# FINAL REPORT

# RDFS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in *E. coli* from Retail Meats (Year 2 - Chicken)

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# 1. Liability statement

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# 2. Executive summary

In accordance with European Directive **2003/99/EC** on the monitoring of zoonoses and zoonotic agents, Member States (MS) are obliged to ensure that procedures are in place to monitor and report on the occurrence of antimicrobial resistance (AMR) in zoonotic organisms. The European Commission Implementing Decision **2013/652/EU**, which came into force **1 January 2014**, outlines the technical requirements for AMR testing, as well as the organisms and livestock species in which AMR must be monitored and reported. Mandatory requirements are set out for MS to monitor and report AMR data for *Salmonella* spp., *Campylobacter jejuni*, indicator commensal *Escherichia coli*, AmpC and extended-spectrum beta-lactamase (ESBL) *E. coli* and carbapenemase producing *E. coli.*

This report outlines the procedures put in place to fulfil these requirements for retail chicken meat in 2016 for AmpC, ESBL and carbapenem resistant *E. coli*, following European Union (EU) guidelines and methods. The requirements (with additional detailed guidance from the EU Reference Laboratory for Antimicrobial Resistance) state that 300 retail chicken should be tested by culture for *E. coli* on MacConkey agar containing 1 mg/L of the cephalosporin antibiotic cefotaxime. *E. coli* isolates cultured from such media are expected to show third generation cephalosporin resistance which may include ESBL or AmpC type resistance, and should be further tested by performing Minimum Inhibitory Concentrations (MICs) to determine their susceptibility to a panel of antibiotics. Samples were also tested for carbapenem resistant *E. coli* on chromID® carba and chromID® OXA-48 agars as recommended by the EU. Furthermore, samples were also plated to CHROMagar™ ESBL for specific detection of ESBL-producing *E. coli* and to MacConkey agar containing 2 mg/L colistin, for detection of colistin resistant *E. coli*. These additional screening agars were added at the request of the FSA. Other additional work was requested by the FSA outside the remit of Decision 2013/652/EU, and included a multiplex PCR to detect *bla*$_{\text{CTX-M}}$, *bla*$_{\text{OXA,}}$ *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes[1] for *E. coli* isolated from CHROMagar™ ESBL, and sequencing of the *bla*$_{\text{CTX-M}}$ genes in CTX-M positive isolates from this agar. Presumptive *E. coli* from MacConkey agar + 2 mg/L colistin were also tested for the presence of plasmid mediated colistin resistance genes *mcr1* and *mcr2.* Finally, viable counts of all samples were also determined on MacConkey agar + 1 mg/L cefotaxime and on CHROMagar™ ESBL.

For this study, as in 2015, the Animal and Plant Health Agency (APHA) worked in conjunction with Hallmark Veterinary Compliance Services, who arranged sampling,

collection and posting of samples to APHA, and have reported separately on the sample details.[2]

The number of samples allocated in each NUTS-3 area was proportional to the population. Samples were allocated to each retailer in proportion to market share based on Defra's 2013 Family Food data. The FSA provided a table showing the proportion of retail chicken purchases by weight for large supermarket chains in the UK from 2013 Family Food data.

All samples were collected from the 11 supermarket chains in the "Big Four" and "Other large supermarket" category. Only fresh chicken with skin on or off was collected. Processed, pre-prepared including goujons, ready-based, marinated, seasoned, herbed, stuffed, “cook in the bag”, breaded, battered chicken, frozen or cooked chicken were all excluded. Samples were collected twice per quarter. To ensure an even distribution the average sample numbers per quarter were 79 (approx. 40 samples per sampling week).

A total of 313 samples were collected between January and December 2016 (three samples were excluded from analysis because of foreign providence) from England (n=272), Scotland (n=20), Wales (n=11), and Northern Ireland (n=10). The types of chicken meat collected were whole chicken (n=158), chicken breast (n=79) and other cuts, including quarters, legs, thighs & drumsticks (n=76).

A bespoke in-house SOP based on published EU methods was written for the purpose of this study and agreed with the FSA before commencement of work, for the previous year’s sampling. This SOP was used for this year also, but with modifications, for example the inclusion of agars for the isolation of carbapenem and colistin resistant *E. coli*. The method involved enrichment of 25 grams of meat in Buffered Peptone Water (BPW), before plating this enrichment broth to the selective agars. The method has the theoretical potential to detect one AmpC or ESBL *E. coli* in 25 grams of meat.

None of the samples were positive on the two carbapenem agars. Of the 313 samples tested, 141 (45.1%, 95% confidence interval 39.6% to 50.6%) grew on MacConkey agar + 1 mg/L cefotaxime. Between 1 and 82 samples were tested from the 11 different supermarket chains, and between 25% and 100% (only one sample tested) of samples from each supermarket gave rise to *E. coli* on MacConkey agar + 1 mg/L cefotaxime.

A total of 95 samples, representing 30.4% (95% confidence interval 25.5% to 35.7%) of samples tested overall, gave rise to growth of presumptive ESBL-producing *E. coli* on CHROMagar™ ESBL. For these 95 isolates from CHROMagar™ ESBL, 92.6% were positive for the $bla_{\text{CTX-M}}$ gene by PCR, whilst 5 / 7 of the remaining isolates were positive for the $bla_{\text{SHV}}$ gene, one was only positive for $bla_{\text{TEM}}$ and one was negative for $bla_{\text{CTX-M}}$, $bla_{\text{OXA,}}$

*bla*$_{SHV}$ and *bla*$_{TEM}$ genes[1]. Most of the *bla*$_{CTX-M}$ positive isolates had the sequence of the *bla*$_{CTX-M}$ gene determined by sequencing of PCR products, and additionally by whole genome sequencing for four isolates. Isolates were mainly sequence type *bla*$_{CTX-M\ 1}$ ( n = 72), with single isolates determined as *bla*$_{CTX-M\ 15}$ or *bla*$_{CTX-M\ 28}$ or *bla*$_{CTX-M\ 32}$ or *bla*$_{CTX-M\ 55}$. The sequence types of the *bla*$_{SHV}$ genes were not determined.

Whilst nine samples gave growth to presumptive *E. coli* on MacConkey agar + 2 mg/L colistin, none of the "sweeps" ~ 10 to 20 colonies tested by RT-PCR were positive for plasmid mediated colistin resistance genes *mcr-1* or *mcr-2*, and as such isolates were not kept for further tests.

Only 12 samples (3.8%, 95% confidence interval 2.2% to 6.6%) from 6/ 11 supermarkets gave rise to presumptive *E. coli* counts on MacConkey agar + 1 mg/L cefotaxime and / or CHROMagar™ ESBL. These counts ranged from 40 cfu/gram (detection limit) to 400 cfu/gram.

Determination of the susceptibility (MICs) of isolates to a panel of relevant antibiotics allowed phenotypic characterisation of third generation cephalosporin resistance. An ESBL phenotype was inferred if the isolates were resistant to cefotaxime and / or ceftazidime but susceptible to cefoxitin and the isolates showed clavulanate synergy with cefotaxime and / or ceftazidime. An AmpC phenotype was inferred if cefotaxime/ clavulanate and ceftazidime / clavulanate synergy was not shown and isolates were resistant to cefotaxime, ceftazidime and cefoxitin.

By MICs, 93/141 of the isolates from MacConkey agar + 1 mg/L cefotaxime were found to have an ESBL phenotype (including 3 isolates that had an AmpC/ESBL phenotype), representing 29.7% (95% confidence interval 24.9% to 35.0%) of samples tested overall; 48/141 were found to have an AmpC phenotype (excluding the 3 isolates that also had an ESBL phenotype), representing 15.3% (95% confidence interval 11.8 % to 19.7%) of samples tested overall. If including the AmpC/ESBL phenotype isolates, 16.3% (95% confidence interval 12.6% to 20.8%) of the samples tested had an AmpC phenotype.

Between 8.3% and 100% (only one sample tested), and between 0% and 33% of the samples tested per supermarket had an ESBL or AmpC phenotype respectively.

None of the 141 isolates from MacConkey agar + 1 mg/L cefotaxime were microbiologically resistant (using EUCAST ECOFFS) to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin. Additionally, none of the isolates were resistant to the antibiotics azithromycin, temocillin and tigecycline.

Only three isolates were resistant to gentamicin and only about 25% of isolates were resistant to chloramphenicol, but as would be expected, all isolates were resistant to the beta-lactam antibiotic ampicillin. All of the isolates designated as ESBL phenotype were resistant to the cephalosporin antibiotics cefepime and ceftazidime, and all but one isolate was resistant to cefotaxime. All of the isolates designated as AmpC were resistant to cefoxitin.
Most or several of the isolates were resistant to the antibiotics sulfamethoxazole (78.0%), tetracycline (74.5%) and trimethoprim (38.3%) and about 25% of the isolates were resistant to the quinolone antibiotics nalidixic acid and / or ciprofloxacin. Interestingly, isolates with an AmpC phenotype tended to show fewer strains resistant to the antibiotics cefepime, ciprofloxacin, nalidixic acid sulfamethoxazole, tetracycline and trimethoprim than isolates with an ESBL phenotype.

Overall results showed that (93) 29.7% and (51) 16.3% of retail chicken samples were positive for ESBL or AmpC phenotype *E. coli* respectively (including the 3 isolates with the combined AmpC/ESBL phenotype), based on results from MacConkey agar + 1 mg/L cefotaxime. Using CHROMagar™ ESBL, 95 (30.4%) of samples were positive for presumptive ESBL phenotype *E. coli*, of which 88 (28.1%) of samples were confirmed to be *bla*$_{\text{CTX-M}}$ positive (mainly *bla*$_{\text{CTX-M 1}}$), and a further 5 isolates were positive for *bla*$_{\text{SHV,}}$ giving 93 (29.7%) of isolates positive for either *bla*$_{\text{CTX-M}}$ or *bla*$_{\text{SHV}}$. Results showed a decrease in the proportion of samples positive for ESBL-producing *E. coli* compared to a previous (2013-2014) UK study, which reported that 65.4% of 159 retail chicken samples were positive for ESBL-producing *E. coli.*[3] This difference was statistically significant using a chi-squared test (odds ratio 0.45 p-value<0.001). Whilst the 2013/14 study used similar methodology, and utilised samples from three regions in England, and from Scotland and Wales, it should be noted that the slightly different sampling criteria and sample processing methods used in the current study could have influenced results. None of the samples were positive for carbapenem resistant *E. coli* or for *E. coli* positive for the plasmid mediated colistin resistance genes *mcr-1* and *mcr-2*, and only 12 meat samples had viable counts (without enrichment) of presumptive ESBL-producing *E. coli* above the detection limit (40 cfu/gram), and these counts were all less than or equal to 400 cfu/gram.

## 3. Lay person's summary

In accordance with European Directive **2003/99/EC** on the monitoring of bacteria that can pass from animals to humans and causes disease (zoonoses and zoonotic agents), Member States (MS) are obliged to ensure that procedures are in place to monitor and report on the occurrence of antimicrobial resistance (AMR) in such bacteria.

The requirements (with additional detailed guidance from the EU Reference Laboratory for Antimicrobial Resistance) state that 300 retail chicken should be tested by culture for the bacterium *E. coli. E. coli* bacteria are a normal part of the gut flora of mammals and as such can be useful "indicator" bacteria for AMR. Whilst some strains of *E. coli* can cause disease, most strains of *E. coli* can be present in healthy animals and humans.

The EU requirements state that samples should be tested on an agar that will select for resistance to antibiotics known as third generation cephalosporins, and such antibiotics are important for treating infections in humans. *E. coli* from this agar normally show two main types of resistance types known as Extended Spectrum β-lactamse (ESBL) or AmpC type resistance. Isolates from this agar were then tested by performing Minimum Inhibitory Concentrations (MICs) to determine the susceptibility / resistance of isolates to a panel of antibiotics.

EU requirements also state that samples should be tested on two agars that will select for resistance to a group of antibiotics known as carbapenems. Carbapenem antibiotics are also really important in human medicine, as they are termed "last resort" antibiotics, used to treat infections when all or almost all other treatment options are non-viable, due to the target bacteria being resistant to most / all other relevant antibiotics. Other agars used at the request of the FSA to test samples included an agar to specifically isolate *E. coli* with ESBL type resistance and an agar to isolate colistin resistant *E. coli.* Colistin is another "last resort" antibiotic, so it is important to monitor if resistance to this type of bacteria is occurring in food samples.

Other additional work requested by the FSA included genetic tests to determine what antibiotic resistance genes were associated with ESBL and colistin resistance in *E. coli* isolates. For colistin resistance, a mobile resistance gene referred to as *mcr*-1 was discovered in 2015, so colistin resistant *E. coli* were tested for this gene. The *mcr*-1 gene is considered particularly important as is encodes resistance to the "last resort" antibiotic colistin, and as it is mobile it has the potential to transfer resistance in the gut to other similar bacteria. Finally, extra work requested by the FSA included peforming counts of antibiotic resistant (AmpC

and ESBL type resistance only) *E. coli* in each sample, as this provides useful information on whether AmpC/ESBL positive meat samples have a low or high number of antibiotic resistant bacteria on them.

The number of samples allocated in each area was proportional to the population and .samples were collected from the 11 supermarket chains in the "Big Four" and "Other large supermarket" category. Only fresh chicken with skin on or off was collected. Samples were collected twice per quarter. To ensure an even distribution the average sample numbers per quarter were 79 (approx. 40 samples per sampling week).

A total of 313 samples were collected between January and December 2016 from England (n=272), Scotland (n=20), Wales (n=11), and Northern Ireland (n=10). The types of chicken meat collected were whole chicken (n=158), chicken breast (n=79) and other cuts, including quarters, legs, thighs & drumsticks (n=76).

Overall results showed that 93 (29.7%) and 51 (16.3%) of retail chicken samples were positive for ESBL or AmpC phenotype *E. coli* respectively. Genetic tests showed that most of the isolates that had ESBL type resistance had a gene known as the $bla_{\text{CTX-M-1}}$ gene. This gene is known to confer resistance to third generation cephalosporin antibiotics and this gene has commonly been found in *E. coli* from chickens and chicken meat in other studies.

None of the samples were positive for *E. coli* resistant to the last resort carbapenem antibiotics or for *E. coli* positive for the plasmid mediated colistin resistance genes *mcr-1*.

Only 12 meat samples had counts of presumptive ESBL-producing *E. coli* above the detection limit of 40 bacteria per gram of meat, and these counts were all less than or equal to 400 bacteria per gram of meat.

Results showed a decrease in the proportion of samples positive for ESBL-producing *E. coli* compared to a previous (2013-2014) UK study, which reported that 65.4% of 159 retail chicken samples were positive for ESBL-producing *E. coli.*[3] The differences were statistically significant. However, whilst the 2013/14 study used similar methodology, and utilised samples from three regions in England, and from Scotland and Wales, it should be noted that the slightly different sampling criteria and sample processing methods used in the current study could have influenced results.

## 4. Glossary

**AmpC phenotype** – A phenotype of resistance to cephalosporin antibiotics such as cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor-β-lactam combinations.

**AmpC enzyme** – Enzyme conferring AmpC type resistance

**AMR** – Antimicrobial resistance

**APHA** – Animal and Plant Health Agency

**BPW** – Buffered Peptone broth, a liquid media widely used to grow bacteria

**CRL** – Community Reference Laboratory

**CTX-M** – group of ESBL enzymes that give bacteria resistance to cephalosporin antibiotics.

**Enterobacteriaceae** – Family of bacteria including many common gut bacteria such as *Escherichia coli* or *E. coli*

**CA-ESBL** - CHROMagar™ ESBL, for isolation of ESBL-producing *E. coli*

**CARBA** - ChromID® CARBA agar, for isolation of carbapenemase resistant *E. coli*

**COL** - Colistin

**CTX** – Cefotaxime

**ECOFF** – Epidemiological Cut Off value (with respect to antibiotic resistance)

**EN** - Norme Européenne /Europäische Norm (European Standard)

**ESBL** – Extended Spectrum β-lactamase. Enzymes that are capable of breaking down many penicillin type antibiotics, including cephalosporin antibiotics

**EU** – European Union

**EUCAST** - European Committee on Antimicrobial Susceptibility Testing

**FSA** – Food Standards Agency

**HCCA** - α-Cyano-4-hydroxycinnamic acid

**ISO** - International Organisation for Standardisation

**MALDI ToF** – Matrix-Assisted Laser Desorption / Ionization Time-of-Flight

**MCA** – MacConkey agar

**MCA-COL** – MacConkey agar + 2 mg/L colistin

**MCA-CTX** - MacConkey agar + 1 mg/L cefotaxime

**MIC** – Minimum Inhibitory Concentration

**MS** – Member States

**NUTS** - Nomenclature of Units for Territorial Statistics

**OXA-48** - ChromID® OXA-48 agar, for isolation of carbapenemase resistant *E. coli*

**PBS** – Phosphate Buffered saline

**QC** – Quality control

**SOP** – Standard Operating Procedure

# 5. Materials and Methods

All the methodology with respect to the work performed is detailed in five internal APHA Standard operating procedures (not included in this report).

These SOPs are:-

- Isolation of background (indicator commensal) and antibiotic resistant *Enterobacteriaceae* from meats and caecal contents according to CRL, EU and / or APHA protocols (CBU 0278).
- Microbank -70ºC Bacterial Storage System (CBU0155).
- Storage of *Salmonella* and *E. coli* Day Cultures (CBU0093).
- Identification of Bacteria by Oxidase (BA050) and Indole Spot Test – a Rapid Method for Bacteria (BA0130) and by MALDI ToF (BAC 0334).
- Minimum Inhibitory Concentration (MIC) – The Sensititre Method (BA0604).
- Oxidase (BA050)
- Indole Spot Test – a Rapid Method for Bacteria (BA0130)
- Real Time PCR for plasmid mediated colistin resistance genes *mcr-1* and *mcr-2* (BAC0415).

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

**Isolation of background (indicator commensal) and antibiotic resistant *Enterobacteriaceae* from meats and caecal contents according to EU and / or APHA protocols.**

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

- **EU method -** Isolation of ESBL, AMPC and carbapenemase producing E. coli from fresh meat - October 2015
- **EU method -** Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AMPC producing *E. coli* in meat and animals - November 2015
- **EU method** - Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli*

Pdf files of the most recent versions of the above EU methods can be found on-line at http://eurl-ar.eu/233-protocols.htm.

The EU method was slightly modified in order to perform viable bacterial counts on the meat samples, as requested by the FSA. However, this modification did not affect overall the work being carried out according to EU methods.

In brief, 25 gram of meat sample collected, transported and stored under conditions as stipulated by the EU protocols, was homogenised in 75 ml of sterile chilled PBS and a small amount (~2 ml) of this homogenate was kept for viable bacterial counts. The remainder of the chilled PBS-meat homogenate was added to 150 ml of 1.66 x sterile BPW (to make 250 ml of single strength BPW), which was incubated at 37 ± 1ºC for 18-22 hours.

The incubated BPW / meat homogenate was used to inoculate (10µl) MacConkey agar containing 1 mg/L cefotaxime (MCA-CTX), chromID® CARBA (CARBA) and chromID® OXA-48 (OXA-48). Samples were also plated to CHROMagar™ ESBL (CA-ESBL), for specific detection of ESBL-producing *E. coli* and to MacConkey agar containing 2 mg/L colistin (MCA-COL), for detection of colistin resistant *E. coli*, and these were additional non-EU stipulated screening agars added at the request of the FSA.

All plates were QC tested prior to use, according to EU or APHA methods as appropriate, as outlined in the SOP.

MCA-CTX and MCA-COL plates were incubated for 18-22 hours at 44 ± 0.5 °C before checking for lactose fermenting colonies. Other media were incubated at 37 ± 1ºC for 18-22 hours, before checking for presumptive *E. coli.*

Lactose fermenters from MCA-CTX were assumed to be presumptive AmpC / EBSL *E. coli*, blue colonies from CA-ESBL were assumed to be presumptive ESBL-producing *E. coli* and pink to burgundy colour colonies from CARBA and OXA-48 agars were assumed to be presumptive carbapenem resistant *E. coli.* A single presumptive *E. coli* from each of these agars was plated again to the agar of origin to ensure purity prior to storage and further tests. This method has the theoretical potential to detect one *E. coli* of interest per 25 grams of meat.

From MCA-COL plates, a sweep of ~ 10 to 20 lactose fermenters (according to SOP BAC0415) was used to prepare a crude DNA sample for detection of *mcr-1* and *mcr-2* plasmid mediated colistin resistance genes by real time PCR. A sweep was taken to increase the sensitivity of detection of the *mcr* genes.

The proportion of positive samples were calculated, and exact binomial 95% confidence intervals for each of the proportions were calculated in Stata 12 (Stata Corporation, College Station, TX, USA).

**Storage of purified presumptive AMPC / EBSL *E. coli* prior to further tests**

Isolates will be stored for up to five years to comply with EU requirements. Isolates were stored in duplicate, on Dorset egg slopes, and / or "bead" culture (frozen in cryogenic material at -70ºC).

In brief, for Dorset egg slopes, a small amount of purified bacterial culture was aseptically transferred using a 10 µl loop from the second agar plate to the Dorset egg slope, which was then stored at room temperature. For "beads," a larger amount of purified bacterial culture was aseptically transferred using a 10 µl loop from the second agar plate to a commercial "beads" tube. The cryogenic liquid and bacterial growth was mixed in the tube, before removing most of the supernatant cryogenic liquid, and then storing the tube at - 70ºC.

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

For lactose fermenters isolated from MCA-CTX at 44ºC, combined use of oxidase and indole tests as described by in-house SOPs, was used to confirm such isolates as *E. coli*. Presumptive *E. coli* from other agars, such as CA-ESBL, CARBA and OXA-48, were identified by MALDI ToF as described by an in-house SOP and based on that previously described.[4]

For the oxidase test and indole tests, a single well isolated colony was taken from MCA-CTX agar, plated onto blood agar and incubated overnight at 37ºC. Growth from the blood agar was then used to perform oxidase and indole tests.

For the oxidase test, in-brief, a portion of bacterial colony to be tested was taken with a sterile plastic loop and rubbed onto filter paper impregnated with oxidase reagent. A deep purple colour developing within 10 seconds was taken to be "oxidase positive". The indole test was performed in the same way, but using filter paper impregnated with James reagent (BioMerieux). Within 10 seconds, a positive reaction was indicated by the presence of a colour change to pink/red. Lactose fermenter colonies from MCA-CTX that grew at 44ºC were confirmed as *E. coli* if oxidase negative and indole positive.

Isolates from non MacConkey agars prior to MALDI ToF were also grown on blood agar. A small amount of bacterial growth was applied to the metal target plate. Growth on the target

plates was overlaid with 1 µl of 70% formic acid to perform a partial protein extraction, and allowed to dry. Each spot was then overlaid with 1 µl of HCCA matrix, and again this was allowed to dry before the target plate was loaded into the MALDI ToF machine.

Using Biotyper software, resulting spectra from the MALDI ToF run were searched against the Bruker database of spectra, and if the resulting score was ≥ 2.000, this was taken as reliable identification to the species level.

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

MICs were performed as described in our in house SOP (BA0604), based on EN ISO 20776-1:2006.

*E. coli* isolates were inoculated into Mueller Hinton broth at a suitable dilution for application to commercially prepared plates containing two fold dilution series of antimicrobial compounds in accordance with Decision 2013/652/EU. After incubation at 37ºC for 18 hours, the plates were examined and growth end points established for each antimicrobial to provide MIC's. Microbiologically resistant and susceptible interpretation for the MIC's were obtained by comparison with ECOFF's published by EUCAST.

For *E.coli,* the presence of carbapenemase producing strains, Extended Spectrum Beta Lactamase producers (ESBL) or AmpC enzyme producers was determined initially by assessing isolate MIC's against the microbiological breakpoints for meropenem, cefotaxime and ceftazidime. Any isolates showing a meropenem MIC's greater than 0.125mg/l, cefotaxime MIC's greater than 0.25mg/l or ceftazidime MIC's greater than 0.5mg/l were tested against a further panel of antimicrobials containing cefotaxime, ceftazidime, cefotaxime / clavulanate, ceftazidime / clavulanate, imipenem, ertapenem, temocillin, cefoxitin, cefepime and meropenem. Consequently, isolates have results reported for all of these confirmatory antimicrobials where an MIC greater than the cut off values stated above was observed for any of the screening compounds (cefotaxime, ceftazidime or meropenem) included in the first panel of antimicrobials.

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

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

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

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

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

**Statistics**

A simple chi-squared tests was used to compare some of the results from this study to a previous similar published study.[3]

# 6. Results

**General considerations**

An excellent working partnership was built up with the company contracted by FSA to supply the meat samples (HallMark Veterinary and Compliance Services) in 2015, and continued in 2016. Communication between the two organisations and all other aspects of the partnership were excellent.

All meat samples arrived within the correct temperature range, as stipulated by the EU requirements.

**Details of the meat samples tested.**

The background details of the meat samples tested were provided as part of the report produced by HallMark for the FSA.[2] Table 1 of the FSA report has been reproduced in the main in this report (Table 1) for convenience (with agreement from HallMark and the FSA). The samples collected by region and the number that were positive for AmpC / ESBL *E. coli* on MCA-CTX are shown in Table 2.

**Samples positive for AmpC / ESBL *E. coli* on MCA-CTX**

Of the 313 samples tested, 141 (45.1%, 95% confidence interval 39.6% to 50.6%) grew on MCA-CTX (Tables 3 and 4). Between 1 and 82 samples were tested from the 11 different supermarket chains, and between 25% and 100% (only one sample tested) of samples from each supermarket gave rise to *E. coli* on MCA-CTX (Table 5).

By MICs, 93/141 of the isolates from MCA-CTX were found to have an ESBL phenotype (Table 4), including 3 isolates that had an AmpC/ESBL phenotype, representing 29.7% (95% confidence interval 24.9% to 35.0%) of samples tested overall, and 48/141 isolates (exluding the three isolates with an AmpC/ESBL phenotype) were found to have an AmpC phenotype, representing 15.3% (95% confidence interval 11.8 % to 19.7%) of samples tested overall, or 16.3% (95% confidence interval 12.6% to 20.8%) of samples tested if isolates with an AmpC/ESBL phenotype are included (Table 5).

**MIC results for isolates from MCA-CTX**

Table 6 shows the individual antibiotic sensitivities for isolates from meat samples positive on MCA-CTX, from which the ESBL and AmpC phenotypes were derived.

The summary interpretation of MIC results for *E. coli* isolates from MCA-CTX for the 141 positive samples is shown in Table 7.

None of the isolates were microbiologically resistant (using EUCAST ECOFFS) to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin. Additionally, none of the isolates were resistant the antibiotics azithromycin, temocillin and tigecycline.

Most or several of the isolates were resistant to the antibiotics sulfamethoxazole, tetracycline and trimethoprim, and about 25% of the isolates were resistant to the quinolone antibiotics nalidixic acid and ciprofloxacin.

Isolates with an AmpC phenotype tended to show fewer strains resistant to the antibiotics cefepime, ciprofloxacin, nalidixic acid sulfamethoxazole, tetracycline and trimethoprim than isolates with an ESBL phenotype (Table 7).

**Counts of presumptive ESBL-producing *E. coli* from MCA-CTX and CA-ESBL**

Of the 313 samples tested, only 12 (3.8%, 95% confidence interval 2.2% to 6.6%) from various supermarkets gave rise to presumptive *E. coli* counts on MacConkey agar + 1 mg/L cefotaxime and / or CHROMagar™ ESBL (Table 8) above the detection limit. These counts ranged from 40 cfu/gram (detection limit) to 400 cfu/gram.

**Presumptive ESBL-producing *E. coli* from CA-ESBL and PCR results**

A total of 95 samples, representing 30.4% (95% confidence interval 25.5% to 35.7%) of samples tested overall, gave rise to growth of presumptive ESBL-producing *E. coli* on CA-ESBL (Table 9). For the 95 isolates from CA-ESBL, 92.6% were positive for the $bla_{\text{CTX-M}}$ gene by PCR, whilst the remaining 7 isolates were mainly positive the $bla_{\text{SHV}}$ gene (n = 5). Most of the $bla_{\text{CTX-M}}$ positive isolates had the sequence of the $bla_{\text{CTX-M}}$ gene determined by PCR and additionally by whole genome sequencing for four isolates. Isolates were mainly sequence type CTX-M 1, with one of each isolate determined as CTX-M 15 or 28 or 32 or 55. The sequence types of the $bla_{\text{SHV}}$ genes were not determined.

**Results of whole genome sequencing**

Four isolates were found to be CTX-M 15 by sequencing of PCR amplicons, and these isolates were whole genome sequenced to determine if they were the pandemic O25: H4, ST131 clonal strain (Table 10). None of the isolates were O25: H4, ST131 (Table 10), although for one isolate, the MLST and O group was not determined, but this isolate was H31 and not H4.

Surprisingly, by WGS, only one of the four isolates was found to be CTX-M 15 and the other three were closely related CTX-M types 1 and 55. Original cultures and DNA preparations for both the WGS and the original PCR were re-tested, and all results agreed with the results from the WGS (Table 10). In previous work, on a few occasions, isolates had two CTX-M types, and it is possible that for three of the isolates CTX-M 15 was detected on first testing, but not subsequent testing. This could relate to the predominant gene type at the time of testing, if more than one CTX-M type was present.

**Plasmid mediated colistin resistance genes *mcr-1* and *mcr-2***

Nine samples gave growth to presumptive *E. coli* on MCA-COL, none of the isolates tested by RT-PCR were positive for plasmid mediated colistin resistance genes *mcr-1* or *mcr-2.*

**Statistics**

Results showed a decrease in the proportion of samples positive for ESBL-producing *E. coli* compared to a previous (2013-2014) UK study, which reported that 65.4% of 159 retail chicken samples were positive for ESBL-producing *E. coli.*[3] This difference was statistically significant using a chi-squared test (odds ratio 0.45 p-value<0.001). Whilst the 2013/14 study used similar methodology, and utilised samples from three regions in England, and from Scotland and Wales, it should be noted that the slightly different sampling criteria and sample processing methods used in the current study could have influenced results.

## 7. Tables

**Table 1.** HallMark summary of sampling strategy

| Summary of Sampling Strategy | Comments and Deviations |
|---|---|
| **Selection of NUTS3 regions**<br><br>Based on the "MYE2: Population Estimates by single year of age and sex for local authorities in the UK, mid-2014" 109 NUTS3 regions to be selected so that they cover all 4 countries in the UK, and comprise at least 80% of the UK population. | No deviation |
| **Sample Numbers**<br><br>The number of samples allocated in each NUTS-3 area to be proportional to the population. To account for potential loss of samples, missing data etc. an additional 5% of samples to be planned in the sampling scheme = 316 chicken samples. | No deviation |
| The number of chicken samples to be collected (includes 5% contingency):<br><br>England - 273<br>Scotland - 21<br>Wales - 11<br>Northern Ireland - 10<br>Total: 315 | Achieved number of samples:<br><br>England – 272<br>Scotland – 20<br>Wales – 11<br>Northern Ireland – 10<br>Total: 313<br><br>**Notes on unassayable samples:** |

| | |
|---|---|
| | **England:** 2 samples unassayable due to foreign provenance. One of these was replaced with an assayable re-sample.<br>**Scotland:** One unassayable sample due to foreign provenance.<br>Unassayable samples are not listed in the final data reporting. |
| Samples to be collected twice per quarter. To ensure an even distribution the average sample numbers per quarter are 79 (approx. 40 samples per sampling week). | No deviations |
| **Food Categories**<br><br>• Whole chicken (50% of samples)<br>• Chicken breast – including diced/sliced (25% of samples)<br>• Other cuts – including quarters, legs, thighs, drumsticks (25% of samples)<br>• Total planned samples: 315<br><br>Collect only fresh chicken with skin on or off. Exclude processed, pre-prepared chicken including goujons, ready-based, marinated, seasoned, herbed, stuffed, "cook in the bag", breaded or battered chicken. Also, exclude frozen or cooked chicken. | 158 Whole chicken – 50%<br>79 chicken breast – 25%<br>76 other cuts: 24%<br><br>Total: 313 (99% of the planned sample number. 104% of the required 300 samples).<br><br>No deviations |

| | |
|---|---|
| **Target numbers for Meat Cuts**<br><br>Whole chicken: 157<br>Chicken breast: 79<br>Other cuts: 79 | Numbers sampled:<br>Whole chicken: 158 (1 replacement for unavailable 'other cuts')<br>Chicken breast: 79<br>Other cuts: 76 (2 unassayable, 1 unavailable)<br><br>Note: 5 samples from Northern Ireland arrived later than 24h with the lab but still under 36h; as they were still at the correct temperature and within the correct use by dates they have been listed as assayable samples. |
| **Selection of Retailers**<br><br>All samples to be collected from the 11 supermarket chains in the "Big Four" and "Other large supermarket" category. They are Tesco, Asda, Sainsbury's, Morrison's, Aldi, Co-op, Marks and Spencer, Waitrose, Iceland, Lidl and Spar. | No deviations |
| **Selection of purchase points within supermarket chains and within a NUTS3 region**<br><br>Within a NUTS3 region, according to availability of the specified retail chains, surveyors are assigned specific retail outlet addresses. | No deviations |

| | |
|---|---|
| **Retailer availability**<br>Experience shows that surveyors find a small percentage of scheduled retail outlets changed or closed (temporarily or permanently). HallMark may need to swap the pre-assigned retailers between NUTS-3 regions in some circumstances. To be done minimising the impact and maintaining the market share %. | No deviations |
| **Selection of specific products within each meat category**<br>Surveyors can freely select a sample from the randomly assigned food category and from the assigned retailer outlet.<br>For a description of the sampling methodology addressing the specific evidence requirement and the objectives outlined above can be found in the Sampling Instructions for Surveyors (attached). Samples must be collected exactly as described in this document. | No deviations |
| **Number of Samples per Retail Chain**<br>Samples allocated to each retailer in proportion to market share based on Defra's 2013 Family Food data. FSA provided a table showing the Proportion of retail chicken purchases by weight for large supermarket chains in the UK from 2013 Family Food data[3] | |

**Table 2**. Number of samples collected by regions and isolates tested by MICs

| NUTS 3 or other political structure used in sample allocation | Total number of samples collected | Number of isolates available for AMR testing (ESBL-, AmpC-producing E.coli) | Number of isolates available for AMR testing (Carbapenemase-producing E.coli) | Number of isolates tested by MICs |
|---|---|---|---|---|
| Aberdeen City and Aberdeenshire | 3 | 2 | 0 | 2 |
| Barking & Dagenham and Havering | 3 | 1 | 0 | 1 |
| Barnet | 2 | 0 | 0 | 0 |
| Barnsley, Doncaster and Rotherham | 5 | 4 | 0 | 4 |
| Bath and North East Somerset, North Somerset and South Gloucestershire | 4 | 1 | 0 | 1 |
| Berkshire | 5 | 0 | 0 | 0 |
| Bexley and Greenwich | 3 | 3 | 0 | 3 |
| Birmingham | 7 | 6 | 0 | 6 |
| Bournemouth and Poole | 2 | 1 | 0 | 1 |
| Bradford | 3 | 1 | 0 | 1 |
| Brent | 2 | 0 | 0 | 0 |
| Bristol, City of | 3 | 2 | 0 | 2 |
| Bromley | 2 | 1 | 0 | 1 |
| Buckinghamshire CC | 3 | 1 | 0 | 1 |
| Calderdale and Kirklees | 4 | 1 | 0 | 1 |
| Cambridgeshire CC | 4 | 3 | 0 | 3 |
| Cardiff and Vale of Glamorgan | 3 | 1 | 0 | 1 |
| Central Hampshire | 3 | 1 | 0 | 1 |
| Central Valleys | 2 | 1 | 0 | 1 |
| Cheshire East | 2 | 2 | 0 | 2 |
| Cheshire West and Chester | 2 | 1 | 0 | 1 |
| Clackmannanshire and Fife | 3 | 2 | 0 | 2 |

| | | | | |
|---|---|---|---|---|
| Cornwall and Isles of Scilly | 3 | 2 | 0 | 2 |
| Coventry | 1 | 1 | 0 | 1 |
| Croydon | 2 | 2 | 0 | 2 |
| Devon CC | 5 | 1 | 0 | 1 |
| Dorset CC | 3 | 2 | 0 | 2 |
| Dudley | 2 | 1 | 0 | 1 |
| Durham CC | 3 | 1 | 0 | 1 |
| Ealing | 2 | 2 | 0 | 2 |
| East Dunbartonshire, West Dunbartonshire and Helensburgh & Lomond | 2 | 1 | 0 | 1 |
| East Kent | 3 | 0 | 0 | 0 |
| East Lancashire | 2 | 0 | 0 | 0 |
| East Merseyside | 3 | 1 | 0 | 1 |
| East of Northern Ireland | 3 | 2 | 0 | 2 |
| East Riding of Yorkshire | 2 | 2 | 0 | 2 |
| East Surrey | 2 | 1 | 0 | 1 |
| East Sussex CC | 3 | 0 | 0 | 0 |
| Edinburgh, City of | 3 | 0 | 0 | 0 |
| Enfield | 2 | 1 | 0 | 1 |
| Essex Haven Gateway | 3 | 1 | 0 | 1 |
| Essex Thames Gateway | 2 | 0 | 0 | 0 |
| Flintshire and Wrexham | 2 | 2 | 0 | 2 |
| Glasgow City | 4 | 2 | 0 | 2 |
| Gloucestershire | 4 | 2 | 0 | 2 |
| Greater Manchester North East | 4 | 2 | 0 | 2 |
| Greater Manchester North West | 4 | 1 | 0 | 1 |
| Greater Manchester South East | 3 | 1 | 0 | 1 |
| Greater Manchester South West | 3 | 2 | 0 | 2 |
| Gwent Valleys | 2 | 2 | 0 | 2 |

| | | | | |
|---|---|---|---|---|
| Hackney and Newham | 4 | 2 | 0 | 2 |
| Haringey and Islington | 3 | 2 | 0 | 2 |
| Harrow and Hillingdon | 3 | 1 | 0 | 1 |
| Hartlepool and Stockton-On-Tees | 2 | 0 | 0 | 0 |
| Heart of Essex | 2 | 0 | 0 | 0 |
| Hertfordshire | 7 | 5 | 0 | 5 |
| Hounslow and Richmond upon Thames | 3 | 1 | 0 | 1 |
| Inverclyde, East Renfrewshire and Renfrewshire | 2 | 2 | 0 | 2 |
| Kensington & Chelsea and Hammersmith & Fulham | 2 | 0 | 0 | 0 |
| Kent Thames Gateway | 2 | 0 | 0 | 0 |
| Lambeth | 2 | 0 | 0 | 0 |
| Leeds | 5 | 3 | 0 | 3 |
| Leicester | 2 | 1 | 0 | 1 |
| Leicestershire CC and Rutland | 4 | 2 | 0 | 2 |
| Lewisham and Southwark | 4 | 2 | 0 | 2 |
| Lincolnshire | 4 | 1 | 0 | 1 |
| Liverpool | 3 | 0 | 0 | 0 |
| Manchester | 3 | 0 | 0 | 0 |
| Merton, Kingston upon Thames and Sutton | 3 | 3 | 0 | 3 |
| Mid Kent | 2 | 0 | 0 | 0 |
| Mid Lancashire | 2 | 0 | 0 | 0 |
| North and North East Lincolnshire | 2 | 0 | 0 | 0 |
| North Hampshire | 2 | 0 | 0 | 0 |
| North Lanarkshire | 1 | 0 | 0 | 0 |
| North Northamptonshire | 2 | 0 | 0 | 0 |
| North Nottinghamshire | 3 | 0 | 0 | 0 |
| North of Northern Ireland | 2 | 1 | 0 | 1 |

| | | | | |
|---|---|---|---|---|
| North Yorkshire CC | 4 | 3 | 0 | 3 |
| Northumberland | 2 | 0 | 0 | 0 |
| Norwich and East Norfolk | 2 | 2 | 0 | 2 |
| Nottingham | 2 | 1 | 0 | 1 |
| Outer Belfast | 2 | 0 | 0 | 0 |
| Oxfordshire | 4 | 1 | 0 | 1 |
| Redbridge and Waltham Forest | 3 | 1 | 0 | 1 |
| Sandwell | 2 | 2 | 0 | 2 |
| Sheffield | 3 | 2 | 0 | 2 |
| Shropshire CC | 2 | 1 | 0 | 1 |
| Somerset | 3 | 2 | 0 | 2 |
| South and West Derbyshire | 3 | 2 | 0 | 2 |
| South Hampshire | 3 | 1 | 0 | 1 |
| South Lanarkshire | 2 | 0 | 0 | 0 |
| South Nottinghamshire | 2 | 0 | 0 | 0 |
| South West Wales | 2 | 2 | 0 | 2 |
| Staffordshire CC | 5 | 3 | 0 | 3 |
| Suffolk | 4 | 2 | 0 | 2 |
| Tyneside | 5 | 2 | 0 | 2 |
| Wakefield | 2 | 1 | 0 | 1 |
| Wandsworth | 2 | 2 | 0 | 2 |
| Warwickshire | 3 | 2 | 0 | 2 |
| West and South of Northern Ireland | 3 | 1 | 0 | 1 |
| West Essex | 2 | 0 | 0 | 0 |
| West Kent | 2 | 2 | 0 | 2 |
| West Northamptonshire | 2 | 1 | 0 | 1 |
| West Surrey | 5 | 3 | 0 | 3 |
| West Sussex (North East) | 2 | 2 | 0 | 2 |

| | | | | |
|---|---|---|---|---|
| West Sussex (South West) | 3 | 0 | 0 | 0 |
| Wiltshire | 3 | 2 | 0 | 2 |
| Wirral | 2 | 0 | 0 | 0 |
| Worcestershire | 4 | 2 | 0 | 2 |

**Table 3.** Samples positive for ESBL phenotype *E. coli* from MCA-CTX (Grey cells indicates isolates have an AmpC/ESBL phenotype)

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1364243 | 11/10/2016 | G | Other Cuts | Barnsley, Doncaster and Rotherham |
| 1364244 | 11/10/2016 | B | Chicken Breast | Barnsley, Doncaster and Rotherham |
| 1364250 | 11/10/2016 | B | Whole Chicken | Barnsley, Doncaster and Rotherham |
| 1364252 | 11/10/2016 | I | Whole Chicken | Sheffield |
| 1364254 | 11/10/2016 | A | Whole Chicken | Sheffield |
| 1364256 | 13/10/2016 | E | Whole Chicken | East Riding of Yorkshire |
| 1364266 | 12/10/2016 | D | Whole Chicken | West Northamptonshire |
| 1364273 | 12/10/2016 | B | Whole Chicken | South West Wales |
| 1364277 | 18/10/2016 | G | Other Cuts | Lewisham and Southwark |
| 1364278 | 18/10/2016 | K | Chicken Breast | Lewisham and Southwark |
| 1364280 | 12/10/2016 | K | Whole Chicken | Ealing |
| 1364281 | 12/10/2016 | L | Chicken Breast | Ealing |
| 1364283 | 11/10/2016 | A | Other Cuts | Leicestershire CC and Rutland |
| 1364284 | 07/12/2016 | G | Other Cuts | Gloucestershire |
| 1364285 | 07/12/2016 | A | Other Cuts | Gloucestershire |
| 1364293 | 12/10/2016 | I | Whole Chicken | Somerset |
| 1364303 | 25/08/2016 | B | Other Cuts | South and West Derbyshire |
| 1364308 | 24/08/2016 | G | Whole Chicken | Leicester |
| 1364310 | 23/08/2016 | K | Whole Chicken | Flintshire and Wrexham |
| 1364311 | 23/08/2016 | L | Whole Chicken | Cheshire East |
| 1364314 | 23/08/2016 | K | Chicken Breast | Cheshire West and Chester |

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1364325 | 23/08/2016 | K | Whole Chicken | Aberdeen City and Aberdeenshire |
| 1364327 | 23/08/2016 | B | Whole Chicken | Clackmannanshire and Fife |
| 1364332 | 24/08/2016 | A | Whole Chicken | Inverclyde, East Renfrewshire and Renfrewshire |
| 1364333 | 24/08/2016 | B | Chicken Breast | Inverclyde, East Renfrewshire and Renfrewshire |
| 1364335 | 24/08/2016 | B | Whole Chicken | East Dunbartonshire, West Dunbartonshire and Helensburgh & Lomond |
| 1364336 | 24/08/2016 | L | Whole Chicken | Glasgow City |
| 1364342 | 25/08/2016 | I | Chicken Breast | North of Northern Ireland |
| 1562692 | 19/01/2016 | K | Whole Chicken | Gwent Valleys |
| 1562695 | 19/01/2016 | K | Chicken Breast | Cardiff and Vale of Glamorgan |
| 1562696 | 19/01/2016 | K | Chicken Breast | Central Valleys |
| 1562930 | 24/05/2016 | B | Chicken Breast | Worcestershire |
| 1563707 | 12/10/2016 | K | Chicken Breast | Bristol, City of |
| 1563719 | 24/05/2016 | I | Chicken Breast | Sandwell |
| 1612887 | 07/12/2016 | K | Other Cuts | Oxfordshire |
| 1612891 | 08/12/2016 | E | Whole Chicken | Buckinghamshire CC |
| 1612903 | 07/12/2016 | C | Chicken Breast | Cornwall and Isles of Scilly |
| 1612904 | 07/12/2016 | C | Whole Chicken | Cornwall and Isles of Scilly |
| 1614173 | 15/04/2016 | K | Whole Chicken | East Surrey |
| 1614175 | 15/04/2016 | B | Chicken Breast | West Kent |
| 1614177 | 12/04/2016 | K | Chicken Breast | Glasgow City |
| 1614185 | 12/04/2016 | K | Whole Chicken | Hounslow and Richmond upon Thames |
| 1614188 | 12/04/2016 | A | Chicken Breast | Redbridge and Waltham Forest |
| 1614191 | 12/04/2016 | A | Whole Chicken | Bexley and Greenwich |

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1614193 | 12/04/2016 | K | Whole Chicken | Bexley and Greenwich |
| 1614194 | 23/02/2016 | G | Chicken Breast | South Hampshire |
| 1614198 | 24/02/2016 | B | Whole Chicken | Bournemouth and Poole |
| 1614199 | 23/02/2016 | G | Other Cuts | Dorset CC |
| 1614201 | 24/02/2016 | E | Chicken Breast | Dorset CC |
| 1614207 | 25/02/2016 | G | Whole Chicken | Merton, Kingston upon Thames and Sutton |
| 1614208 | 25/02/2016 | G | Chicken Breast | Merton, Kingston upon Thames and Sutton |
| 1614210 | 23/02/2016 | F | Chicken Breast | Haringey and Islington |
| 1614212 | 23/02/2016 | I | Chicken Breast | Haringey and Islington |
| 1614214 | 14/07/2016 | I | Other Cuts | West Sussex (North East) |
| 1614219 | 06/07/2016 | G | Chicken Breast | Warwickshire |
| 1614229 | 24/02/2016 | K | Whole Chicken | Norwich and East Norfolk |
| 1614245 | 20/01/2016 | K | Chicken Breast | West Surrey |
| 1614250 | 21/01/2016 | A | Whole Chicken | Croydon |
| 1614251 | 21/01/2016 | L | Whole Chicken | Bromley |
| 1614255 | 19/01/2016 | K | Chicken Breast | Suffolk |
| 1614257 | 19/01/2016 | K | Whole Chicken | Cambridgeshire CC |
| 1614261 | 19/01/2016 | B | Other Cuts | Suffolk |
| 1614262 | 19/01/2016 | A | Other Cuts | Cambridgeshire CC |
| 1614268 | 20/01/2016 | K | Other Cuts | Hertfordshire |
| 1614270 | 20/01/2016 | A | Whole Chicken | Hertfordshire |
| 1614271 | 20/01/2016 | I | Whole Chicken | Hertfordshire |
| 1614688 | 24/05/2016 | B | Other Cuts | Enfield |

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1614704 | 25/05/2016 | C | Chicken Breast | Birmingham |
| 1614705 | 25/05/2016 | G | Other Cuts | Staffordshire CC |
| 1614707 | 25/05/2016 | L | Other Cuts | Shropshire CC |
| 1614710 | 25/05/2016 | J | Chicken Breast | Birmingham |
| 1614712 | 25/05/2016 | K | Chicken Breast | Staffordshire CC |
| 1614714 | 25/05/2016 | K | Whole Chicken | Birmingham |
| 1614718 | 24/05/2016 | K | Whole Chicken | Sandwell |
| 1614719 | 24/05/2016 | B | Whole Chicken | Dudley |
| 1614723 | 24/05/2016 | F | Chicken Breast | Lincolnshire |
| 1614735 | 12/04/2016 | I | Whole Chicken | Calderdale and Kirklees |
| 1614739 | 12/04/2016 | K | Whole Chicken | Greater Manchester North East |
| 1614749 | 14/04/2016 | K | Whole Chicken | Tyneside |
| 1614753 | 13/04/2016 | K | Whole Chicken | East of Northern Ireland |
| 1614756 | 23/02/2016 | K | Whole Chicken | Leeds |
| 1614760 | 23/02/2016 | C | Chicken Breast | North Yorkshire CC |
| 1614762 | 23/02/2016 | K | Chicken Breast | Leeds |
| 1614763 | 23/02/2016 | I | Other Cuts | North Yorkshire CC |
| 1614764 | 23/02/2016 | A | Other Cuts | Leeds |
| 1614765 | 23/02/2016 | K | Other Cuts | North Yorkshire CC |
| 1614766 | 23/02/2016 | G | Chicken Breast | Bradford |
| 1614904 | 07/07/2016 | L | Other Cuts | West Sussex (North East) |
| 1614908 | 12/07/2016 | I | Other cuts | Greater Manchester North West |
| 1614911 | 06/07/2016 | I | Chicken Breast | Wandsworth |

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1614913 | 12/07/2016 | K | Whole Chicken | Greater Manchester South East |
| 1614914 | 07/07/2016 | L | Chicken Breast | Greater Manchester South West |
| 1614924 | 24/05/2016 | I | Whole Chicken | Worcestershire |

**Table 4.** Samples positive for AmpC phenotype *E. coli* from MCA-CTX (Grey cells indicates isolates have an AmpC/ESBL phenotype)

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1364251 | 11/10/2016 | G | Whole Chicken | Barnsley, Doncaster and Rotherham |
| 1364255 | 13/10/2016 | D | Whole Chicken | East Riding of Yorkshire |
| 1364258 | 13/10/2016 | K | Other Cuts | Wakefield |
| 1364265 | 11/10/2016 | G | Other Cuts | Coventry |
| 1364274 | 12/10/2016 | B | Other Cuts | South West Wales |
| 1364282 | 11/10/2016 | L | Other Cuts | Leicestershire CC and Rutland |
| 1364288 | 08/12/2016 | F | Whole Chicken | Wiltshire |
| 1364290 | 12/10/2016 | K | Whole Chicken | Bristol, City of |
| 1364300 | 25/08/2016 | E | Chicken Breast | South and West Derbyshire |
| 1364305 | 24/08/2016 | K | Chicken Breast | Nottingham |
| 1364308 | 24/08/2016 | G | Whole Chicken | Leicester |
| 1364309 | 23/08/2016 | A | Chicken Breast | Flintshire and Wrexham |
| 1364310 | 23/08/2016 | K | Whole Chicken | Flintshire and Wrexham |
| 1364313 | 23/08/2016 | E | Other Cuts | Cheshire East |
| 1364323 | 23/08/2016 | B | Chicken Breast | Aberdeen City and Aberdeenshire |
| 1364328 | 23/08/2016 | K | Other Cuts | Clackmannanshire and Fife |
| 1364345 | 25/08/2016 | D | Whole Chicken | West and South of Northern Ireland |
| 1562691 | 19/01/2016 | K | Other Cuts | Gwent Valleys |
| 1562692 | 19/01/2016 | K | Whole Chicken | Gwent Valleys |
| 1563611 | 12/10/2016 | L | Chicken Breast | Bath and North East Somerset, North Somerset and South Gloucestershire |
| 1563680 | 24/05/2016 | A | Whole Chicken | Barking & Dagenham and Havering |
| 1563700 | 12/10/2016 | C | Chicken Breast | Somerset |

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1612881 | 08/12/2016 | K | Chicken Breast | Durham CC |
| 1612913 | 07/12/2016 | C | Other Cuts | Wiltshire |
| 1614174 | 15/04/2016 | B | Whole Chicken | West Kent |
| 1614189 | 13/04/2016 | I | Whole Chicken | East of Northern Ireland |
| 1614192 | 12/04/2016 | K | Other Cuts | Bexley and Greenwich |
| 1614203 | 23/02/2016 | K | Other Cuts | Central Hampshire |
| 1614206 | 25/02/2016 | B | Chicken Breast | Merton, Kingston upon Thames and Sutton |
| 1614222 | 06/07/2016 | B | Chicken Breast | Warwickshire |
| 1614226 | 06/07/2016 | D | Chicken Breast | Devon CC |
| 1614231 | 24/02/2016 | K | Chicken Breast | Essex Haven Gateway |
| 1614238 | 24/02/2016 | G | Whole Chicken | Norwich and East Norfolk |
| 1614243 | 20/01/2016 | G | Chicken Breast | West Surrey |
| 1614244 | 20/01/2016 | F | Whole Chicken | West Surrey |
| 1614249 | 21/01/2016 | D | Whole Chicken | Croydon |
| 1614254 | 19/01/2016 | G | Whole Chicken | Cambridgeshire CC |
| 1614267 | 20/01/2016 | B | Chicken Breast | Hertfordshire |
| 1614269 | 20/01/2016 | K | Whole Chicken | Hertfordshire |
| 1614681 | 24/05/2016 | G | Whole Chicken | East Merseyside |
| 1614708 | 25/05/2016 | I | Other Cuts | Birmingham |
| 1614709 | 25/05/2016 | B | Other Cuts | Birmingham |
| 1614713 | 25/05/2016 | K | Other Cuts | Birmingham |
| 1614716 | 25/05/2016 | B | Whole Chicken | Staffordshire CC |
| 1614738 | 12/04/2016 | K | Other Cuts | Greater Manchester North East |

| Sample number | Date tested | Super-market code | Food Category | Sampling Location (NUTS3) |
|---|---|---|---|---|
| 1614745 | 14/04/2016 | K | Chicken Breast | Tyneside |
| 1614917 | 06/07/2016 | B | Other Cuts | Wandsworth |
| 1614919 | 07/07/2016 | I | Other Cuts | Greater Manchester South West |
| 1614920 | 05/07/2016 | G | Whole Chicken | Harrow and Hillingdon |
| 1614926 | 19/01/2016 | G | Whole Chicken | Hackney and Newham |
| 1614927 | 19/01/2016 | I | Chicken Breast | Hackney and Newham |

**Table 5.** Number of samples per supermarket tested and that gave rise to *E. coli* on MCA-CTX with resistance phenotypes

| Supermarket Code | Total number of samples tested | No. positive on McC-CTX agar (%)* | ESBL phenotype confirmed by MICs (%)* | AmpC phenotype confirmed by MICs (%)* | AmpC/ESBL phenotype confirmed by MICs (%)* |
|---|---|---|---|---|---|
| A | 24 | 12 (50.0) | 10 (41.7) | 2 (8.3) | 0 |
| B | 51 | 22 (43.1) | 13 (25.5) | 9 (17.7) | 0 |
| C | 18 | 6 (33.3) | 4 (22.2) | 2 (11.1) | 0 |
| D | 12 | 5 (41.7) | 1 (8.3) | 4 (33.3) | 0 |
| E | 12 | 5 (41.7) | 3 (25.0) | 2 (16.7) | 0 |
| F | 16 | 4 (25.0) | 2 (12.5) | 2 (12.5) | 0 |
| G | 34 | 19 (55.8) | 11 (32.4) ** | 9 (26.5) ** | 1 (2.9) |
| I | 49 | 16 (32.7) | 11 (22.4) | 4 (8.2) | 0 |
| J | 1 | 1 (100) | 1 (100) | 0 (0) | 0 |
| K | 82 | 42 (51.2) | 29 (35.4) ** | 15 (18.3) ** | 2 (2.4) |
| L | 14 | 9 (64.3) | 7 (50.0) | 2 (14.3) | 0 |

* - %’s are based on total numbers of samples tested per supermarket

** - These values include isolates with an AmpC/ESBL phenotype

**Table 6.** MIC results of 20 antibiotics against all isolates from MCA-CTX

| Sample no | AMP | AZM | FEP | CTX | FOX | CAZ | CHL | CIP | NAL | CST | ETP | IPM | MEM | GEN | TMC | TET | TGC | SUL | TMP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1364243 | R | S | R | R | S | R | S | R | S | S | S | S | S | S | S | R | S | R | R |
| 1364244 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364250 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364251 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1364252 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364254 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1364255 | R | S | S | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1364256 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364258 | R | S | S | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1364265 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1364266 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364273 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364274 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1364277 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | R |
| 1364278 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1364280 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364281 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1364282 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1364283 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364284 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364285 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364288 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1364290 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |

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| 1364293 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364300 | R | S | S | R | R | R | S | R | R | S | S | S | S | R | S | R | S | R | R |
| 1364303 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | S | S | R | R |
| 1364305 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | R | S | S | S |
| 1364308 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1364309 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | R | R |
| 1364310 | R | S | R | R | R | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364311 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364313 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364314 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364323 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1364325 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364327 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364328 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1364332 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364333 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1364335 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364336 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1364342 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1364345 | R | S | S | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1562691 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1562692 | R | S | R | R | R | R | S | R | S | S | S | S | S | R | S | R | S | R | S |
| 1562695 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1562696 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1562930 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1563611 | R | S | S | R | R | R | S | R | R | S | S | S | S | S | S | S | S | S | S |
| 1563680 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1563700 | R | S | S | R | R | R | S | R | R | S | S | S | S | S | S | S | S | S | S |

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| 1563707 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1563719 | R | S | R | R | S | R | S | R | R | S | S | S | S | R | S | S | S | S | S |
| 1612881 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1612887 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1612891 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1612903 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1612904 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1612913 | R | S | S | R | R | R | S | R | R | S | S | S | S | S | S | S | S | S | S |
| 1614173 | R | S | R | R | S | R | R | R | R | S | S | S | S | S | S | R | S | R | R |
| 1614174 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1614175 | R | S | R | R | S | R | R | R | R | S | S | S | S | S | S | R | S | R | R |
| 1614177 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614185 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614188 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614189 | R | S | S | R | R | R | S | R | R | S | S | S | S | S | S | S | S | S | S |
| 1614191 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614192 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614193 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | S | S | R | R |
| 1614194 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614198 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614199 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614201 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | S | S | R | R |
| 1614203 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614206 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1614207 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1614208 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614210 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614212 | R | S | R | R | S | R | R | R | R | S | S | S | S | S | S | R | S | R | R |

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| 1614214 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | R |
| 1614219 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614222 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614226 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614229 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614231 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1614238 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614243 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614244 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | R | R |
| 1614245 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614249 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | R | S | S | R |
| 1614250 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614251 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1614254 | R | S | R | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614255 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614257 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614261 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1614262 | R | S | R | R | S | R | S | R | S | S | S | S | S | S | S | R | S | R | R |
| 1614267 | R | S | S | R | R | R | S | R | R | S | S | S | S | S | S | S | S | S | S |
| 1614268 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1614269 | R | S | S | R | R | R | S | R | S | S | S | S | S | S | S | R | S | S | S |
| 1614270 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614271 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614681 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614688 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1614704 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1614705 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614707 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | S | S | R | R |

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| 1614708 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614709 | R | S | S | R | R | R | S | R | R | S | S | S | S | S | S | S | S | S | S |
| 1614710 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614712 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | R |
| 1614713 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614714 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | R |
| 1614716 | R | S | S | R | R | R | S | R | R | S | S | S | S | S | S | S | S | R | R |
| 1614718 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614719 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614723 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614735 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614738 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614739 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614745 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614749 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614753 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614756 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | S | S | R | R |
| 1614760 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1614762 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614763 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614764 | R | S | R | S | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1614765 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614766 | R | S | R | R | S | R | S | R | R | S | S | S | S | S | S | R | S | R | S |
| 1614904 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614908 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614911 | R | S | R | R | S | R | S | S | S | S | S | S | S | R | S | R | S | R | R |
| 1614913 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | R |
| 1614914 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |

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| 1614917 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614919 | R | S | S | R | R | R | S | S | S | S | S | S | S | S | S | S | S | S | S |
| 1614920 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1614924 | R | S | R | R | S | R | S | S | S | S | S | S | S | S | S | R | S | R | S |
| 1614926 | R | S | R | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |
| 1614927 | R | S | S | R | R | R | S | S | S | S | S | S | S | R | S | R | S | R | S |

AMP – ampicillin (R > 8 mg/L); AZM – azithromycin (R > 16 mg/L); FEP – cefepime (R > 0.125 mg/L); CTX – cefotaxime (R > 0.25 mg/L); FOX – cefoxitin (R > 8); CAZ – ceftazidime (R > 8 mg/L); CHL – chloramphenicol (R > 16 mg/L; CIP – ciprofloxacin (R > 0.064 mg/L); NAL - nalidixic acid (R > 16 mg/L); CST – colistin (R > 2 mg/L); ETP – Ertapenem (R > 0.064 mg/L); IPM – Imipenem (R > 0.5 mg/L); MEM – Meropenem (R > 0.125 mg/L); GEN – gentamicin (R > 2 mg/L); TMC - temocillin (R > 32mg/L); TET – tetracycline (R > 8); TGC - tigecycline (R > 0.5); SUL – sulfamethoxazole (R > 64 mg/L); TMP - trimethoprim (R > 2 mg/L).

Interpretative criteria according to tables 1and 4 in Commission Implementing Decision 2013/652/EU.

**Table 7.** Summary of resistance phenotypes for all isolates from MCA-CTX

| Antibiotic | No. resistant[a] / No. tested | |
|---|---|---|
| | ESBL* | AmpC** |
| Ampicillin | 93/93 | 48/48 |
| Azithromycin | 0/93 | 0/48 |
| Cefepime | 93/93 | 15/48 |
| Cefotaxime | 92/93 | 48/48 |
| Cefoxitin | 3/93* | 48/48 |
| Ceftazidime | 93/93 | 48/48 |
| Chloramphenicol | 3/93 | 0/48 |
| Ciprofloxacin | 25/93 | 9/48 |
| Colistin | 0/93 | 0/48 |
| Ertapenem | 0/93 | 0/48 |
| Gentamicin | 10/93 | 13/48 |
| Imipenem | 0/93 | 0/48 |
| Meropenem | 0/93 | 0/48 |
| Naladixic Acid | 22/93 | 8/48 |
| Sulfamethoxazole | 91/93 | 19/48 |
| Temocillin | 0/93 | 0/48 |
| Tetracycline | 85/93 | 19/48 |
| Tigecycline | 0/93 | 0/48 |
| Trimethoprim | 47/93 | 7/48 |

Orange highlight denotes the four different cephalosporin antibiotics which were tested.
Grey highlight denotes the three carbapenem antibiotics ertapenem, imipenem and meropenem and colistin (all last resort antibiotics).
Green highlight denotes a lower proportion of ESBL versus AmpC or AmpC versus ESBL isolates resistant for stated antibiotic.

*a* Microbiologically resistant using EUCAST ECOFFS.
* Includes the 3 isolates with an AmpC/ESBL phenotype which show resistance to cefoxitin
** Does not include the 3 isolates with an AmpC/ESBL phenotype

**Table 8.** Viable count of presumptive *E. coli* if above detection limit

| Sample number | Supermarket code | Product text description | Dispatch date | Viable counts [a] (cfu/g) on agars | |
|---|---|---|---|---|---|
| | | | | MCA-CTX | CA-ESBL |
| 1364265 | G | British Chicken Wings | 10-Oct-2016 | 40 | 160 |
| 1364273 | B | Whole Chicken | 11-Oct-2016 | 0 | 120 |
| 1364303 | B | Free Range corn-fed British chicken thighs | 24-Aug-2016 | 0 | 40 |
| 1364325 | K | Scottish Medium Whole Chicken | 22-Aug-2016 | 0 | 120 |
| 1364332 | A | Scottish Small Fresh Whole Chicken | 23-Aug-2016 | 0 | 40 |
| 1612891 | E | British Small Chicken | 7-Dec-2016 | 0 | 280 |
| 1614244 | F | British Chicken medium | 19-Jan-2016 | 40 | 0 |
| 1614257 | K | British Whole Chicken | 18-Jan-2016 | 400 | 0 |
| 1614723 | F | British Chicken 2 Breast Fillets skin-on | 23-May-2016 | 120 | 160 |
| 1614739 | K | British small whole chicken | 11-Apr-2016 | 0 | 80 |
| 1614748 | B | British Chicken | 13-Apr-2016 | 0 | 40 |
| 1614765 | K | British chicken thighs | 22-Feb-2016 | 0 | 40 |

*a* – Limit of detection = 40 cfu/gram of meat

**Table 9**. PCR results (*bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes) and CTX-M gene sequence for *E. coli* from CA-ESBL

| Sample number | Supermarket code | Product text description | PCR results for *bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes | CTX-M gene sequence |
|---|---|---|---|---|
| 1364243 | G | British chicken thighs | CTX,TEM | CTXM-15 / 15 WGS |
| 1364250 | B | British chicken medium | CTX | CTXM-1 |
| 1364251 | G | British Chicken Medium | CTX | CTXM-1 |
| 1364252 | I | British Whole Chicken | CTX | CTXM-1 |
| 1364254 | A | 100% British Small whole chicken | CTX,TEM | CTXM-1 |
| 1364255 | D | Class A Fresh British Whole Medium Chicken | CTX | CTXM-1 |
| 1364258 | K | British Chicken Thighs | CTX | CTXM-1 |
| 1364265 | G | British Chicken Wings | CTX,TEM | CTXM-1 |
| 1364266 | D | Class A fresh british whole small chicken | CTX | CTXM-1 |
| 1364273 | B | Whole Chicken | CTX | CTXM-1 |
| 1364275 | B | British Chicken Boneless Thigh Fillets | CTX | ND |
| 1364277 | G | British Chicken Thighs | CTX,TEM | CTXM-1 |
| 1364278 | K | British Chicken Mini Filletts | CTX | CTXM-1 |
| 1364281 | L | British Chicken Breast Fillet | CTX | CTXM-1 |
| 1364283 | A | Chicken thigh fillets | CTX | CTXM-1 |
| 1364285 | A | 100% British Chicken Thigh Fillets | CTX | ND |
| 1364290 | K | Whole chicken | CTX | ND |
| 1364293 | I | British Corn Fed Whole Chicken | CTX,TEM | CTXM-1 |
| 1364303 | B | Free Range Corn-fed British chicken thighs | CTX | CTXM-1 |
| 1364310 | K | British Small Whole Chicken without giblets | CTX | CTXM-1 |
| 1364311 | L | British Chicken without giblets | CTX | CTXM-1 |
| 1364314 | K | British Chicken Mini Fillets | CTX | CTXM-1 |
| 1364323 | B | British Chicken Breast Mini Filets | CTX | CTXM-1 |
| 1364325 | K | Scottish Medium Whole Chicken | CTX | CTXM-1 |
| 1364328 | K | Chicken Drumsticks | CTX | CTXM-1 |
| 1364332 | A | Scottish Small Fresh Whole Chicken | CTX,TEM | CTXM-1 |
| 1364333 | B | British Chicken Breast Fillets | CTX,TEM | CTXM-1 |
| 1364335 | B | Scottish Chicken Medium | CTX | CTXM-1 |
| 1364342 | I | Northern Irish Chicken Whole Breast Fillets | CTX | CTXM-1 |
| 1562692 | K | British Chicken Fresh Class A | CTX | ND |
| 1562695 | K | Chicken Breast Mini-Fillets | CTX | CTXM-1 |
| 1562696 | K | British Chicken Breast Mini-Fillets | CTX | CTXM-1 |
| 1562930 | B | British chicken breast mini fillets | CTX | CTXM-1 |
| 1563611 | L | British 2 Part Boned Breasts | CTX,TEM | CTXM-1 |
| 1563700 | C | British Chicken Breast Fillets | CTX | ND |
| 1563707 | K | Chicken Breast Portions | CTX | CTXM-1 |
| 1563719 | I | British chicken whole breast fillets | TEM | ND |
| 1612878 | K | British Small Whole Chicken | SHV | ND |

| Sample number | Supermarket code | Product text description | PCR results for *bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes | CTX-M gene sequence |
|---|---|---|---|---|
| 1612885 | G | British Chicken | CTX | ND |
| 1612887 | K | Briitish Chicken Thigh Fillets | CTX | ND |
| 1612891 | E | British Small Chicken | CTX | ND |
| 1612903 | C | British Chicken Breast Fillets | CTX | ND |
| 1612904 | C | British Whole Chicken | CTX | ND |
| 1612913 | C | British Chicken Thigh Fillets | CTX | ND |
| 1614173 | K | Free Range Whole Chicken | CTX,TEM | CTXM-1 |
| 1614175 | B | British Chicken Breast Mini Fillets | TEM,SHV | ND |
| 1614177 | K | Chicken Breast Portions | CTX | CTXM-10 |
| 1614178 | K | Whole Chicken | CTX | CTXM-1 |
| 1614180 | I | British Chicken Thighs | CTX | CTXM-1 |
| 1614185 | K | British Medium Whole Chicken | CTX | CTXM-1 |
| 1614188 | A | Chicken Breast Fillets | CTX | CTXM-1 |
| 1614191 | A | Small Whole Chicken. | CTX,TEM | CTXM-1 |
| 1614192 | K | British Chicken Thighs | TEM,SHV | ND |
| 1614193 | K | British Medium Whole Chicken. | CTX | CTXM-1 |
| 1614194 | G | British Chicken Breast Fillets | CTX | CTXM-1 |
| 1614198 | B | Free Range Corn-fed British Whole Chicken | CTX | CTXM-1 |
| 1614201 | E | British Chicken Free Range Breast Fillets | CTX | CTXM-1 |
| 1614208 | G | British Chicken breast fillets | CTX | CTXM-1 |
| 1614210 | F | Corn Fed Chicken Breast Fillets | CTX | CTXM-1 |
| 1614212 | I | British Chicken Whole Breast Fillets | CTX,TEM | CTXM-1 |
| 1614219 | G | British Chicken Breast Fillets | CTX | CTXM-1 |
| 1614229 | K | British Whole Chicken | CTX | CTXM-1 |
| 1614233 | B | British Chicken Fresh Class A | SHV,TEM | ND |
| 1614243 | G | British Diced Breast | Neg | ND |
| 1614244 | F | British Chicken medium | CTX,TEM | CTXM-15 / 55 WGS |
| 1614245 | K | British Chicken Breast Fillets skinless | CTX | CTXM-1 |
| 1614249 | D | Fresh Whole Chicken without giblets | SHV | ND |
| 1614250 | A | Small Whole Chicken | CTX,TEM | CTXM-1 |
| 1614255 | K | Chicken Breast Fillets skinless | CTX,TEM | CTXM-1 |
| 1614257 | K | British Whole Chicken | CTX,TEM | CTXM-1 |
| 1614261 | B | British Chicken Boneless Thigh Fillet | CTX | CTXM-1 |
| 1614262 | A | Chicken Thighs | CTX | CTXM-1 |
| 1614268 | K | Free Range Chicken Thigh Fillets | CTX | CTXM-28 |
| 1614270 | A | Small Whole Chicken | CTX,TEM | CTXM-15 /1 WGS |
| 1614683 | I | British Chicken whole breast fillets | CTX | CTXM-1 |
| 1614685 | G | British chicken diced breast fillets | CTX | CTXM-1 |
| 1614688 | B | British Chicken Wings | CTX | CTXM-1 |
| 1614718 | K | British small whole chicken | CTX | CTXM-1 |

| Sample number | Supermarket code | Product text description | PCR results for *bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes | CTX-M gene sequence |
|---|---|---|---|---|
| 1614719 | B | British chicken | CTX | CTXM-1 |
| 1614723 | F | British Chicken 2 Breast Fillets skin-on | CTX | CTXM-1 |
| 1614734 | E | British Chicken Mini Fillets | CTX,SHV | CTXM-15 / 1 WGS |
| 1614735 | I | British Small whole chicken | CTX | CTXM-1 |
| 1614739 | K | British small whole chicken | CTX | CTXM-32 |
| 1614741 | G | Chicken Wings | CTX,TEM | CTXM-1 |
| 1614749 | K | British Small Whole Chicken | CTX | CTXM-1 |
| 1614751 | D | British whole small chicken | CTX | CTXM-1 |
| 1614753 | K | Northern Irish medium whole chicken | CTX | CTXM-1 |
| 1614756 | K | British whole chicken | CTX | CTXM-1 |
| 1614762 | K | British 2 chicken breast fillets | CTX | CTXM-1 |
| 1614764 | A | Chicken thigh fillets | CTX | CTXM-1 |
| 1614765 | K | British chicken thighs | CTX | CTXM-1 |
| 1614766 | G | British Chicken diced breast fillet | CTX | CTXM-1 |
| 1614911 | I | British Chicken Mini Fillets | CTX | CTXM-1 |
| 1614914 | L | British chicken breast fillets | CTX | CTXM-1 |
| 1614924 | I | British small whole chicken | CTX | CTXM-1 |

NA – Not applicable; ND – Not determined; WGS – result by whole genome sequencing as part of previously discussed ResAlert.

**Table 10.** Results of whole genome sequencing using DTU (and APHA) pipelines for *E. coli* that were CTX-M 15 by sequencing of PCR amplicons

| Sample Name | Species | MLST and O group | Plasmids | pMLSTs | Resistance Genes | APHA Resistance genes (differences DTU) * | Virulence Genes | Resistant to ** |
|---|---|---|---|---|---|---|---|---|
| Bead LREC 3258<br>Meat sample 01364243 October | Escherichia coli | ST-38<br><br>O7:H18<br>(99.74%:96.65%) | Col(BS512)<br>IncY | NA | tet(A)<br>sul2<br>dfrA14<br>blaCTX-M-15<br>blaTEM-1B<br>strA<br>strB | + qnrS1 | eilA<br>iss<br>gad | |
| Bead LREC 2665<br>Meat sample 01614734 April | Escherichia coli | ST-10<br><br>O13:H48<br>(99.83%:100%) | IncX3<br>ColRNAI<br>p0111<br>Col(MG828) | IncI1 [ST-3] | aadA1<br>sul2<br>QnrS1<br>dfrA1<br>tet(A)<br>blaSHV-12<br>blaCTX-M-1 | + ant3-1a<br>+ sat2A | iss<br>astA | |
| Bead LREC 2354<br>Meat sample 01614244 January | Escherichia coli | ST-3776<br><br>O166:H45<br>(99.53%:99.82%) | IncX1<br>ColRNAI | IncF [Unknown ST] | aac(3)-IId<br>aadA17<br>sul3<br>QnrS1<br>dfrA14<br>tet(A)<br>blaCTX-M-55<br>blaTEM-1B<br>lnu(F) | - sul3 | eilA<br>iss | AMP, CTX, CAZ, SUL, TMP, FOX. |
| Bead LREC 2346<br>Meat sample 01614270 January | Escherichia coli | Unknown ST<br><br>O?:H31<br>(ND:98.4%) | p0111<br>Col(MG828)<br>ColRNAI | IncI1[ST-3]<br>IncHI1 [Unknown ST]<br>IncF [F18:A6*:B1] | aadA5<br>sul2<br>dfrA17<br>blaTEM-1B<br>blaCTX-M-1<br>tet(A) | | air<br>lpfA<br>iss<br>eilA<br>tsh<br>iroN | AMP, CTX, CAZ, SUL, TET, TMP, FEP. |

* + and gene – gene detected by APHA but not by DTU pipeline;- and gene – gene detected by DTU but not APHA pipeline. Consensus otherwise. ** Resistant using EFSA cut-offs. Serotyping (% match to gene)

A Bacterial Analysis Platform: An Integrated System for Analysing Bacterial Whole Genome Sequencing Data for Clinical Diagnostics and Surveillance. Thomsen MCF, Ahrenfeldt J, Cisneros JLB, Jurtz V, Larsen MV, Hasman H, Aarestrup FM and Lund O. PLoS ONE, 2016, 11(6): e0157718. doi:10.1371/journal.pone.0157718

## 8. Discussion

Many different studies have shown that ESBL-producing *E. coli* can be detected on raw poultry meat in countries, such as the Netherlands, where one study showed that 94% of chicken meat samples were positive for ESBL-producing *E. coli*,[5] in Germany in 2012, where 60% of 120 chicken meat samples purchased were positive for mainly CTX-M-1 ESBL-producing Enterobacteriaceae,[6] in Portugal,[7] and in the UK[7, 8].

In theA UK study carried out by Dhanji et al,[7] found that 29.5% of 210 chicken meat samples imported in 2008 were positive for oxyimino-cephalosporin-resistant *E. coli*. Of the 141 isolates tested, 30% and 27% were positive for CTX-M groups 2 and 8 ESBL-producing genes respectively, whilst 42% were positive for AmpC CMY-type enzymes, and 1% produced a group 2 CTX-M along with a CMY enzyme.[7] In a more recent study in which retail chicken meat samples were collected in 2013-2014 from 5 different regions in the UK, 65.4% of 159 samples were positive for mainly *bla*$_{\text{CTX-M-1}}$ ESBL-producing *E. coli*, whilst *bla*$_{\text{CTX-M-15}}$ ESBL-producing *E. coli* was not detected.

The 2014 EFSA summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food , did not report on any carbapenem resistant *E. coli* detected in chickens or turkeys in Europe.[9] However, one study has shown that 65.09% and 11.32% of retail chicken in Egypt was positive for ESBL-producing or *bla*$_{\text{NDM}}$ carbapenem resistant Enterobacteriaceae respectively.[10]

One of the problems of comparing results from different studies is that each study may use a slightly different enrichment technique and / or final isolation agar, and as such results are not truly comparable. For current and on-going EU studies however, all participants are using identical methodologies and sample sizes, so results will be comparable across member states.

For the UK study conducted in 2013-2014, enrichment of meat samples in BPW, followed by plating to CHROMagar ESBL and CHROMagar CTX was employed.[3] For the EU survey reported here, samples were also enriched in BPW, and then plated to MCA-CTX and CA-ESBL.[3] As such the isolation methods between the two surveys was similar. The sample collection for the 2013-2014 survey, although not identical to that performed in the current study, did involve purchase of meat samples from local retailers in each of five UK regions (London, East Anglia, the North West, Scotland and Wales) in numbers that were representative of UK market share, and samples were purchased on five different occasions over a ~ 7 month period.[3] Comparing the findings of the 2013-2014 study[3] and this study, it

was encouraging that a drop from 65.4% to 29.7% of retail chicken samples being positive for ESBL-producing *E. coli* in the UK was observed. This difference was statistically significant using a chi-squared test (odds ratio 0.45 p-value<0.001), however, it should be noted that the slightly different sampling criteria and sample processing methods used in the current study could have influenced results.

In Denmark, cephalosporin antibiotics have not been used in poultry for more than 10 years, and it has been considered that the high prevalence of AmpC/ESBL-producing bacterial detected in Danish broiler meat might be caused by practices upstream in the production pyramid, since the breeding company supplying birds until recently used cephalosporin antibiotics as a prophylactic measure.[11] In 2012, the use of third generation cephalosporins in chicks destined for broiler parent flocks in UK was stopped voluntarily, as was any occasional use for commercial generations of laying hens and broilers.[12] It is likely that this would take a while to have an effect on cephalosporins resistance in *E. coli* from chicken meat, for example until the progeny of these parents were placed. Some persistent resitance on farm should also have reduced further over time.

In Quebec Canada, higher rates of ceftiofur resistance in *Salmonella* Heidelberg isolates from chicken meat than from humans, prompted broiler chicken hatcheries to voluntarily interrupt the extra-label in-ovo use of ceftiofur during 2005-2006.[13] This ban was associated with a decrease in the prevalence of ceftiofur resistance from 2004 to 2006 among retail chicken (62% to 7%; p<0.001) and human (36% to 8%; p<0.0001) *Salmonella* Heidelberg isolates and retail chicken *E. coli* isolates (34% to 6%; p<0.0001), which was reversed when ceftiofur use was reintroduced.[13] The study concluded that changes in ceftiofur resistance *E. coli* and *Salmonella* Heidelberg in retail chicken meat appeared to be related to changing levels of ceftiofur use in hatcheries.[13] Whilst it is not possible to categorically state that the drop from 65.4% to ~ 30% of retail chicken samples being positive for ESBL-producing *E. coli* in the UK between the years 2013-2014 and 2016 is directly related to the voluntary cessation of third generation cephalosporins in 2012 in chicks destined for broiler parent flocks in UK, it would seem probable, based on similar findings seen in the Canadian study.[13]

In this study and the UK study of 2013-2014, the predominant CTX-M sequence type was CTX-M1.[3] This therefore differs from the CTX-M group 2 and 8 *E. coli* isolates found on imported chicken in an earlier study.[7]

The predominant *E. coli* strain associated with human infections is the pandemic O25-ST131 CTX-M-15-producing clone.[14, 15] Whilst four isolates of *E. coli* that were $bla_{\text{CTX-M15}}$ were

detected by PCR, whole genome sequencing of these isolates showed that none were the pandemic O25-ST131 CTX-M-15-producing clone and only one of the four isolates was confirmed as CTX-M-15 by WGS. As such, the evidence would suggest that at present in the UK, ESBL-producing *E. coli* from retail chicken differs from the predominant strain in humans and retail chicken is not a source of the human pandemic O25-ST131 CTX-M-15-producing clone. This was also the overarching finding of recent work performed by PHE,[16] in that ESBL-producing *E. coli* from humans (blood, faeces, and including sewage) were largely distinct from those present in raw meat, live animals and farm slurry, with ST131 hugely dominant as a cause of human disease. Even after excluding ST131, the next *E. coli* type in rank, overall and in each of the human sources, was ST38 (9.4% in sewage isolates, 8.0% in human faeces and 5.8% in bacteraemia isolates, ~ 40% of all ST38 isolates were CTX-M 15), and no ST38 isolates were found in the meat, slurry or scanning animal surveillance isolates, again suggesting that it is a 'human-adapted' strain.[16] Although one isolates in this study was CTX-M 15 ST38, WGS results showed that the isolate was different from human ST38 isolates recovered as part of the recent PHE project.[16]

With respect to the degree of contamination of chicken samples with presumptive AmpC/ESBL *E. coli*, whilst overall 45.1% of samples were positive on MCA-CTX agar for AmpC/ESBL-producing *E. coli*, only 3.8% of samples had counts above the detection limit on CA-ESBL or MCA-CTX, suggesting that for most positive samples, the levels of AmpC or ESBL producing *E. coli* on the samples was very low.

None of the 141 isolates from MCA-CTX were microbiologically resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin. This correlates with the findings of the 2014 EFSA report for *E. coli* from chickens and turkeys, in that none of the isolates were resistant to carbapenem antibiotics, and only low numbers of isolates were resistant to colistin.[9] Whilst nine of the samples gave rise to lactose fermenting colonies on MCA-COL, these were negative for plasmid mediated colistin resistance genes *mcr-1* and *mcr-2*. Additionally, none of the isolates were resistant to the antibiotics azithromycin, temocillin and tigecycline. Most or several of the isolates were resistant to the antibiotics sulfamethoxazole (78.0%), tetracycline (74.5%) and trimethoprim (38.3%), and about 25% of the isolates were resistant to the quinolone antibiotics nalidixic acid and ciprofloxacin.

# 9. Conclusions

- Of the 313 UK retail chicken samples tested, 93 (29.7%) and 51 (16.3%) (including AmpC/ESBL phenotype isolates) were positive for ESBL or AmpC phenotype *E. coli* respectively, based on results from MCA-CTX agar and MICs to determine AmpC or ESBL phenotype.

- Using CA-ESBL, 95 (30.4%) of samples were positive for presumptive ESBL phenotype *E. coli*, of which 93 (29.7%) were confirmed by PCR to be $bla_{\text{CTX-M}}$ (mainly CTX-M 1) or $bla_{\text{SHV}}$ positive. This therefore shows a good correlation of results between MCA-CTX coupled with phenotypic testing for ESBLs, and use of an ESBL selective agar (CA-ESBL), coupled with PCR.

- The predominant CTX M types recovered from retail chicken (mainly CTX-M-1) differ to those causing human disease.

- None of the samples were positive for carbapenem resistant *E. coli.*

- None of the samples were positive for *E. coli* with plasmid mediated colistin resistance genes *mcr-1* and *mcr-2*.

- Only 12 meat samples (3.8%) had viable counts (without enrichment) of presumptive AmpC or ESBL-producing *E. coli* above the detection limit (40 cfu/gram), and these counts were all less than or equal to 400 cfu/gram.

- Results showed a decrease in the proportion of samples positive for ESBL-producing *E. coli* compared to a previous (2013-2014) UK study that reported that 65.4% of 159 retail chicken samples were positive for ESBL-producing *E. coli.* This difference was statistically significant using a chi-squared test (odds ratio 0.45 p-value<0.001), however, it should be noted that the slightly different sampling criteria and sample processing methods used in the current study could have influenced results.

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