, IncFII, IncX4	UK
CH-02898536-COL-b-22	624	*ant(3")-Ia , aph(3")-Ib , aph6-Id, bla$_{TEM-1c}$, mcr-1.1, parC, sat2A, sul2,dfrA1, tetA(B)*	Col-156, IncFIB, IncFII, IncX4	UK
TU-03135215-COL3-22	533	*bla$_{TEM-1B}$, mcr-1.1, parC, tetA*	Col-156, Col-RNAI, IncFIB, IncFIC, IncFII, IncX4, Col440I	Poland
TU-03135215-COL5-22	533	*bla$_{TEM-1B}$, mcr-1.1, parC, tetA*	Col-156, Col-RNAI, IncFIB, IncFIC, IncFII, IncX4, Col440I	Poland
CH-00864920(a)-COL-22	1286	*bla$_{TEM-135}$, mcr-1.1, qnrB5, tetA*	Col-MG828, IncHI2A, IncX4, p0111, Col440I	Netherlands
CH-00864920(b)-COL-22	1286	*blaTEM-135, mcr-1.1, qnrB5, tetA*	Col-RNAI, IncHI2A, IncX4, p0111, Col440I	Netherlands
CH-03038322(a)-COL-22	117	*ant(3")-Ia, aph(3")-Ic, bla$_{TEM-1B}$, mcr-1.1, parC, sat2A, sul2, dfrA1, tetA(B)*	Col-pVC, IncFIB, IncFIC, IncFII, IncI2, Col440I	Netherlands

2022 Isolate ref	MLST	AMR genes	Plasmid type	Country of origin *
CH-03038322(b)-COL-22	117	*aadA5, aph(3")-Ia , aac(3)-IId, HERA-3, catB4, mcr-1.1, sul3, dfrA1, tetA(B)*	Col-156, IncBOKZ, IncFIB, IncFIC, IncFII, IncI1, IncI2, Col440I	Netherlands
TU-00865017(a)-COL-22	398	*aph(6)-Id, bla$_{TEM-1B}$, floR, mcr-1.1, qnrS1, tetA*	Col-RNAI, IncX4, Col440I	Poland
TU-00865017(b)-COL-22	6803	*ant(3")-Ia , bla$_{TEM-135}$, cmlA1, mcr-1.1, qnrB19, qnrS1, sul3, dfrA15 , tetA*	Col-RNAI, IncFIB, IncFII, IncX, IncY, Col440I	Poland

Table 21 WGS results for mcr-1 positive isolates from previous APHA surveys.

2020 survey isolate ref	MLST	AMR genes	Plasmid type	Country of origin
2798047 Chicken breast 5-8-20	162	*aadA5, blaTEM-1B, catA1,dhfrA17, qnrB19 mcr-1.1, tetB*	Col(pHAD28), IncFIB(AP001918), IncFIC(FII), IncHI2, IncHI2A, IncI2(Delta), IncX1	Poland
563345 Whole chicken 3-8-20	1011	*aph(6)-Id, aph(3")-Ib blaACC-1a*	Col(BS512), Col(Ye4449) IncFIB(AP001918), IncFIC(FII)	Poland
563345 Whole chicken 3-8-20	10	*aadA1, aph(3")-Ib aph(6)-Id, blaTEM-1B dhfrA1, sul2*	Col(BS512), ColpVC IncFIB(AP001918) IncFIC(FII), IncI1-I (Gamma) p0111	Poland
2798073 Chicken breast 6-10-20	744	*aph(3")-Ia/Ib, aph(6)-Id blaTEM-1B, catA1 mcr-1.1, sul2, tetB*	IncFIA, IncFIB(AP001918) IncFIC(FII), IncQ1, IncX4	Poland
2798073 Chicken breast 6-10-20	93	*aadA1, aph(3")-Ib, aph(6)-Id, blaTEM-1, catA1, dhfrA1/8 mcr-1.1, sul2, tetA/B*	IncFIA, IncFIB(AP001918) IncFIC(FII), IncI1-I(Gamma), IncQ1, IncX4	Poland
2798073 Chicken breast 6-10-20	744	*aadA1, aph(3")-Ia/Ib, aph(6)-Id, blaTEM-1B, catA1, dhfrA1, mcr-1.1, sul2, tetB*	ColpVC IncFIA, IncFIB(AP001918), IncFIC(FII), IncI1-I(Gamma), IncQ1, IncX4	Poland
T00512133 Turkey Crown joint 9-12-20	8778	*blaTEM-1B, dfrA36,mcr-1.1, mdf(A), qnrS1, qnrB19, sul2.*	Col(pHAD28), IncX1/4.	UK
T00512133 Turkey Crown joint 9-12-20	8778	*blaTEM-1B, dfrA36, mcr-1.1, mdf(A),qnrS1, qnrB19, sul2.*	Col(pHAD28), IncX1/4.	UK
T00512133 Turkey Crown joint 9-12-20	8778	*blaTEM-1B, dfrA36, mcr-1.1, mdf(A), qnrS1, qnrB19, sul2.*	Col(pHAD28), IncX1/4.	UK
T00512101 Turkey Crown joint 16-12-20	889	*aadA1/2, aph(3")-Ib, aac(3)-IId, aph(6)-Id, catA1, cmlA1, blaTEM-1B, blaOXA-1 dfrA1/12/15/36, floR, mcr-1.1, df(A),qnrS1, sul1,2,3, tet(A).*	Col(MG828), Col156, IncFIA, IncFIB(AP001918), IncFII, IncHI2/A, IncI1-I(Alpha), IncI2(Delta), IncQ1, IncR, IncX4, IncY, p0111.	UK
T00512101	58	*aadA1/2, aph(3")-Ia/Ib, aph(6)-Id,catA1, cmlA1, blaTEM-*	Col(MG828), Col156, Col440I, IncFIA,	UK

2020 survey isolate ref	MLST	AMR genes	Plasmid type	Country of origin
Turkey Crown joint 16-12-20		*B/106/126/135/220, blaOXA-1,dfrA1/12/15/36, floR, mcr-1.1, mdf(A), qnrS1, sul1,2,3, tet(A).*	IncFIB(AP001918), IncFIC(FII), IncFII, IncHI2/A, IncI1-I(Alpha), IncR, IncX1/4, p0111.	
T00512101 Turkey Crown joint 16-12-20	58	*aadA1, aph(3")-Ib, aph(6)-Id, catA1, cmlA1, blaTEM-1B/106/126/135/220, blaOXA1, dfrA1/12/36, floR, mcr-1.1, mdf(A), qnrS1, sul1,2,3, tet (A).*	Col(MG828), Col156, Col440I, IncFIA, IncFIB(AP001918), IncFIC(FII), IncI1-I(Alpha), IncR, IncX1/4, p0111.	UK
T00512003 Turkey breast 4-01-21	744	*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).*	IncFIB(AP001918), IncFII, IncFII(pSE11), IncI2, IncQ1, p0111.	Germany
T00512003 Turkey breast 4-01-21	93	*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).*	IncFIB(AP001918), IncFII(pSE11), IncI2, IncQ1.	Germany
T00512003 Turkey breast 4-01-21	744	*aadA5, aph(3')-Ia, aph(3")-Ib, aph(6)-Id, catA1, blaTEM-1B, dfrA17, mcr-1.1, mdf(A), sul1,2, tet(B).*	IncFIB(AP001918), IncFII, IncFII(pSE11), IncI2, IncP6, IncQ1, p0111.	Germany

6. *E. coli* - discussion

AMR surveys of retail meat have been conducted at APHA since 2016 for chicken, and since 2020/2021 for turkey (alternate years) using the same methodology and thereby allowing the results to be compared directly. The 2022 survey findings for chicken meat have found that the proportions of retail samples that are positive for presumptive ESBL- and/or AmpC-producing *E. coli* have only marginally dropped compared to 2020 (from 13% of samples to 11.8%). The most significant decrease between the APHA surveys occurred between 2016 and 2018 where the prevalence of ESBL and/or AmpCs in retail chicken decreased from 45% to 13.6 %. These results were published in 2020 by Randall *et al*, 2017 and it was considered that significant reductions in antimicrobials used in the UK poultry meat sector since 2014 and 2017 may have caused this drop [24, 25]. In contrast the prevalence of ESBL-and/or AmpC-producing isolates in turkey has stayed the same 2022 and 2020/2021 survey at 11%.

In the EU AMR harmonised monitoring surveys in food-producing animals there has been a continuing decreasing trend of the prevalence of ESBL- and AmpC-producing organisms since the surveys began in 2016. For all MS combined the prevalence has dropped from 39.8% in 2018, to 31.5% in 2021. The total drop of prevalence in broiler meat for all MS combined has been 51% in total over the whole period [26]. Turkey meat is not monitored in the EU AMR monitoring survey, but in the caecal contents of fattening turkeys the prevalence of ESBL- and/or AmpC-producing isolates has decreased from over 40% in 2016 to 34.2% in 2020. These decreasing trends are statistically significant and generally paralleled with statistically significant decreases observed in most reporting countries.

One observation in this 2022 UK survey is the increased prevalence of AmpC-producing *E. coli* in turkey samples compared to the previous survey, with an increase from zero prevalence in 2020-21 to 3.3% in 2022. This increase in AmpC-producing *E. coli* has not been observed in chicken samples, which has been decreasing year on year the survey has been conducted. Interestingly 6 of the AmpC-producing *E. coli* from turkeys (out of 10) had the same MIC profile and the same MLST type ST88 (from 2 different retailers) which may suggest that these isolates could be a clone (further analysis would be required to determine this). Another possible reason why less AmpC-producing isolates were obtained in turkeys in 2020-21 was that the survey was conducted over a shorter sampling period from October 2020 to February 2021 (a delay due to Covid) and less samples (*n*=210) were tested, so we may have missed detecting AmpC producers in that survey. For the EU AMR harmonised surveys, a decrease was seen in the average prevalence of AmpC producers in fattening turkeys for all MS from 7.9 % in 2018 to 5.3% in 2020. UK data provided by Northern Ireland reported a prevalence of 0.4% in fattening turkeys in 2020 [27].

AmpC beta-lactamases in *E. coli* can be due to upregulation and thus overexpression of chromosomally- encoded existing AmpC genes or to enzyme production by plasmid-encoded transferable genes. WGS of the AmpC-producing isolates from the survey revealed that six out of the ten AmpC producers from turkey meat contained the chromosomal mutation which causes the gene to be overexpressed, whilst three of the turkey isolates possessed the plasmid-encoded gene $bla_{\text{cmy-2}}$. The underlying mechanism of the AmpC resistance was not elucidated in one of the isolates displaying that was displaying an AmpC phenotype. The monitoring of AmpC producers in turkey meats needs to be continued to determine if the increase in prevalence is real and warrants investigation. The AmpC-producing isolates (from turkeys and broilers) had similar

resistances to ESBLs with resistance to three different cephalosporins (but not to cefepime) nor to carbapenems which is the normal enzymatic activity phenotype In *E. coli* AmpC isolates [28, 29]. The *bla*$_{cmy-2}$ AmpC gene type was shown by WGS to be present in one third of the AmpC isolates. This gene type has a broad geographic spread and is one of the main causes of beta lactam resistance at present [30] [31]. There has been a worldwide increase in *Klebsiella pneumoniae* and *E. coli* isolates in humans and animals with the plasmid encoded *bla*$_{cmy-2}$ AmpC B-lactamase gene in clinical isolates especially in Asia [32]. This increase is thought to have occurred by dissemination of plasmids harbouring the *bla*$_{cmy-2}$ via the food production chain. Antimicrobial usage in food animals is suspected [33-35].

6.1 Resistance to 'last resort' antibiotics

As in previous years, none of the samples gave rise to isolates on the two agars that selected for carbapenem-resistant *E. coli,* suggesting that in the UK retail chicken meat samples are not contaminated with carbapenem-resistant *E. coli.* With respect to the colistin, we have isolated colistin-resistant *E. coli* from eight meat samples where the *E. coli* isolates harbour the *mcr-1* gene. *mcr* genes have become globally disseminated over the past few years in *E. coli* and other Enterobacteriaceae.

Resistance to colistin is of great concern to governments, medics and scientists across the world. Colistin is a Polymyxin antimicrobial, and as such is included in the five antimicrobials listed by the WHO as 'critically important and of 'highest priority' for human medicine. This antimicrobial has been used extensively in farm animals all over the world, including in Europe [36] although usage has dramatically reduced in recent years since the European Medicines Agency (EMA) 2016 recommendations to reduce colistin use in animals to decrease the risk of antimicrobial resistance. These recommendations also included that colistin-medicines should be reserved for treatment of animals where no effective alternative treatments exist [37].

6.2 Colistin *mcr-1*- positive *E. coli*

The isolation of low levels of *mcr-1* harbouring *E. coli* in UK retail chicken and turkey meat (1.3% for both meat types) is not surprising since they have been isolated previously in UK retail chicken and turkey meat in 2020 and 2021/21 respectively and their incidence does not appear to have increased (from 0.95% in chicken and 1.4 % in turkey). In the EU AMR harmonised monitoring survey in 2020/2021 eight out of 32 MS reported colistin- resistant *E. coli* isolates from broiler caeca and seven of the 13 countries reported it in turkeys [26].

Three countries showed a decrease in prevalence in broilers (Italy, Portugal, Romania) and increasing trends were observed in Bulgaria and Cyprus. In turkeys, no statistically significant trends were observed [26].

Performing MICs for the *mcr-1*- positive *E. coli* of our retail meat survey confirmed that all were colistin resistant, but none were resistant to cefotaxime. In the original Chinese report on the emergence of *mcr*-1, the study reported the co-existence of CTX-M genes and *mcr-1* [38]. In this study, WGS has not detected any ESBL genes in any of the *mcr-1*- positive isolates and this correlates with the sensitivity to cefotaxime by MIC testing. Other MIC results correlated well with the WGS results.

In this study, we have conducted WGS of all *mcr-1*- positive *E. coli* isolated from the survey and compared them to the *mcr*- positive isolates of the previous surveys. These *mcr-1*- positive isolated have been found to possess a range of AMR genes encoding resistance to aminoglycosides, chloramphenicol, penicillins (but not cephalosporins), quinolones, sulphonamides, tetracycline and trimethoprim, and that they harbour many of the same genes as seen in the isolates from previous surveys. Other genes to note are the presence of the gene for macrolide resistance (*mph*) which was observed in an *E. coli* isolate from Polish chicken meat in 2020 and from turkey meat of German origin in 2021. Also, *flo*R gene was identified in one isolate from chicken isolate in this survey (from UK origin meat) and a *mcr*-1 positive *E. coli* turkey isolate, also of UK origin from the 2020-21 turkey survey. This plasmid mediated gene encodes for resistance to the antimicrobial florfenicol (a derivative of chloramphenicol) that is used for the treatment of animal diseases. This gene has been reported in *Klebsiella pneumoniae* in isolates in China [39]. Lastly, in this survey *par*C gene mutation has been observed in some of the quinolone resistant *mcr*-1 positive isolates. This gene has not been observed in *mcr*-1 resistant isolates in previous surveys. If *par*C gene mutation was not present then the isolates possessed the quinolone resistance genes q*nr*S1, or *qnr*B5 instead.

The diverse range of AMR genes present in the *mcr-1* positive *E. coli* also mirrors the large array of plasmid DNA sequences present. Isolates present in chicken and turkey meat from Netherlands, Lithuania and Poland contained DNA of eight different plasmids or Inc types. To date, *mcr* genes have been detected in plasmids of diverse incompatibility (Inc) types, with IncI2, IncHI2, and InX4 being the most abundant types identified [40]. In 2020, we conducted additional in-depth analysis of the DNA sequences (long and short read WGS) of some of the *mcr-1 E. coli* isolates from the retail chicken survey and

elucidated that in two of them that the *mcr-1* gene resided on an Inc-X4 plasmid, and that other AMR genes were not present with the *mcr-1* gene.

These retail surveys have shown that *E. coli* harbouring *mcr*-1 gene in meats are very diverse. Long read sequencing would be required to elucidate the location of the *mcr-1* gene in the genome and if other resistance genes are present on any mobilizable elements.

6.3 Antimicrobial resistances of *E. coli* ESBLs

As in previous years the ESBL and/or AmpC-producing *E. coli* isolates from UK retail chicken and turkey were found to be MDR, with chicken and turkey isolates being resistant to up to seven and six different classes of antimicrobials respectively. MDR in commensal *E. coli* and ESBL *E. coli* from humans and animals is not uncommon. All *E. coli* isolates from chicken and turkey in this survey were resistant to common antimicrobials such as ampicillin, sulfamethoxazole or tetracycline (and chicken ESBL isolates were also resistant to trimethoprim). Antimicrobials such as ampicillin, sulfamethoxazole and tetracycline have been widely used for many years in veterinary medicine to treat infections in production animals [1]. The WHO categorises ampicillin as a 'critically important antimicrobial' (CIA), while sulfamethoxazole, trimethoprim and tetracycline are categorised as 'highly important antimicrobials' [10].

Resistance to 'highest priority' CIAs was very low for colistin and for azithromycin (turkey isolates were sensitive to azithromycin), but resistance to third-generation cephalosporin cefepime was high with 100% resistant (and resistance was observed as expected to ampicillin and cefotaxime). All isolates however were sensitive to cefoxitin, which is the first year in *E. coli* isolates from broilers where this has been observed.

In the EU harmonised survey of 2020, indicator *E. coli* (no data reported for ESBLs from meat) resistance to ampicillin, sulfamethoxazole, trimethoprim and tetracycline was the most common resistance trait observed, with large differences in resistance levels between countries were observed in food-producing animals [41]. EFSA reported statistically significant decreasing temporal trends in resistance to ampicillin, ciprofloxacin, cefotaxime, tetracycline and colistin in Europe, as well as increasing trends in isolates which are completely sensitive, and progress towards lower levels of resistance in several countries and in the EU group as a whole.

When looking at changes in resistance to antimicrobials in the ESBL and/or AmpC-producing *E. coli* from across our surveys, we are seeing small reductions in the resistance to most antibiotics, but not to any of the cephalosporins (except cefoxitin which has dropped). For the fluoroquinolones, there has been no change to *E. coli* resistant to ciprofloxacin in chicken since 2018, but a decline in resistance to nalidixic acid has been observed. We have seen a reduction in resistance to the cephalosporin cefepime in turkeys.

In 2021, amikacin was added to the harmonised panel for the monitoring of AMR in indicator and ESBL *E. coli*. While amikacin is not used in food-producing animals in the UK, it can be used in people to treat urinary tract infections, bacteraemia and intra-abdominal infections caused by Gram negative bacteria. The addition of amikacin to the harmonised panel is intended to improve the detection of 16S RNA methyltransferases (RMTases) which confer high-level resistance to amikacin [22, 42]. RMTases have been increasingly found in association with carbapenemases, AmpC or ESBL enzymes and fluoroquinolone resistance in Enterobacterales from humans in Europe [43, 44].

In our study, we did identify one ESBL and AmpC-producing *E. coli* from turkey meat that had a low level of resistance to the aminoglycoside amikacin (MIC=16, 1 doubling dilution over the ECOFF and clinical break point R>8). WGS analysis did not reveal the presence of any recognised genes that confer resistance to aminoglycosides.

Resistance to amikacin in Enterobacterales can be caused by multiple mechanisms, including 16S rRNA methyltransferases [45] or possession of aminoglycoside modifying enzymes. The most common cause is the aminoglycoside *N*-acetyltransferase AAC(6′)-Ib, that acetylates amikacin, tobramycin, kanamycin and netilmicin but not gentamicin [46]. This gene can be found in association with integrons, transposons, plasmids as well as on the chromosomes of gram-negative bacteria [47]. Recently a novel aminoglycoside acetyltransferase gene (*aac* (6')-Iao) and a 16S rRNA methyltransferase (RMTase) gene labelled *rmtl* has been recently discovered in human fecal microbiota using metagenomics [48]. Rmtl conferred high resistance to 4,6-disubstituted 2-DOS aminoglycosides and shared the closest amino acid identity of 32% with ArmA from Klebsiella pneumonia, whilst AAC(6')-Iao showed 48% identity to AAC(6')-Ian from a clinical isolate of *Serratia marcescens*. The identification of a low-level amikacin resistant *E. coli* isolate in the absence of any specific resistance mechanism identified through WGS could be related to aspects such as cell wall permeability or efflux; the inherent test variation of one doubling

dilution in the MIC test may also be relevant when assessing the correlation between phenotypic and genotypic data.

6.4 Molecular characterisation of *E. coli* strains using MLST types

The sequence types (STs) of the *E. coli* isolates from chicken and turkey isolates were diverse with 20 and 10 different STs identified in isolates from chicken and turkey. Only four *E. coli* STs (ST58, ST117, ST155 and ST752) were common in both chicken and turkey.

The AmpC-producing isolates fell into five different STs, with ST88 being the most common with 8 out of 12 isolates (see figure 1). None of the isolates possessed the common ST types found in humans such as ST131, ST405 and ST10/38 [49] except for ST88 which is globally distributed in humans and animals [50]. ST88 was the common ST type of the AmpC isolates.

6.5 *E. coli* ESBL gene types

Our retail surveys have shown that there has been a change in the $bla_{\text{CTX-M}}$ gene types (CTX-M type) in ESBL-producing *E. coli* across the years. In 2018 CTX-M-1 was the dominant ESBL gene type in chickens (in 84% of ESBL isolates) but in 2022 this has been totally replaced with CTX-M-15 and CTX-M-55 and SHV-12 ESBL types. This is not unexpected as horizontal transfer of the ESBL genes (*bla*) on plasmids occurs between bacteria of the same or closely related species in the intestinal microbiota of animals and humans, and therefore CTX-M types are changing temporally across the world [51-55].

In this 2022 survey, a large number of the chicken isolates and turkey isolates possessed ESBL variant SHV-12 (32% and 48% respectively). This is the first year where this variant has been identified. In previous years the majority of ESBLs possessed a CTX-M gene variant. SHV-ESBLs are usually encoded by self-transmissible plasmids and mobile genetic elements and have become widespread throughout the world in *Klebsiella pneumoniae* and *E. coli* and in other Enterobacteriacae [23]. Studies in broilers and retail poultry meats in different European countries are reporting the presence of the ESBL gene $bla_{\text{SHV-12}}$ alongside CTX-M-1, [56-60]. In our study, for chicken isolates, SHV-12 is equally dominant with ESBL type CTX-M-55 (at 32% and 34% respectively) followed by CTX M-15 (18%). In turkeys SHV-12 is dominant (48%) with CTX-M-15 at 24% and CTX-M-55 (24%).

For many years the dominant CTX-M types in chickens and humans have not been the same and studies have concluded that the ESBLs in chicken meats are not a major source

of ESBLs in humans [49, 61]. This study suggests that 18% of chicken and 24% of turkey ESBL isolates are now the same CTX-M type as humans, which is CTX-M-15. This suggests that there may be a link between animals and humans. This CTX-M type has been the dominant type in humans since 2003 and is widely disseminated across many countries including Europe and UK [49, 61, 62]. None of the ESBL CTX-M-15 variants from our retail survey were ST131, which is associated with the human pandemic O25-ST131 CTX-M-15-producing clone [63, 64].

7. *Salmonella* spp. results

7.1 *Salmonella* counts pre-enrichment and detection post-enrichment.

Of the 608 chicken and turkey samples tested, only eight (1.3%) were found to be positive for *Salmonella* spp. using the ISO 6579:1 2017 method for the detection of *Salmonella* in the food chain. Six of the *Salmonella* isolates were from chicken samples (1.9%, 95% CI [0.3-3.5%]) and two (0.7%, 95% CI [0-1.6%]) from turkey samples (Table 22). The five fresh turkey samples that had been previously frozen (under the derogation period) were negative for *Salmonella* spp.. The *Salmonella* serotyping results are shown in Table 23. Four isolates were serotyped as *S*. Agona, three of these were from chicken and one from a turkey sample. The other serovars were *S*. Infantis, *S*. Mbandaka, and *S*. Paratyphi B variant Java which were from chicken samples, and one *S*. London from a turkey sample.

The miniaturised most probable number (MPN) technique did not allow an estimation of the levels of *Salmonella* spp. organisms present in the eight meat samples as the results were all negative for *Salmonella* spp. The same homogenates had been used for both methods, but the eight samples where *Salmonella* spp. had been detected, were negative by the mini MPN method.

Table 22 Salmonella spp. detected in chicken and turkey samples.

Meat	No tested	No *Salmonella* positive (%) after enrichment	Counts (MPN method)
Chicken	306	6 (1.9)	0
Turkey	302	2 (0.7)	0

Why the MPN method failed to detect *Salmonella* spp. is likely due to the low levels of salmonella organisms in the meat samples. Differing amounts of test material was used following the ISO standard methods, which is 25g of meat for the direct detection method, but only 0.2g of sample for the MPN method (per well). The mini MPN method is not appropriate for enumeration of *Salmonella* spp. in low levels and the ISO advises to use the conventional MPN for samples with low numbers. The standard MPN method involves enriching 25g of meat in BPW, whilst the mini MPN method only uses 0.2g of sample for detection. The advantage of the mini MPN is that it takes less time to conduct and uses fewer resources.

Table 23 Salmonella serovars and AMR detected in chicken and turkey samples. NT=not tested, AMP=Ampicillin, GEN=Gentamicin, TET=Tetracycline, - no AMR

***Salmonella* serovar**	**Meat type and cut**	**Country of origin**	**Meat ID**	**AMR by MIC testing**
Agona	Chicken leg	UK	CH-03038552	-
Infantis	Whole chicken	UK	CH-00865002	-
Mbandaka	Chicken breast	UK	CH-03038387	-
Agona	Whole chicken	UK	CH-00865019	-
Agona	Chicken leg	UK	CH-00864702	AMP
Paratyphi B variant Java	Chicken breast	Netherlands	CH-03038363	NT
Agona	Turkey breast	UK	TU-00825387	AMP, GEN, TET
London	Turkey Crown	UK	TU-00825279	-

7.2 AMR phenotypes of *Salmonella* (as determined by MIC testing)

Microbroth dilution testing was conducted using the same panel of 20 antimicrobials as used for *E. coli*. Only seven of the eight *Salmonella* spp. isolates could be tested as one was found to be a *S*. Paratyphi B variant Java and therefore was moved to a higher containment laboratory where MIC testing was not possible. MIC testing showed that only two isolates were resistant to antimicrobials, and no isolates possessed a ESBL phenotype. The two isolates that had resistances were both *S*. Agona (see Table 24). One was from chicken which was resistant to ampicillin only (CH-00864702), and the other was from turkey (sample TU-00825387), and was resistant to ampicillin, gentamicin and

tetracycline. *S*. Agona is in the top 20 most frequent *Salmonella* serovars that caused human outbreaks in 2021 in the EU, which may indicate a link to the consumption of contaminated chicken [65].

Table 24 MIC results of *Salmonella* spp. isolates against panel of 15 antimicrobials. The table shows that only *S.* Agona possessed a resistance phenotype. There would be no changes to the *Salmonella* interpretations if current EUCAST ECOFFs are used instead of the ones stipulated by the EU decision 2020/1729/EU. Interpretative criteria were EUCAST ECOFFs as stipulated by EU decision 2020/1729/EU (see Table 14). R denotes resistance and S denotes sensitivity. See glossary for key to antimicrobials.

Sample	Serotype	AMP	CTX	CAZ	CIP	NAL	CST	MEM	GEN	AMK	AZI	TGC	CHL	SUL	TMP	TET	No. of antibiotic classes resistant to
CH-03038552-Salm-22	Agona	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
CH-00865002-Salm-22	Infantis	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
CH-03038387-Salm-22	Mbandaka	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
CH-00865019-Salm-22	Agona	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
CH-00864702-Salm-22	Agona	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1
TU-00825387-Salm-22	Agona	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R	3
TU-00825279-Salm-22	London	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0
Total no of isolates resistant	–	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1	–
Percentage of isolates resistant	–	29	0	0	0	0	0	0	14	0	0	0	0	0	0	14	–

7.3 AMR Genotype as determined by WGS and comparison to AMR phenotype.

WGS of the eight *Salmonella* isolates showed that they harboured between 1 and 6 AMR genes (see Table 25). The AMR phenotype matched the AMR genotype for all isolates tested except for the presence of *aac6*, which is the gene which encodes for gentamicin resistance. This gene however is redundant in *Salmonella* and explains why resistance not observed by MIC testing. For the *S*. Paratyphi B variant Java, where MIC testing could not be conducted, whole genome sequencing revealed the presence of genes that confer resistance to ampicillin ($bla_{\text{TEM-1B}}$), lincomycin (*lnu*G), streptothricin (*sat*2a) and trimethoprim (*dfr*A1).

Additional AMR genes identified in the *Salmonella* spp. isolates were *fosA* which confers resistance to an old antibiotic called fosfomycin (*fosA*). This gene was present in two *S*. Agona chicken isolates (CH-03038552 and CH-00864702). Lincomycin (*InuG*) and Spectinomycin, Streptomycin (*aadA*) resistance genes were also present in the *S*. Agona isolate CH-00864702. As these antimicrobials are not part of the MIC panel phenotypic resistance to these were not determined.

Table 25 AMR genes identified by whole genome sequencing of Salmonella spp. detected in chicken and turkey samples. NT= Not tested. Key to AMR genes and resistance encoded:
***aac3-IId* (Gentamicin), *aac6-Iy* (Gentamicin), *aadA* (Spectinomycin, Streptomycin), *ant3* (aminoglycosides), *dfrA* (Trimethoprim), *fosA* (fosfomycin), *InuG* (Lincomycin), sat2A (streptothricin), *TEM-1b* (Ampicillin), *tetA* (Tetracycline),**

***Salmonella* serovar**	**Meat ID**	**AMR by MIC testing**	**WGS Genes**
Agona	CH-03038552	none	*aac6-Iy, fosA*
Infantis	CH-00865002	none	*aac6-Iy*
Mbandaka	CH-03038387	none	*aac6-Iaa*
Agona	CH-00865019	none	*aac6-Iy*
Agona	CH-00864702	AMP	*aac6-Iy, aadA, fosA, InuG, TEM-1b,*

Salmonella serovar	Meat ID	AMR by MIC testing	WGS Genes
Paratyphi B variant Java	CH-03038363	none	*aac6-ly, dfrA1, lnuG, sat2A, TEM-1b,*
Agona	TU-00825387	AMP, GEN, TET	*aac6-ly, aac6-laa, ant3, aac3-lld, TEM-1b, tetA*
London	TU-00825279	none	*aac6-ly*

8. *Salmonella* spp. isolates - discussion

The prevalence of *Salmonella* spp. in retail meat in 2022 was shown to be 1.9 % and 0.7% in UK retail chicken and turkey meat respectively. There has been a lack of recent studies on the prevalence of *Salmonella* spp. in UK retail meat. In 2011, there was a study of fresh retail chicken meat from Republic of Ireland, of which 25% of the samples were produced in the United Kingdom, 5.1% of which were positive for *Salmonella* spp. [66]. A more recent survey in 2020 showed that 6.3% of frozen ready to cook- chicken products (primarily breaded) of UK origin were positive for *Salmonella* spp. [67]. The levels from both these studies are higher than in our 2022 survey; we cannot however directly compare the studies, as they are not the same time period, same sample type, same methodology or survey design. The frozen poultry products were either comminuted and reformed or non-comminuted but reformulated and contained other ingredients. This study did enumerate the *Salmonella* in these products using the full MPN method, and found that the average MPN/g of the samples (from all countries) was 3.5 MPN/g, and that 60% of the samples had a MPN <1.0 cfu/g. As we have used the less sensitive mini MPN detection method in our survey, which has a limit of detection of <1 cfu/g, compared to <0.02 cfu/g for the full MPN method, this could explain why we failed to enumerate salmonella organisms in our samples. The full MPN method was not employed due to its laborious intensity to perform.

Salmonella serovars identified included Agona (*n*=3), Infantis *(n*=1), Mbandaka, and *S*. Paratyphi B variant Java from chicken, and *S.* Agona and *S.* London from turkey. None of these isolates were shown to be ESBL- or carbapenemase-producers. Interestingly the *S*. Paratyphi isolated was ST28, which is same ST type as the *Salmonella* Paratyphi B variant Java that has been common in poultry in the

Netherlands [68]. The origin of the chicken breast sample was the Netherlands. Van Pelt *et al*. 2003 reported an increase in resistance to flumequine (a quinolone) and to ciprofloxacin in *S*. Paratyphi variant Java isolates in 2002; our WGS analysis did not reveal resistance genes to these antibiotics [68].

The majority of the *Salmonella* spp. isolates (*n*=6) were 100% sensitive to the panel of antimicrobials except for two isolates. A *S*. Agona isolate from turkey meat had resistance to ampicillin, gentamicin and tetracycline, and a *S*. Agona from chicken was resistant to ampicillin only. As ampicillin, sulfamethoxazole and tetracycline are antimicrobials that have been commonly used in veterinary medicine these resistances are not unexpected.

In the EFSA EU monitoring AMR survey of 2020-2021, resistance to ampicillin, tetracyclines and sulfonamides in *Salmonella* from broilers, turkeys and derived meat ranged from moderate to very high in most MS, and resistance to third generation cephalosporins was rare [26]. Resistance was observed (in some countries) to nalidixic acid and ciprofloxacin, especially *S*. Kentucky isolates from broilers and turkeys, and an increasing resistance to ciprofloxacin was seen in *S*. Enteritidis isolates. Some *Salmonella* serovars from poultry sources, such as *S*. Kentucky and *S*. Infantis had comparatively elevated levels of combined resistance to ciprofloxacin and cefotaxime.

The *S*. Infantis isolate was shown to be sensitive to all the antibiotics (same panel of antimicrobials as used in the EU harmonised monitoring survey) and therefore unlikely to be linked to the MDR strain circulating in broiler flocks since 2013 [69]. In the EU AMR monitoring survey, the proportion of presumptive ESBL- or AmpC-producers was shown to be very low or low among *Salmonella* isolates recovered from all food producing animal populations and carcases of broilers, and none were carbapenemase-producing. The only *Salmonella* spp. that was found to be carbapenem resistant were isolates from cases of human infection. [26].

9. *Campylobacter* spp. results

9.1 Campylobacter (detection and enumeration)

The direct culture test method used in this study could detect *Campylobacter* spp. in samples with 10 colony forming units per gram (CFU/g) or higher, and up to seven

Campylobacter colonies from each positive sample were identified to species level. The enumeration method used in this study allowed for quantification of *Campylobacter* spp. when more than 45 CFU/g were present. Samples contaminated with less than 45 CFU/g were considered positive, but the levels of contamination were not quantifiable. Chicken broiler carcasses contaminated with ≥1000 *Campylobacter* CFU/g are considered highly contaminated and this level of contamination is considered a threshold to assess the relative risk of exposure to people [70]. This study considered both the presence of all *Campylobacter* positive samples of chicken and turkey meat and those samples that were considered highly contaminated.

9.2 Detection of *Campylobacter* spp. in chicken meat samples

Campylobacter spp. were detected in 145 of the 305 chicken samples tested giving a prevalence of 47.5% (Table 26). *C. jejuni* was detected in 143 chicken samples (46.9%) whilst *C. coli* was detected in 17 samples (5.6%), and both species were detected in 15 samples (4.9%). The majority of *Campylobacter*-positive (n=145) chicken samples originated from UK production (97.9%) and there were three positive samples (2.1%) produced in the Netherlands. The proportion of *Campylobacter* positive samples varied between the cuts of chicken meat. There was a prevalence of 80% in chicken wing samples, 64% in legs, 60% in whole birds and lowest prevalence in breast (22%). The proportion of *Campylobacter* positive samples was significantly higher in samples with skin (60.3%) compared to samples without skin (21.8%) (Chi^2=40.175, P-Value < 0.001).

Table 26 Chicken meat sample types tested for Campylobacter spp.

Sample category	Sample description	Number of samples	Number of positives	% *Campylobacter* positive (95% confidence interval)
Pieces (skinless)	drumsticks, leg quarters, thighs, thigh steaks	2	1	50.0 (0.0 – 100.0)
Leg (with skin)	Leg (with skin)	66	42	63.6 (52.0 – 75.2)
Breast (skinless)	Breast steak/joint	99	21	21.2 (13.2 – 29.3)

Sample category	Sample description	Number of samples	Number of positives	% *Campylobacter* positive (95% confidence interval)
Breast (with skin)	Breast steak/joint	12	3	25.0 (0.5 – 49.5)
Wings (with skin)	Wings (with skin)	10	8	80.0 (55.2 – 100.0)
Whole bird (with skin)	Whole bird	116	70	60.3 (51.4 – 69.2)
All samples (with skin)	As above	204	123	60.3 (53.6 – 67)
All samples (skinless)	As above	101	22	21.8 (13.7 – 29.8)
All samples	As above	305	145	47.5 (41.9 – 53.1)

9.3 Counts of *Campylobacter* spp. in chicken meat samples

A total of 17 (5.6%) chicken meat samples had high levels of contamination (≥1000 CFU/g) (Table 27) and in total 69 (22.6%) samples were contaminated with >100 CFU/g (Figure 2). It is noted that of the 96.5% of the samples contaminated with more than 100 CFU/g contained skin. Highly contaminated samples (≥1000 CFU/g) were restricted to whole bird (10.4%) and leg samples (7.7%). The proportion of chicken wings positive with lower levels of contamination appeared higher than for other sample types, however the sample size was small (*n*=9).

Until recently, the major retailers have published data on the incidence of highly contaminated carcases at retail, this was in response to an agreed FSA/poultry industry target of less than 7% of chickens at retail being highly contaminated [3]. The published data is generally suggestive of the major retailers meeting this target. In this survey, when results for whole birds are stratified by retailer and independent butcher retailers, an estimate for the proportion of highly contaminated birds can be presented with a 95% confidence interval attached. Due to the low sample size, there is a wide degree of uncertainty, however three of the five birds sampled from independent butchers (60.0%) are highly contaminated, which is in excess of the FSA target of 7% (Figure 3). The proportion of highly contaminated whole chickens at retail was lower (10.3%, n=95) although the confidence interval straddled the FSA target threshold of 7%. No highly contaminated whole chickens were detected at two of the larger retailers (sampled more than 10 times).

Table 27 Chicken meat samples positive for Campylobacter spp. with counts >1000 colony forming units per gram (CFU/g) (high level contamination). Mixed-both *C. coli* and *C. jejuni* detected. CC – County Council.

Sample Number	Sampling Date	Food Category	Location	Species present	CFU/g meat
802849	15/02/2022	Chicken whole	Belfast	*C. jejuni*	1700
803064	05/04/2022	Chicken leg	Wirral	*C. jejuni*	7900
803256	24/01/2022	Chicken whole	Kent Thames Gateway	*C. jejuni*	1090

Sample Number	Sampling Date	Food Category	Location	Species present	CFU/g meat
862053	07/12/2022	Chicken leg	West Northamptonshire	*C. jejuni*	3400
864796	13/09/2022	Chicken whole	Staffordshire CC	*C. jejuni*	4500
864839	20/10/2022	Chicken leg	Greater Manchester Northeast	*C. jejuni*	2600
864850	20/10/2022	Chicken leg	Greater Manchester Northeast	*C. jejuni*	20100
864986	16/08/2022	Chicken whole	Leeds	*C. jejuni*	1130
865022	11/04/2022	Chicken leg	Dorset CC	*C. jejuni*	11600
865023	16/06/2022	Chicken whole	West Sussex (Northeast)	*C. jejuni*	5800
872225	15/03/2022	Chicken whole	Cambridgeshire CC	*C. jejuni*	5300
872268	14/02/2022	Chicken whole	Berkshire	Mixed	5600
872269	14/02/2022	Chicken whole	Berkshire	*C. jejuni*	7400
872482	24/01/2022	Chicken whole	West Surrey	Mixed	2800
872493	25/01/2022	Chicken whole	Cornwall and Isles of Scilly	*C. jejuni*	16700
3012850	20/10/2022	Chicken whole	Durham CC	*C. jejuni*	25700
3038407	16/06/2022	Chicken whole	East Merseyside	Mixed	7500

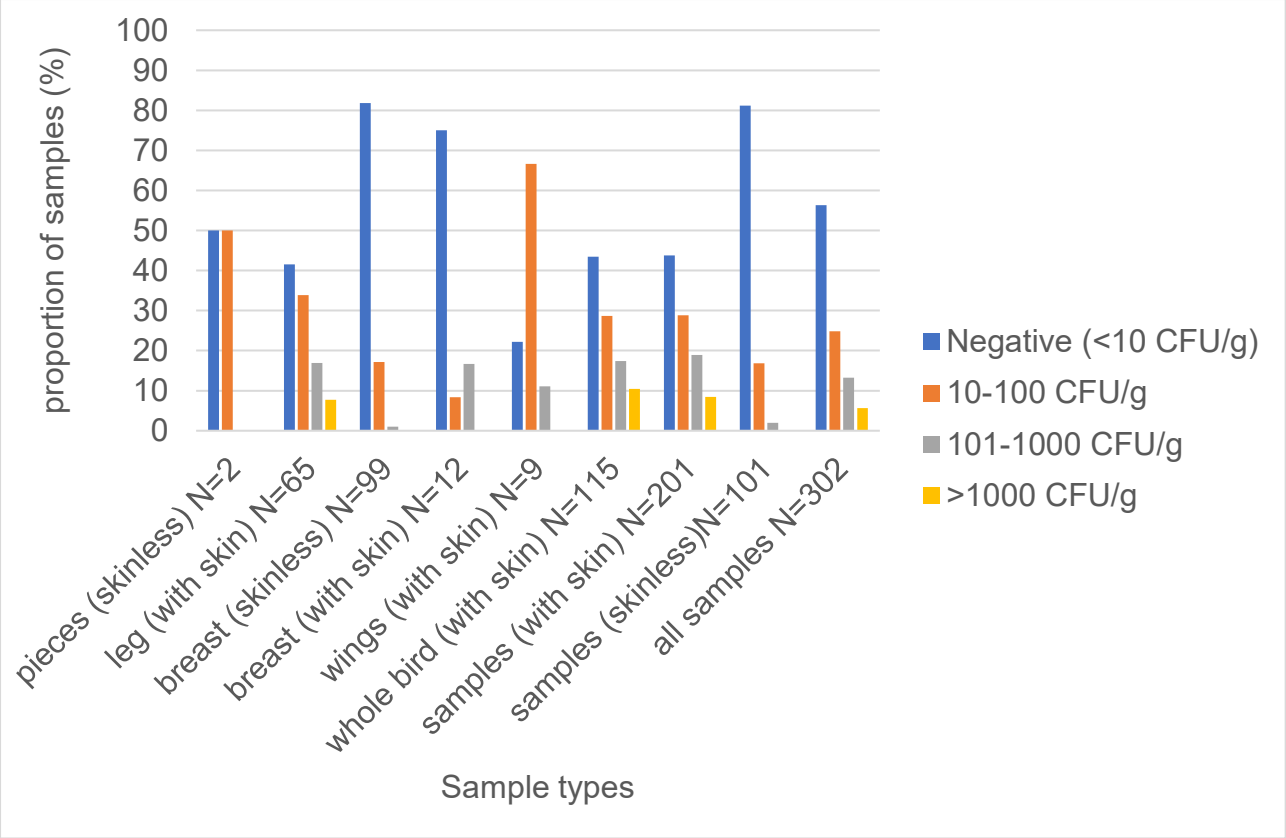


Figure 2 Distribution of Campylobacter spp. in chicken meat samples.

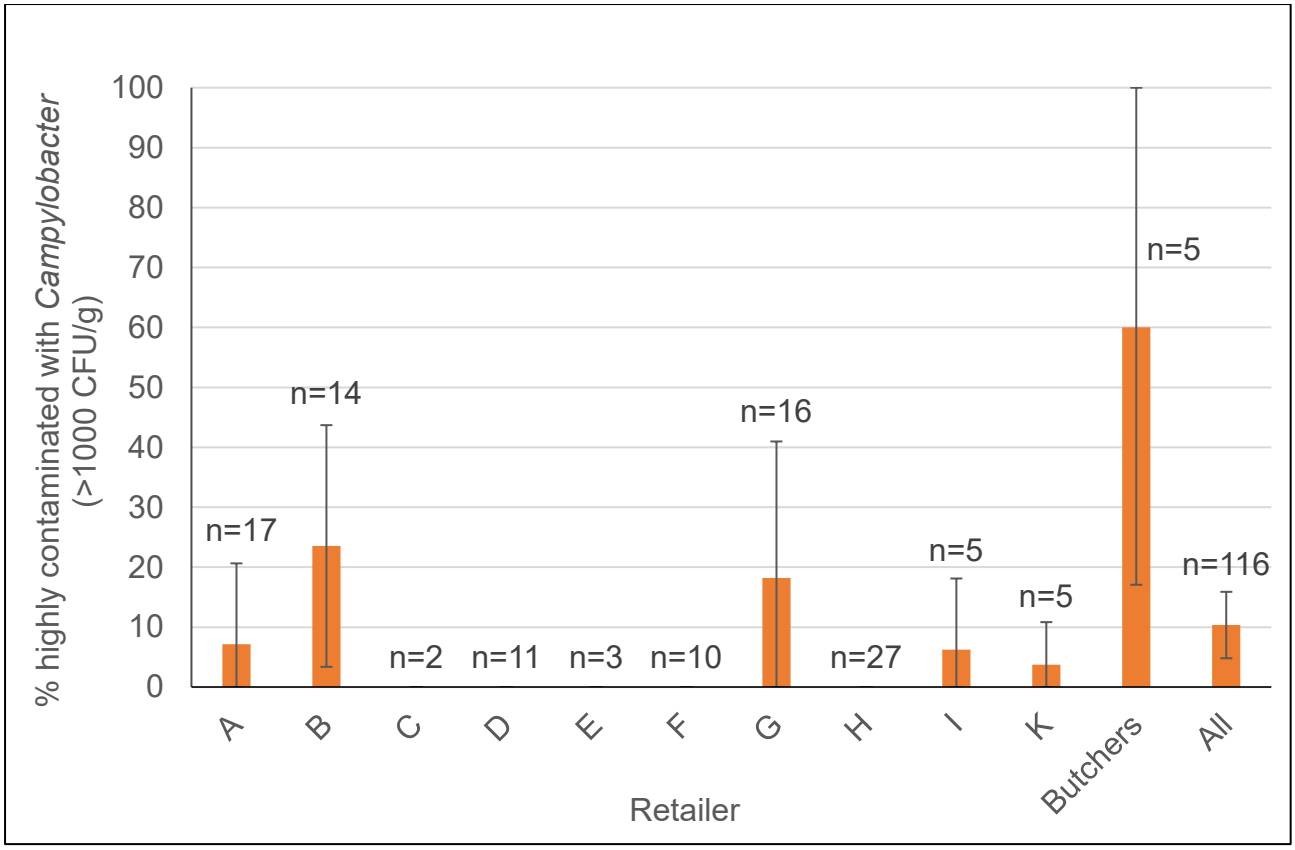


Figure 3 Proportion of whole chicken samples from different retailers that are highly contaminated with Campylobacter (>1000 CFU/g).

9.4 Detection of *Campylobacter* spp. in turkey samples

Campylobacter spp. was detected in 15 of the 302 turkey samples, giving a prevalence of 5.0% (Table 28). *C. jejuni* was identified as the predominant *Campylobacter* species in turkey with a prevalence of 4.3% (13/302) whilst *C. coli* was identified in 0.7 % of the samples (2/302). The proportion of *Campylobacter* spp. -positive samples varied depending on the meat cut of turkey tested with 12.5% of turkey leg samples (n=56) positive, as were turkey crown samples with skin (n=24). Just one of the 13 whole birds tested positive (7.7%), and no breast meat samples (skin or skinless) tested positive. The proportion of *Campylobacter* positives in samples with skin (10.4%) was significantly higher than for samples without skin (1.6%) (Chi^2=8.341, P-Value = 0.004).

Table 28 Turkey meat sample types tested for Campylobacter.

Sample category	Sample description	Number of samples	Number of positives	%*Campylobacter* positive (95% confidence interval)
Mixed other pieces (with skin)	Thigh joint/drumsticks	10	1	10.0 (0.0 - 28.6)
Mixed other pieces (skinless)	thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks.	181	2	1.1 (0.0 - 1.6)
Breast (with skin)	Breast steak/joint	12	0	0.0 (0.0 – 0.0)
Breast (skinless)	Breast steak/joint	4	0	0.0 (0.0 – 0.0)
Turkey leg (with skin)	Leg	56	7	12.5 (3.8 – 21.2)
Crown (with skin)	Crown/joint/butterfly	24	3	12.5 (0.0 – 25.7)
Crown (skinless)	Crown/joint/butterfly	2	1	50.0 (0.0 – 100.0)
Whole bird (with skin)	Whole bird	13	1	7.7 (0.0 – 22.2)
All samples (with skin)	As above	115	12	10.4 (4.8 – 16.0)
All samples (skinless)	As above	187	3	1.6 (0.0 – 3.4)
All samples	As above	302	15	5.0 (2.5 – 7.4)

9.5 Counts of *Campylobacter* spp. in turkey samples

Of the 15 *Campylobacter* positive turkey samples detected, 9 were not quantifiable with estimated levels between 10-45 CFU/g, and two samples had counts between 46 CFU/g and 100 CFU/g. (Table 29). Only four samples were contaminated with more than 100 CFU/g, and they all contained skin (Figure 4), whilst no turkey samples considered highly contaminated were detected.

Table 29 Counts of Campylobacter per gram (CFU/g) in turkey meat samples. TMOP- turkey mixed other pieces included thighs, diced thigh, breast strips, breast fillets, breast stir fry and drumsticks. CC – County Council.

*** Count considered an estimate (between 10 and 45 CFU/g)**

Sample Number	Sampling Date	Food Category	Location	Species present	CFU/g meat
511796	10/05/2022	Turkey leg	East Kent	*C. jejuni*	220
825238	19/12/2022	Turkey crown joint	City of Edinburgh	*C. jejuni*	30*
825279	19/12/2022	Turkey crown joint	Cardiff and Vale of Glamorgan	*C. jejuni*	10*
861857	13/07/2022	Turkey leg	Enfield	*C. jejuni*	30*

Sample Number	Sampling Date	Food Category	Location	Species present	CFU/g meat
864703	19/10/2022	Turkey crown joint	Barking & Dagenham and Havering	*C. jejuni*	84
864718	24/11/2022	TMOP	Devon CC	*C. jejuni*	40*
864762	17/08/2022	Turkey leg	Mid Lancashire	*C. jejuni*	10*
864861	17/10/2022	Turkey leg	East Sussex CC	*C. jejuni*	100
864881	21/11/2022	Turkey crown joint	Leicester	*C. jejuni*	220
864883	21/11/2022	Turkey leg	Leicestershire CC and Rutland	*C. jejuni*	150
2898827	25/01/2022	TMOP	Cornwall and Isles of Scilly	*C. jejuni*	10*
2996023	19/12/2022	Turkey whole bird	Central Valleys	*C. jejuni*	140
3038463	05/04/2022	TMOP	Greater Manchester Southeast	*C. coli*	12
3038578	20/07/2022	Turkey Leg	Sheffield	*C. jejuni*	10*
3135102	21/11/2022	Turkey Leg	Leicestershire CC and Rutland	*C. coli*	30*

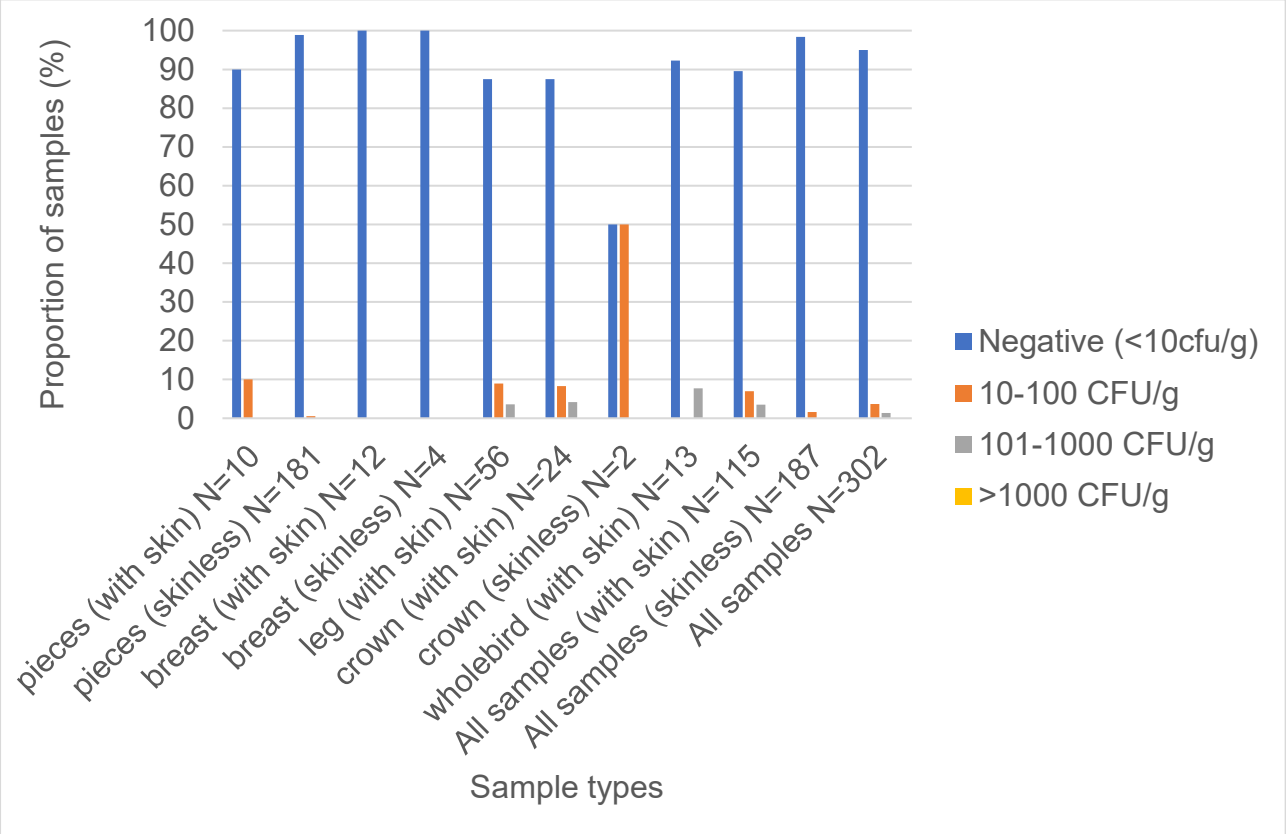


Figure 4 Distribution of Campylobacter in turkey meat samples.

9.6 Further characterisation of *Campylobacter* spp. recovered from chicken meat

A single isolate of *C. jejuni* (n=141) recovered from each of the *C. jejuni*-positive chicken samples was characterised by MIC and WGS. A single *C. coli* isolate (n=16) from each of the *C. coli* positive samples was characterised. Isolates (*C. jejuni* =2, *C. coli* =1) from three positive samples were not characterised as they could not be retrieved from frozen storage.

Ciprofloxacin resistance was observed in 65.4% of *C. jejuni* isolates from chicken meat and tetracycline resistance observed in 73.8% (Table 30). Considering the proposed resistance threshold for ertapenem, resistance was observed in 17.0% of *C. jejuni* isolates. Of the 16 characterised *C. coli* isolates from chicken meat, 81.3% were resistant to tetracycline and 68.8% were resistant to ertapenem. Resistance to ciprofloxacin was seen in 31.3% of isolates. MDR (specifically to ciprofloxacin, ertapenem, tetracycline) was detected in 11.4% of *C. jejuni* and 25% of *C. coli*. No AMR was observed in 21.3% of *C. jejuni* isolates from chicken and 6.3% of *C. coli* isolates were fully sensitive.

Campylobacter jejuni resistant to tetracycline, ciprofloxacin and ertapenem were detected in 34.3%, 30.3% and 7.9% of the chicken meat samples respectively (Table 31). *C. coli* that were resistant to ciprofloxacin, tetracycline and ertapenem were observed in 1.6%, 4.3% and 3.6% of chicken meat samples respectively (Table 32). MDR *C. jejuni* was detected in 5.3% of samples (Table 31), and MDR *C. coli* was detected in 1.3% of samples (Table 32).

Table 30 Summary of resistance phenotypes of C. jejuni and C. coli from chicken meat (only one isolate per species per sample). EUCAST ECOFFs used as stipulated by the EU decision 2020/1729/EU. See Table 12 for interpretative criteria used. #Three isolates missing

Antimicrobial	Number of *C. jejuni* isolates resistant (n=141#)	Percentage *C. jejuni* resistant (%)	Number of *C. coli* isolates resistant (n=16#)	Percentage *C. coli* resistant (%)
Chloramphenicol (CHL)	0	0	0	0.0
Ciprofloxacin (CIP)	91	64.5	5	31.3
Ertapenem (ERT)	24	17.0	11	68.8
Erythromycin (ERY)	0	0.0	0	0.0
Gentamicin (GEN)	0	0.0	0	0.0
Tetracycline (TET)	104	73.8	13	81.3
CIP+TET	85	60.3	5	31.3
ERT+TET	23	16.3	5	31.3
CIP+ERT+TET	16	11.4	4	25.0
TET only	3	14.2	3	18.8
CIP only	6	4.3	0	0.0
ERT only	1	0.7	2	12.5
Fully sensitive	30	21.3	1	6.3

Table 31 Summary of resistance phenotypes of C. jejuni present in turkey meat samples and chicken meat samples. EUCAST ECOFFs as stipulated by the EU decision 2020/1729/EU. See Table 12 for interpretative criteria used.

*** Discounts the positive samples that did not have an isolate characterised.**

Antimicrobial	**Number of turkey samples with *C. jejuni* phenotype**	**% of turkey samples with *C. jejuni* phenotype (n=301*)**	**Number of chicken samples with *C. jejuni* phenotype**	**% of chicken samples with *C. jejuni* phenotype (n=303*)**
Chloramphenicol (CHL)	0	0	0	0
Ciprofloxacin (CIP)	7	2.3	91	30.3
Ertapenem (ERT)	1	0.3	24	7.9
Erythromycin (ERY)	0	0	0	0
Gentamicin (GEN)	0	0	0	0
Tetracycline (TET)	9	3.0	104	34.3
CIP+TET	6	2.0	85	28.1
ERT+TET	1	0.3	23	7.6
CIP+ERT+TET	1	0.3	16	5.3
TET only	3	1.0	3	1.0
CIP only	0	0	6	2.0
ERT only	0	0	1	0.3
No resistance detected	294	97.6	111	36.6

Table 32 Summary of resistance phenotypes of C. coli present in turkey meat samples and chicken meat samples. EUCAST ECOFFs used as stipulated by the EU decision 2020/1729/EU. See Table 12 for interpretative criteria used.

Antimicrobial	Number of turkey samples with *C. coli* phenotype	% of turkey samples with *C. coli* phenotype (n=302)	Number of chicken samples with *C. coli* phenotype	% of chicken samples with *C. coli* phenotype (n=304*)
Chloramphenicol (CHL)	0	0	0	0
Ciprofloxacin (CIP)	1	0.3	5	1.6
Ertapenem (ERT)	2	0.7	11	3.6
Erythromycin (ERY)	0	0	0	0
Gentamicin (GEN)	0	0	0	0
Tetracycline (TET)	2	0.7	13	4.3
CIP+TET	1	0.3	5	1.6
ERT+TET	2	0.7	5	1.6
CIP+ERT+TET	1	0.3	4	1.3
TET only	0	0.0	3	1.0
CIP only	0	0	0	0.0
ERT only	0	0	2	0.7
No resistance detected	300	99.4	288	94.7

9.7 Further characterisation of *Campylobacter* spp. recovered from turkey meat

In total 21 *Campylobacter*, were characterised. A selection of *C. jejuni* (n=19) recovered from the 13 *C. jejuni*-positive samples were further characterised by MIC and WGS, six positive samples contributed two *C. jejuni* isolates. Four of these samples shared the same MIC profile between each isolate, whilst two samples provided different profiles between the isolates. In addition, a *C. coli* (n=2) isolate from each of the *C. coli*-positive samples was characterised.

Ciprofloxacin and tetracycline resistance were observed in 47.3% and 57.9% of *C. jejuni* isolates collected from turkeys respectively (Table 33). Resistance to ertapenem was observed in 10.5% of *C. jejuni* isolates. Of the two characterised *C. coli* isolates both were resistant to tetracycline and ertapenem, and one was resistant to ciprofloxacin. MDR (to ciprofloxacin, tetracycline and ertapenem) was detected in *C. jejuni* (10.5%) and *C. coli* (50.0%). No AMR was observed in 36.8% of *C. jejuni* isolates from turkey. At a sample level, tetracycline resistant *C. jejuni* was detected in just 3% of samples, ciprofloxacin resistance detected in 2.3% and

ertapenem resistance in 0.3% (Table 31). Only 0.3% of turkey meat samples contained an MDR *C. jejuni* or an MDR *C. coli* (Table 31 and Table 32).

Table 33 *Campylobacter* resistance phenotypes from Turkey meat

Antimicrobial	Number of *C. jejuni* resistant (out of 19)	Percentage *C. jejuni* resistant (%)	Number of *C. coli* resistant (out of 2)	Percentage of *C. coli* resistant (%)
Chloramphenicol (CHL)	0	0	0	0.0
Ciprofloxacin (CIP)	9	47.3	1	50.0
Ertapenem (ERT)	2	10.5	2	100.0
Erythromycin (ERY)	0	0.0	0	0.0
Gentamicin (GEN)	0	0.0	0	0.0
Tetracycline (TET)	11	57.9	2	100.0
CIP+TET	8	42.1	1	50.0
ERT+TET	2	10.5	2	100.0
CIP+ERT+TET	2	10.5	1	50.0
TET only	3	15.8	0	0.0
Fully sensitive	7	36.8	0	0.0

9.8 Multi-locus sequence typing (MLST) and detection of resistance genes from chicken meat samples

A total of 14 *Campylobacter* spp. isolates from chicken meat samples were characterised by WGS, and each isolate came from a different meat sample. Nine different MLST sequence types (STs) were identified from these isolates (Table 34). The PubMLST database [71] confirmed that eight of these STs had previously been isolated from people. The remaining ST(7743) was restricted to chicken samples in the PubMLST database. Three chicken meat samples harboured ST464 and three contained ST9987, whilst ST6175 was isolated from two chicken samples.

Each isolate was assigned to a MLST clonal complex (CC) where possible [71]. Isolates within a CC all share common sequences for at least four loci of the MLST system. Many of the sequence typed isolates were assigned to complexes that are frequently found in chicken and people (21, 353, 828, 354, 464), however ST9897 was not assigned to a clonal complex. A review of submissions to the PubMLST

website can provide a crude indication (based on submission of isolates) on the emergence of new sequence types, and ST464 (CC464) and ST6175 (CC21) are notable for increasing numbers in recent years, whilst ST9897(unassigned CC) is notable as it has not been submitted prior to 2019.

9.9 MLST of isolates from turkey meat samples

A total of six *Campylobacter* isolated from turkey meat samples were characterised by WGS, and four isolates came from four different meat samples, one meat sample contributed two isolates. Five different STs were identified from turkeys isolates sequenced (Table 34), and the PubMLST database confirms that three of these STs have previously been isolated from people. The remaining STs identified in the turkey isolates had not been listed before on PubMLST. Two isolates were unassigned to any clonal complex, with ST9897 also observed in the chicken meat samples. The three clonal complexes identified are commonly found in poultry and people, CC21, CC464, and CC828.

9.10 Detection of resistance genes by WGS

No resistance genes for chloramphenicol, gentamicin or erythromycin were identified in the 20 isolates characterised, this finding was complementary to the observed phenotypes (Table 34 and Table 35). The genotype predictions for AMR to quinolones were also complementary to the phenotype, all the isolates phenotypically resistant had a single mutation in *gyrA* gene in the quinolone resistance determinant region (p.T86I), which is the most common mutation for this phenotype. The same *tet-O* gene for tetracycline resistance was detected in the tetracycline resistant isolates, however two isolates considered resistant by phenotype did not have any resistance gene detected. The MIC for one of these isolates was 2mg/l which is on the threshold for resistance.

The ResFinder pipeline determined that 80.0% of the isolates had the bla_{OXA-61} gene, 5.0% had $bla_{OXA-185}$ gene, and 15% did not have a recognised bla_{OXA} gene. This gene confers resistance to beta-lactam antibiotics such as ampicillin [72]. In this study it was not possible to correlate the gene presence to beta-lactam resistance as no beta-lactams were included in the MIC panel. Ertapenem resistance did not correlate with bla_{OXA} gene, although it is noteworthy that two of the three isolates

with no *bla*$_{OXA}$ gene were very susceptible to ertapenem with an MIC of <0.125mg/ml.

Two ST464 *C. jejuni* isolates from a single turkey sample had the same quinolone and tetracycline resistance genes but only one had a *bla*$_{OXA}$ gene (Table 35). The variant with the *bla*$_{OXA}$ gene had an MIC of 4mg/ml for ertapenem, whilst the isolate without the gene had an MIC of 1.

Considering only genotypic results 14 of the 20 isolates characterised could be considered MDR, with resistance to beta-lactams, tetracycline and fluoroquinolone.

Table 34 Chickens: characterisation of C. jejuni (n=13) and C. coli (n=1) using WGS to determine MLST; Multi locus sequence type. C; resistance to ciprofloxacin, E; resistance to ertrapenem, T; resistance to tetracycline. MIC; minimum inhibitory concentration *Mutation in the quinolone resistance determining region; *gyrA* (p.T86I) **Tetracycline phenotype MIC=2mg/l ***Tetracycline phenotype MIC>64mg/l

Sample ref	MLST	Clonal complex	AMR genes	Species	Resistance phenotype
803255	6175	21	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/T
861781	464	464	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T
862057	262	21	*betaL-g1046a_OXA-61*	*C. jejuni*	E/T**
864608	8141	446	*tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/T
864796	400	353	*tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/T

Sample ref	MLST	Clonal complex	AMR genes	Species	Resistance phenotype
864827	6175	21	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/T
864856	9897	u/a	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T
864932	828	828	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C.coli*	C/E/T
865001	7743	661	*betaL-g1046a_OXA-61quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T***
865003	354	354	*betaL-g1046a_OXA-61 tetra-g1924_tet-O*	*C. jejuni*	C/T
865051	9897	u/a	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T
865077	464	464	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T
872225	9897	u/a	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/T

Sample ref	MLST	Clonal complex	AMR genes	Species	Resistance phenotype
872573	464	464	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T

Table 35 Turkeys: characterisation of *C. jejuni* (n=5) and *C. coli* (n=1) using WGS to determine MLST, presence of resistance genes and mutations in *gyrA*, and MIC testing for phenotypic resistance. MLST; Multi locus sequence type. C; resistance to ciprofloxacin, E; resistance to ertrapenem, T; resistance to tetracycline.* Mutation in the quinolone resistance determining region; *gyrA* (p.T86I)

Sample ref	MLST	Clonal complex	Resistance genes	Species	Resistance phenotype
2898827	9897	u/a	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T
511796	464	464	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T
511796	464	464	*tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/E/T

Sample ref	MLST	Clonal complex	Resistance genes	Species	Resistance phenotype
3038578	12342	u/a	*betaL-g2096_OXA-185 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/T
864718	6175	21	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. jejuni*	C/T
3038463	12393	828	*betaL-g1046a_OXA-61 tetra-g1924_tet-O quino-g2384_gyrA_campy_Chr**	*C. coli*	C/E/T

10. Campylobacter discussion

10.1 Presence of *Campylobacter* spp. on chicken and turkey meat

Campylobacter spp. were detected in 47.5% of chicken meat samples, with the highest proportion of positive samples observed in wing portions (80.0%), leg portions (63.6%) and whole birds (60.3%). *Campylobacter* spp. contamination was less frequent in non-skinned breast portions (21.2%). In general, detectable contamination was more prevalent in skin on meat samples (60.3%) relative to non-skin samples (21.8%).

A survey of retail chicken meat in the UK in 2017 reported an overall prevalence of 27.9% in fresh meat and a similar distribution of *Campylobacter* contamination amongst the various sample types [73] The samples collected in the current study were distributed amongst retailers based on market share and in total 5.6% of all

samples tested were contaminated with high-levels (>1000 CFU/g) of *Campylobacter*. This included a sub-set of 115 whole chickens of which 10.4% (95% CI: 4.8%-16.0%) were highly contaminated with *Campylobacter*. This finding may be indicative of an increase in contamination since 2019, when FSA published data for weighted market shares of whole chickens, and reported that between 5.2%-5.9% of whole chickens were highly contaminated[3]. The uncertainty associated with the present estimate prevents any definitive conclusion.

The FSA, poultry producers and retailers have agreed an indicative target for highly contaminated whole chicken at retail of 7% or less, again this is lower than the estimate in the current study, but uncertainty prevents drawing any definitive conclusion. The frequency of highly contaminated chickens at butcher retailers was high at 60.0% (95% CI: 17.1%-100.0%). Similar findings have been previously reported [74] and drivers behind this observation are not established. Potential influencing factors might include variations in supply chain and/or product characteristics and preparation.

Due to low numbers of imported meat samples tested it was not possible to draw conclusions regarding contamination rates in UK and non-UK produced chicken.

Campylobacter contamination in turkey meat samples appears to be less than for chicken meat. Just 5% of turkey meat samples were contaminated with *Campylobacter* and none were considered highly contaminated. Similar findings were reported in a five-month study of turkey meat at retail in the UK in 2020/21[4].

As observed for chicken meat, contamination was least frequent in turkey breast meat samples. Skin on turkey meat samples is more likely to be contaminated than non-skin samples.

In poultry processing, contamination of the skin is a result of environmental contamination prior to and during slaughter and further contamination with gastro-intestinal contents whilst progressing along the slaughter line. Colonisation of a broiler flock at time of slaughter is associated with the production of the carcases with the high levels of contamination (>1000 CFU/g) [75]. A national monitoring programme for *Campylobacter* in broilers (2012-2017) suggests *ca*. 70% of UK flocks are colonised with *Campylobacter* on entry to the abattoir [76]. Although there

are no published data on the prevalence of *Campylobacter* spp. in turkey flocks at slaughter in the UK, studies of turkey production in Spain and Canada have reported that 74.9% and 47.4% of flocks were positive at slaughter respectively [77, 78]. The concentration of *Campylobacter* within the caecal content is correlated with high levels of contamination in UK broilers [76], however there no similar quantitative data available for turkeys at slaughter.

Turkeys are slaughtered at much heavier weights than broilers and variation in the processing practices may be another differentiator for levels of contamination between the two types of meat. Different systems may be used to chill the carcase, water immersion chilling for turkeys and air chilling for broilers. A study by Berrang et al., suggested that *Campylobacter* counts on immersion chilled carcases were marginally lower than for air chilled carcases [79]. Regardless of the species, the contamination primarily accumulates on the skin, and this is patently reflected in the detection rates between meat with skin on and meat with no skin. In a survey of chicken carcases between 2012-2017, the availability and size of the neck skin flap on a carcase was associated with the higher levels of contamination present on carcases [76].

The predominant *Campylobacter* species detected in either chicken or turkey meat in the study was *C. jejuni*, a finding which is mirrored in detections from the caecal contents of broiler chicken in UK [76] and is also seen in the isolations of *Campylobacter* from cases of campylobacteriosis in people [80]. A sub-selection of *Campylobacter* isolated from chicken and turkey meat in this study were multi-locus sequence typed (MLST) to reveal variants of *Campylobacter* that are frequently found in poultry and associated with disease in people. Although turkey meat is contaminated with *Campylobacter* types associated with disease in people, there is smaller scale of production (Latest poultry and poultry meat statistics - GOV.UK) and consumption in the UK, relative to chicken meat. When coupled with the lower frequency and level of contamination present in the turkey meat sampled, this would suggest that the handling and consumption of broiler meat is a more significant transmission pathway for *Campylobacter* spp., including AMR variants to UK residents.

10.2 AMR in *Campylobacter* contamination found on chicken and turkey meat

The resistance of *C. jejuni* and *C. coli* was investigated against a panel of antimicrobials as specified within the European Union (EU) decision (EU 2020/1729) on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria [11]. This specification was established to fulfil the Zoonoses Directive (2003/99/EC)[81] that requires MS to ensure that monitoring provides comparable data on the occurrence of antimicrobial resistance in zoonotic agents and, in so far as they present a threat to public health..

The panel of antimicrobials for monitoring (Table 14) includes groups that can be used for treatment of infection in people, erythromycin as an example of macrolides, ciprofloxacin as an example of fluoroquinolones, gentamicin as an example of aminoglycosides and also tetracycline[82, 83]. In the latest revision of the monitoring programme chloramphenicol has been included to indicate isolates of high-drug efflux potential which could have elevated MICs to a range of antimicrobial groups [84]. In addition, ertapenem has been included as an example of the carbapenems, which are not licenced for use in livestock in the UK and are reserved as last resort therapies in human medicine.

The World Health Organisation (WHO) has identified that these groups of antimicrobials are either critically important for public health (includes macrolides, quinolones, aminoglycosides and carbapenems or highly important for public health (tetracycline and amphenicols) [85]. The macrolides and quinolones are given the highest priority. The European Medicines Agency (EMA) also recognises the importance of these antimicrobial groups in animals and recommend that carbapenems are avoided, fluoroquinolones are restricted, and that macrolides, amphenicols and aminoglycosides are used with caution and that tetracyclines used with prudence [86].

UK surveys of retail chicken meat undertaken between 2014 and 2020 have identified high levels of resistance to tetracycline and ciprofloxacin [87]. In general for chicken meat samples tested in 2017, 38% of the *C. jejuni* recovered were resistant to ciprofloxacin and 59% were resistant to tetracycline [73]. In the current study (2022) resistance was more frequently detected, with ciprofloxacin resistance

detected in 65.4% of *C. jejuni* isolates and tetracycline resistance in 73.8%. In *C. jejuni* from whole chickens sampled during four individual retail surveys between 2015-2020, ciprofloxacin resistance ranged between 41% and 54% and tetracycline resistance between 52% and 68% [74].

In turkey meat, the prevalence of resistant isolates to ciprofloxacin and tetracycline is lower in 2022 relative to the incidence reported in the 2020/21 study [4]. In the study of 2020/21, 60% of isolates were resistant to ciprofloxacin and in the current (2022) study 47.3% of isolates are resistant. Likewise for tetracycline as 65% of isolates were resistant in 2020/21 and 57.9% are resistant in 2022. These levels of resistance are similar to that reported in the harmonised monitoring of *C. jejuni* from broiler and turkey flocks at slaughter and are in general agreement with figures available for clinical isolates studied in the past decade [2, 88, 89]. In general, there is no clear evidence to suggest that the prevalence of resistance to these antimicrobials is declining in *C. jejuni* contamination on poultry meat, this is despite the successful efforts of the British Poultry Council to antimicrobial stewardship group to reduce the extent of antimicrobial use in poultry meat production. It is interesting to note that despite a higher level of AMU in turkey production relative to chicken meat production, the prevalence of resistance in *C. jejuni* from turkeys is consistently lower than for broilers. The reasons behind this are unclear as MLST typing in this study (2022) and in 2020/2021 suggest that similar types are present in turkey production systems compared with broiler production [4]. Further monitoring is recommended to monitor the long-term prevalence trends for AMR in *C. jejuni* and research is needed to understand factors driving the persistence of resistance to fluoroquinolones in the absence of direct selective pressure.

Resistance to ciprofloxacin is of particular concern as it is one of the remaining treatment options available for campylobacteriosis in people and is classified as a highest priority critically important antimicrobial (HP-CIA) by the WHO.

Campylobacter coli is now included for mandatory monitoring due to its potential to accumulate resistance, its high prevalence in some host species in particular countries and its potential to transfer resistance *via* zoonoses or horizontal transfer to other bacteria [90]. In this 2022 study, 81.3% of *C. coli* isolates from chicken meat harboured resistance to tetracycline and 31.3% were resistant to ciprofloxacin. The

prevalence of tetracycline resistance appears to have increased in *C. coli* since 2017 when 60.0% of isolates were resistant and ciprofloxacin resistance may have reduced as 46.7% *C. coli* were resistant in 2017 [73].

Erythromycin is also used as a treatment option for campylobacteriosis and is considered a HP-CIA. It is encouraging that no erythromycin resistance was observed in any *Campylobacter* spp., whereas 9% and 14% resistance was observed in *C. jejuni* and *C. coli* from chicken meat samples in 2017 [73].

It is also encouraging to report that no resistance was detected to chloramphenicol, which is an indicator for presence of super-efflux pumps.. Efflux pumps can mitigate against the effects of antimicrobials by decreasing their intracellular concentrations and this can result in elevated MICs for bacteria against diverse range of antimicrobials including ciprofloxacin and erythromycin[90]. Reports of resistance to gentamicin are extremely rare [87] and the absence of any resistant in *C. jejuni* or *C. coli* in this study is welcome continuation of this trend.

Tetracycline is a priority antimicrobial of concern and its prevalence in the current study remains high as reported elsewhere. At present specific interventions are focussed more on the HP-CIA's and the threat of emerging resistances.

The European Food Standard Agency (EFSA) recommended inclusion of a carbapenem antimicrobial to the panel for harmonised monitoring in *Campylobacter* in 2019 [84] , as this grouping is not used in animal production but is reserved as a last-resort antibiotic for systemic infections in people. There are very limited data available on carbapenem resistance in *Campylobacter* and the antimicrobial was included in the monitoring specification although a definitive ECOFF between wild type and resistance type was not specified at the time of publication [11].

The EU summary report for 2021 included data on the susceptibility of *C. jejuni* and *C. coli* to ertapenem for the first time, and used a non-validated threshold of >0.5mg/l for resistance relative to wild-type for both species [91]. Using this threshold, unexpectedly elevated levels (29.2%) of ertapenem-resistant *C. coli* were identified in calves at slaughter in the EU in 2021. A limited number of MS reported ertapenem susceptibility data for isolates of *C. jejuni* and *C. coli* from chicken meat. Although the sampling distribution is not uniform or consistent for samples and countries, the

available data indicate resistance to ertapenem in between 10-25% of *C. jejuni* and 10-50% of *C. coli* isolates.

In this study ertapenem resistance, as defined by the >0.5mg/l threshold, was present in 17.0% of *C. jejuni* isolates from chicken meat and 68.8% of *C. coli* isolates. In turkey meat isolates, 10.5% of *C. jejuni* were resistant and the two characterised *C. coli* isolates were also resistant. Although the UK data for ertapenem resistance are comparable to those of the limited reports across Europe, care is needed with interpretation, given these thresholds are not validated. Indeed, EUCAST has recently proposed a tentative ECOFF for *C. jejuni* of >0.125mg/ml. Applying this threshold to the *C. jejuni* data in this study suggests that 59.6% of chicken meat isolates and 68.4% of turkey meat isolates were resistant.

Ertapenem resistant *Campylobacter* has been observed in Georgia, where *C. coli* from poultry and humans have been resistant at the 0.5mg/l threshold, however no *C. jejuni* were resistant [92]. In Portugal, *C. coli* with MIC of >32mg/ml for ertapenem has been reported [93] and some *C. jejuni* have an MIC of 32mg/ml as reported by Lehours in 2018, although most are <0.5mg/ml [94]. In the current study 2.8% of *C. jejuni* from broilers had an MIC >4mg/ml. It is difficult to interpret the significance of the ertapenem findings, due to the limited published data. Harmonised monitoring of caeca from broilers and turkeys will provide more data for the UK when the 2022 VARRS report is published by the Veterinary Medicines Directorate (due autumn of 2023).

Mechanisms of ertapenem resistance are considered to include production of carbapenemases and beta-lactamases (*bla*$_{OXA}$) to degrade the antimicrobial, efflux pumps (*cme*ABC) to reduce intracellular concentrations of the antimicrobial and porins to block access of the antimicrobial to the cell [95]. In this study no specific resistance gene was found to correlate with the resistance status for ertapenem in the 20 campylobacters analysed, more work is needed to identify regions associated with MIC for ertapenem. This would be an advance and welcome addition to the existing AMR predictive WGS pipelines, as demonstrated by the excellent correlation between WGS genotype and phenotype for all other antimicrobials as demonstrated in this study.

In summary these results indicate that resistance to fluoroquinolones is persisting within the poultry production systems despite the antibiotic stewardship initiatives. Given the HP-CIA status for fluoroquinolones, further monitoring and research to understand the drivers behind this persistence is imperative to mitigate against the public health risks of disseminating this resistance in the food-chain. Erythromycin is the main treatment option for complex cases of campylobacteriosis and is considered a HP-CIA. *Campylobacter* that are co-resistant to both ciprofloxacin and erythromycin are a public health concern It is reassuring that no such isolates were detected in the survey but vigilance via continued monitoring is required.

These findings deepen and expand our knowledge of AMR in chicken and turkey produce for the UK 5-year National Action plan 2019-2024, thereby closing data gaps and improving understanding of the hazards and risks from AMR in particular from foods that we commonly consume.

11. Conclusions

11.1 E. coli

- The proportion of retail chicken and turkey samples positive for presumptive ESBL-producing and AmpC-producing *E. coli* was approximately 12.0%. This is similar to prevalence in 2020/2021, so no decline compared to the large decline in ESBL prevalence seen in chicken meat between the 2014 and 2016 surveys (of 45.1%).

- None of the meat samples tested prior to a pre-enrichment incubation had background or ESBL- and/or AmpC-producing *E. coli* counts above the EU detection levels, indicating low numbers of these bacteria on meat samples.

- Carbapenemase-producing *E. coli* were not detected in any chicken or turkey samples tested.

- Further analysis of AMR in the *E. coli* ESBLs from chicken meat indicated no change in percentage of isolates resistant to ciprofloxacin compared to ESBLs isolated in the previous retail survey of 2020, however there was a drop in percentage of isolates resistant to nalidixic acid from 62% to 35%. The percentage of turkey ESBL isolates resistant to both ciprofloxacin and nalidixic

acid had reduced compared to findings in the 2020/2021 survey (79 to 43%, and 58 to 9% respectively for these antimicrobials).

- AmpC-producing *E. coli* were isolated from turkey meat in 2022 but had not been previously isolated in the 2020-2021 survey. This increase in prevalence was statistically significant and four (out of 7) isolates may be related (i.e. same ST type, same retailer and had same AMR profile). Further DNA analysis would be a recommendation. All AmpCs producers were resistant to three different cephalosporins (but not to cefepime as expected), nor to carbapenems. Two were also resistant to ciprofloxacin.

- The ESBL genes in *E. coli* from retail chicken meat were mainly CTX-M-55 (34%) and SHV-12 (32%) plus 18% of CTX-M-15. A large shift has occurred since 2018 when the ESBL gene types of chicken isolates was first examined, and which showed that CTX-M-1 was the dominant type. For turkey isolates, the predominant ESBL type now is SHV-12 (48%).

- WGS demonstrated that the ESBL-producing *E. coli* from chicken with CTX-M-15 were not ST131, which is the ST associated with the human pandemic O25-ST131 CTX-M-15-producing clone [63, 64].

- Eight (1.3%) of the UK retail chicken or turkey samples tested were positive for plasmid-mediated colistin resistance encoded by *mcr-1.* This is the third time that *mcr-1* has been reported in chicken and turkey meat, and countries of origin include Poland, Netherlands, Lithuania, Germany and United Kingdom. The *mcr-1* positive *E. coli* were resistant to many antimicrobials, especially the quinolone antimicrobials and ampicillin. These isolates showed similarity (based on AMR genes, ST and plasmid DNA) to *mcr-1*- positive *E. coli* isolated from poultry and turkey meat in previous surveys.

- In view of the isolation of *mcr-1* from retail chicken and turkey meat, future ongoing monitoring of AMR retail meats in the UK would seem prudent.

11.2 Salmonella

- There was a very low prevalence of *Salmonella* spp. in both chicken (n=6, 1.9%) and turkey (n=2, 0.7%) meat samples, and the numbers of organisms

were below the detection level of the mini-MPN method used for enumeration. The full MPN method is recommended as it is a more sensitive technique for enumeration of low numbers of organisms.

- Most *Salmonella* isolates were sensitive (6 out of 8) to all 14 antimicrobials they were tested against, and none were ESBLs.

11.3 Campylobacter spp.

- The prevalence of *Campylobacter* spp. was low in turkey meat samples (5.0%) but more common in chicken meat samples (47.5%). High levels of contamination (>1000 CFU/g) were observed in 5.6% of chicken meat samples but not those from turkey meat.

- The frequency of contamination was higher in meat samples with skin relative to meat samples without skin. In chicken meat samples with skin 60.3% were contaminated but only 21.8% of samples without skin were contaminated. In turkey meat samples this was 10.4% and 1.6% respectively.

- Ciprofloxacin and tetracycline resistance was frequently detected in both *C. jejuni* and *C. coli* recovered from turkey and chicken meat samples. Using the classification scheme as described in EU summary reports for on AMR in zoonotic and indicator bacteria [91], the prevalence of resistance to ciprofloxacin is classed as either ‘high (20-50%)’ or ‘very high (50-70%)’. The levels of tetracycline resistance would be classified as ‘very high’ or ‘extremely high’ (>70%).

- Resistance to chloramphenicol, erythromycin and gentamicin was not detected in any isolates. Susceptibility to ertapenem varied amongst isolates, although the interpretation of a resistant isolate is still under discussion.

- MLST typing of a sub-set of *Campylobacter* spp. isolates from chicken and turkey meat identified multiple sequence types (STs) that have previously been identified in human isolations of this organism, indicating handling and consumption of poultry meat is a potential transmission pathway for exposure to both *Campylobacter* and AMR.

- Results obtained in this study compared favourably to results from other countries that participated in EU monitoring surveys in 2020, as published by EFSA.
- This work has contributed to the food safety commitments within the 5 year National Action Plan (NAP) on AMR, which is on-going until 2024. The evidence presented will inform the risk assessment for both campylobacteriosis and AMR in chicken and turkey meat which is regularly consumed in the UK. The data is aligned to the current EU harmonised monitoring specification and ensures that the key antimicrobials of concern to public health are monitored for efficacy against *C. jejuni* and *C. coli*.

12. Glossary

Acronym	Definition
AMP	Ampicillin
AmpC-producer	A bacteria which produces a AmpC beta-lactamase enzyme
AmpC beta-lactamase	Enzyme conferring resistance to cephalosporin antibiotics
AmpC phenotype	Antimicrobial resistance profile type with resistance typically to cephalosporin antimicrobials including cefoxitin and also to beta-lactamase inhibitor-beta-lactam combinations
AMK	Amikacin
AMR	Antimicrobial resistance
AMX	Amoxicillin
APHA	Animal and Plant Health Agency
AZI	Azithromycin
BPW	Buffered Peptone broth, a liquid media widely used to grow bacteria
CARBA	ChromID® CARBA agar, for isolation of carbapenemase resistant *E. coli*
CAZ	Ceftazidime
CA-ESBL	CHROMagar™ ESBL, for isolation of ESBL-producing *E. coli*
CC	clonal complex defined as a group of STs
CFU/g	colony forming unit per gram
CHL	Chloramphenicol
CI	Confidence Interval
CIP	Ciprofloxacin
COL	Colistin
CRL	Community Reference Laboratory
CTX-M .	group of ESBL enzymes that give bacteria resistance to cephalosporin antimicrobials
CST	Colistin
CTX	Cefotaxime
ECOFF)	Epidemiological Cut Off value (with respect to antimicrobial resistance
Enterobacteriaceae	Family of bacteria including many common gut bacteria such as *Escherichia coli* or *E. coli*
EN	Norme Européenne /Europäische Norm (European Standard)
ERT	Ertapenem

Acronym	Definition
ERY	Erythromycin
ESBL .	Extended Spectrum beta-lactamase. Enzymes that are capable of breaking down many penicillin type antimicrobials, including cephalosporin antimicrobials
ESBL-phenotype	Antimicrobial resistance profile type with resistance typically to cephalosporin antimicrobials but excluding resistance to cefoxitin and beta-lactamase inhibitor-beta-lactam combinations
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EURL	European Union reference laboratories.
FEP	Cefepime
FOX	Cefoxitin
FSA	Food Standards Agency
GEN	Gentamicin
IPM	Imipenem
ISO	International Organisation for Standardisation
MALDI-ToF	Matrix-Assisted Laser Desorption / Ionization Time-of-Flight
mCCDA	modified charcoal cefoperazone deoxycholate agar
MEM	Meropenem
MDR	Multi-drug resistant
McC	MacConkey agar
McC-COL	MacConkey agar + 2 mg/L colistin
McC-CTX	MacConkey agar + 1 mg/L cefotaxime
MIC	Minimum Inhibitory Concentration
MLST	Multi-locus sequence typing
MS	Member States
NAL	Nalidixic acid
NUTS	Nomenclature of Units for Territorial Statistics
OXA-48	ChromID® OXA-48 agar, for isolation of carbapenemase resistant *E. coli*
pAmpC	Plasmid-encoded Ambler class C beta-lactamases
PCR	Polymerase Chain Reaction
PHENOTYPE	observable characteristics of bacterium due to gene expression

Acronym	Definition
RESISTANCE PHENOTYPE	relating to an antimicrobial and its resistance to it
ST	referring to sequence type as determined by MLST
SUL	Sulfamethoxazole
TET	Tetracycline
TGC	Tigecycline
TMC	Temocillin
TMP	Trimethoprim
WGS	Whole Genome Sequencing
WHO	World Health Organisation

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