RDFS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in E. coli from Retail Meats in UK (2018 - Year 4, chicken)

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1. Liability statement

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2. Lay person's summary

This report presents results of the EU harmonised surveillance of antimicrobial resistance (AMR) in *E. coli* from retail chicken meats in the UK in 2018.

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 meats should be tested by culture for the bacterium *Escherichia coli. E. coli* bacteria are a normal part of the gut flora of mammals and as such can be useful "indicators" of AMR in gut bacteria. Whilst some strains of *E. coli* can cause disease, most strains of *E. coli* do not cause observable disease in healthy animals and humans.

The EU requirements state that samples should be tested on an agar (growth medium) supplemented with a third generation cephalosporin, a group of antimicrobials which are important for treating infections in humans. *E. coli* growth on this agar suggests antimicrobial resistance known as Extended Spectrum β-lactamase (ESBL) resistance and/or AmpC resistance. ESBL resistance is also referred to as ESBL-phenotype, AmpC resistance is also referred to as AmpC-phenotype. The degrees of susceptibility/resistance of *E. coli* recovered from this agar must then be determined to a pre-defined panel of antimicrobials by Minimum Inhibitory Concentrations (MICs) tests.

EU requirements also state that samples should be tested on two other supplemented agars which select bacteria which are resistant to carbapenems, another a group of antimicrobials which are very important in human medicine. Carbapenems are termed "last resort" antimicrobials, because they are used to treat severe infections when all or almost all other treatment options have failed, because the infecting bacteria are resistant to most / all other relevant antimicrobials.

At the request of the FSA, other agars used to test samples included an agar to specifically isolate *E. coli* with ESBL-only type resistance only (rather than for *E. coli* with both an AmpC and an ESBL type resistance as per one of the EU specified agars), and an agar to isolate colistin resistant *E. coli.* Colistin is another “last resort” antimicrobial, so it is important to monitor if resistance in *E. coli* to colistin is occurring in food samples.

Other additional work requested by the FSA included identification of the specific antimicrobial resistance genes associated with ESBL-phenotype or colistin resistance in *E. coli* isolates. Colistin resistance in *E. coli* isolates may involves a number of resistance genes such as *mcr-1, mcr-2* and *mcr-3*. These *mcr* genes are considered particularly important as they are usually carried on genetic elements know as plasmids. As plasmids are “mobile” (can pass from one bacteria to some others), the resistance genes located on them can potentially be shared with other bacteria within the gut. Finally, extra work requested by the FSA included counts of the number of antimicrobial resistant (AmpC and ESBL type resistance only) *E. coli* in each meat sample.

In total, 309 samples of fresh chicken were collected and tested. Samples were collected each month of 2018 from 10 supermarket chains, in proportion to the human population in each participating country, i.e. England (n=269 samples), Scotland (n=19 samples), Wales (n=11 samples), and Northern Ireland (n=10 samples).

The samples included whole chicken (n=125), chicken breast (n=112) and other cuts, i.e. quarters, legs, thighs & drumsticks (n=72). Of the samples collected, 66.3% and 33.7% had skin on or off respectively. Breast samples, rather than thighs or whole chicken, were the main chicken meat sample type from which skin was removed. Two samples were labelled as originating from Poland, but all other samples were stated to be of UK origin.

None of the samples gave rise to bacterial growth (meaning the test results were negative and the bacteria were therefore not resistant to carbapenem antimicrobials) on the two agars that selected for carbapenem resistant *E. coli.* None of the isolates which were resistant to the last resort antimicrobial colistin were positive for the colistin resistance genes *mcr-1, 2* or *3.*

Forty-two (13.6%) of samples gave rise to *E. coli* on MacConkey agar + 1 mg/L cefotaxime, a third generation cephalosporin. These positive results imply the *E. coli* were resistant to cefotaxime. Of these, by MIC determinations, 26 (8.4%) were found to express ESBL-phenotype resistance (including three isolates that also expressed AmpC-phenotype resistance) and 16 (5.2%) were found to express AmpC-phenotype resistance (excluding three isolates that also expressed ESBL-phenotype resistance as these are accounted for above).

It was interesting to note that the proportions of chicken samples with *E. coli* with AmpC+ESBL and ESBL-phenotype were higher for skin off rather than skin on samples. This difference was statistically significant for samples tested on the ESBL specific agar. It is possible that the mechanical process of removing skin from chicken samples causes cross contamination of samples, but this was not tested for.

Using MIC tests, the isolates from the AmpC/ESBL specific agar were tested for the degree of resistance to a total of 19 antimicrobials. Based on the MIC results, isolates were determined as resistant or sensitive to a particular antimicrobial using cut-offs known as ECOFFs (Epidemiological Cut Offs published by EUCAST). The ECOFF distinguishes between organisms without and with phenotypically expressed resistance mechanisms for a bacterial species to an antimicrobial.

Isolates with an AmpC, ESBL or ESBL+AmpC-phenotype were resistant (using ECOFFs) to an average of 6.3, 7.0 or 8.8 of these 19 antimicrobials respectively, and as such the isolates with a combined ESBL+AmpC-phenotype were on average resistant to more antimicrobials. None of the isolates were resistant to the antimicrobials azithromycin, meropenem, temocillin or tigecycline, as was previously observed in the 2016 survey of AMR *E.coli* in retail chicken meat. One AmpC isolate from the AmpC/ESBL specific agar showed resistance to ertapenem and imipenem using ECOFFs, but the isolate was borderline clinically resistant, i.e. just over the clinical breakpoint threshold. This isolate lacked any detectable ertapenem or imipenem resistance genes by whole genome sequencing (WGS) and on retest was microbiologically sensitive to both ertapenem and imipenem.

Genetic tests (PCR) showed that most of the isolates from the ESBL agar carried the *bla*$_{\text{CTX-M-}}$s gene which confers resistance to third generation cephalosporin

antimicrobials, and has been frequently detected in *E. coli* from chickens and chicken meat in previous studies, including the 2016 survey.

Only two meat samples had bacterial counts of presumptive AmpC/ESBL-producing *E. coli* above the detection limit of 20 *E. coli* colony forming units (cfu) per gram of meat, and these counts were both 20 cfu/gram.

The 2016 survey of AMR in retail chicken meat reported a decrease in the proportion of retail chicken samples positive for ESBL-producing *E.* coli (to 29.7%) compared to a proportion reported in a previous (2013/14) UK study, which reported 65.4% of 159 retail chicken samples as positive for ESBL-producing *E. coli.*[1] The drop from 65.4% in the 2013/14 study to 29.7% in the 2016 study was statistically significant, albeit sampling and isolation methods were similar but not identical for the two studies.

The UK survey of retail chicken in 2018 demonstrated a further significant reduction in the numbers of samples positive for ESBL-producing *E. coli* to 8.4% using identical methods to the survey in 2016. This reduction in the level of antimicrobial resistant *E. coli* on chicken meat since 2013/14 may be linked to the banning by the British Poultry Council of the use of third and fourth generation cephalosporins in flocks used for poultry meat production in the UK in 2012 as part of antimicrobial stewardship. All meat samples since 2013, excluding two samples from 2018, were stated to be of UK origin, although for the earlier published study for 2013/14 samples, the origin of 7.5% of the chicken meat samples was not stated.

3. Project 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 UK retail chicken meat in 2018 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 meat samples should be tested by culture for E. coli on MacConkey agar containing 1 mg/L of the cephalosporin antimicrobial cefotaxime. E. coli isolates cultured from such media are expected to show third generation cephalosporin resistance which may include ESBL and / or AmpC type resistance, and should be further tested by performing Minimum Inhibitory Concentrations (MICs) to determine their susceptibility to a panel of antimicrobials. Samples were also tested for carbapenem-resistant *E. coli* on chromID® carba and chromID® OXA-48 agars as recommended by the EU. Furthermore, at the request of the FSA (non-harmomised testing) 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*. Other additional work was requested by the FSA (non-harmomised testing) 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[3] 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 *mcr-1, mcr-2* and *mcr-3.*

Finally, viable counts of all samples for *E. coli* were determined on MacConkey agar + 1 mg/L cefotaxime and on CHROMagar™ ESBL.

For this study, as in previous years, 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.

A total of 315 chicken samples were collected across four countries (England, Scotland, Wales and Northern Ireland) in proportion to their human population size. To account for potential missing data, HallMark added an extra 5% of samples into the sampling plan. The 2018 poultry sampling plan used “proportionate stratified sampling” to allocate samples to NUTS-3 areas and the samples were distributed in proportion to population size.

In agreement with the FSA, the types of chicken to be sampled included both whole chicken carcasses and chicken joints/portions such as quarters, legs, thighs, drumsticks, breasts. 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.

Of the 315 samples planned, ten were rejected because their temperatures, when tested on arrival at the laboratory, were above that stated in the EU guidelines, but six of these were re-sampled giving 311 samples. A further two were not collected due to unavailability.

A total of 309 retail chicken meat samples were collected and tested between January and December 2018 from England (n=269), Scotland (n=19), Wales (n=11), and Northern Ireland (n=10) from ten different supermarket chains. The types of chicken meat collected were whole chicken (n=125), chicken breast (n=112) and other cuts, including quarters, legs, thighs & drumsticks (n=72). Of the samples collected, 66.3% and 33.7% had skin on or off respectively, and breast samples were the main sample type from which skin was removed. Two samples were stated as originating from Poland, but all other samples were stated to be of UK origin.

A bespoke APHA in-house SOP based on published EU methods was written for the purpose of this and previous studies, and agreed with the FSA before commencement of work. 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-producing *E. coli* in 25 grams of meat.

None of the samples were positive on the two carbapenem agars. Whilst thirteen samples gave growth to presumptive *E. coli* on MacConkey agar + 2 mg/L colistin, none of the "sweeps" of ~ 10 to 20 colonies tested by RT-PCR from this agar were positive for *mcr-1,* mcr-*2* or mcr-*3.*

Of the 309 samples tested, 42 (13.6%, 95% confidence interval 9.97% to 17.92%) grew on MacConkey agar + 1 mg/L cefotaxime. Between 8 and 85 samples were tested from the 10 different supermarket chains. Between 2.6% and 30.7% samples from each supermarket gave rise to *E. coli* on MacConkey agar + 1 mg/L cefotaxime, and these differences were not significant (p=0.08).

A total of 31 samples, representing 10.0% (95% confidence interval 6.92% to 13.94%) of samples tested overall, gave rise to growth of presumptive ESBL-producing *E. coli* on CHROMagar™ ESBL. For these 31 isolates from CHROMagar™ ESBL, 27 (87.1%) were positive for the *bla*$_{\text{CTX-M}}$ gene by PCR, whilst three of the four remaining isolates were positive for the *bla*$_{\text{SHV}}$ gene (two also with *bla*$_{\text{TEM}}$) and one was positive for *bla*$_{\text{TEM}}$ only[3]. All of the *bla*$_{\text{CTX-M}}$ positive isolates had the sequence of the *bla*$_{\text{CTX-M}}$ gene determined by sequencing. Isolates were all *bla*$_{\text{CTX-M 1}}$ (n=26), except one isolate that was *bla*$_{\text{CTX-M 55}}$. The sequence types of the *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes were not determined.

The proportion of chicken samples positive on MacConkey agar + 1 mg/L cefotaxime and CHROMagar™ ESBL was higher for skin off rather than skin on samples. This difference was significant for samples tested on CHROMagar™ ESBL. This could be because mechanical removal of the skin causes cross contamination of samples, but this was not tested for.

Only two samples (0.65%, 95% confidence interval 0.08% to 2.32%) pre-enrichment from two different supermarkets gave rise to presumptive AmpC/ESBL *E. coli*-

producing counts of 20 cfu/gram (detection limit) on MacConkey agar + 1 mg/L cefotaxime and none of the samples gave rise to counts above the detection threshold on CHROMagar™ ESBL.

Determination of Minimum Inhibitory Concentrations (MICs) of isolates to a panel of relevant antimicrobials, coupled with interpreting strains as sensitive or resistant using ECOFFs (published by EUCAST), 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, 26/42 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 8.4% (95% confidence interval, 5.57% to 10.09%) of samples tested overall; 16/42 were found to have an AmpC-phenotype (excluding the three isolates that had an ESBL-phenotype), representing 5.2% (95% confidence interval, 2.99% to 8.27%) of samples tested overall. If including the AmpC + ESBL-phenotype isolates, 6.1% (95% confidence interval, 3.74% to 9.44%) of the samples tested had an AmpC-phenotype. Between 2.6% and 15.4%, and between 0% and 15.4% of the samples tested per supermarket had an ESBL or AmpC-phenotype respectively.

None of the 42 isolates from MacConkey agar + 1 mg/L cefotaxime were microbiologically resistant (when ECOFFs were applied to the MIC results) to the ‘last resort’ carbapenem antimicrobial meropenem or to colistin. One AmpC phenotype isolate was just above the EUCAST ECOFFS for the carbapenem antimicrobials ertapenem and imipenem, and as such was microbiologically resistant, but this isolate was not clinically resistant (using EUCAST clinical breakpoints) and was not positive for any carbapenem resistance genes, based on WGS results. The microbiological resistance detected to ertapenem and imipenem was only just over the breakpoint threshold. On retest the isolate was shown to be

microbiologically sensitive to both ertapenem and imipenem. Additionally, none of the isolates were resistant to the antibiotics azithromycin, temocillin or tigecycline.[5]

Only two and three isolates were resistant to chloramphenicol and gentamicin respectively, but all isolates were resistant to the beta (β)-lactam antimicrobial ampicillin and cefotaxime. All of the isolates designated as ESBL-phenotype were also resistant to the cefepime and ceftazidime, and all of the isolates designated as AmpC were also resistant to cefoxitin. Only 50% of the AmpC-phenotype isolates were resistant to cefepime.

Most of the ESBL-phenotype isolates were resistant to sulfamethoxazole and tetracycline, although a lower proportion of the AmpC-phenotype antimicrobials were resistant to these two antimicrobials. About 50% of the ESBL-phenotype isolates were resistant to the quinolone antimicrobials ciprofloxacin and nalidixic acid, but only 25% of the AmpC-phenotype isolates were resistant to these two antimicrobials. Overall 19.0% of isolates were resistant to trimethoprim.

The isolates were tested to a total of 19 antimicrobials for which EUCAST ECOFFS were applied (excludes cefotaxime or ceftazidime with clavulanic acid). Isolates with an AmpC, ESBL or ESBL + AmpC-phenotype were resistant to an average of 6.3, 7.0 or 8.8 of these 19 antimicrobials respectively (based on the re-tested isolate being sensitive to ertapenem and imipenem), and as such the isolates with a combined ESBL + AmpC-phenotype were resistant to more antimicrobials.

In summary, the results of the UK retail survey in 2018 (compared to 2016 survey) showed that 8.4% (29.7%) and 6.1% (16.3%) of retail chicken meat samples were positive for ESBL or AmpC-phenotype *E. coli* respectively (including the three isolates with the combined AmpC/ESBL-phenotype) on MacConkey agar + 1 mg/L cefotaxime. Using CHROMagar™ ESBL (2016 results), 10% (30.4%) of samples were positive for presumptive ESBL-phenotype *E. coli*, of which 8.7% (28.1%) of samples were confirmed to be $bla_{\text{CTX-M}}$ positive (mainly $bla_{\text{CTX-M 1}}$).

None of the samples were positive for carbapenem-resistant *E. coli* on the two carbapenem selective agars, or for *E. coli* positive for the plasmid mediated colistin resistance genes *mcr-1, 2* and *3*. Only two meat samples had viable bacterial counts (without enrichment) of 20 cfu/gram presumptive ESBL-producing *E. coli.*

In 2016, results showed a significant decrease (65.4% to 29.7%) in the proportion of chicken meat samples positive for ESBL-producing *E. coli* compared to a previous (2013/14) UK study that used similar, but not identical methodology.[1] A further decrease was observed in 2018 (compared to 2016) using identical methods to the 2016 survey. This difference was significant for a reduction in the proportion of chicken samples positive on both MacConkey agar + 1 mg/L cefotaxime and CHROMagar™ ESBL (p-value, < 0.0001 in both cases), and in the proportion of samples between 2016 and 2018 confirmed as positive for AmpC or ESBL-phenotype *E. coli* (p-value, < 0.0001 in both cases).

This drop in the level of antimicrobial resistant *E. coli* on retail chicken meat since 2013/14 may be linked to the banning by the British Poultry Council of third- and fourth -generation cephalosporins in in flocks used for poultry meat production in the UK in 2012 as part of antimicrobial stewardship.[2] All meat samples since 2013, excluding two samples from 2018, were stated to be of UK origin, although for the earlier published study for 2013/14 samples, the origin of 7.5% of the chicken meat samples was not stated.

4. Glossary

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

AmpC enzyme – Enzyme conferring AmpC type resistance

AMR – Antimicrobial resistance

APHA – Animal and Plant Health Agency

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

CRL – Community Reference Laboratory

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

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

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

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

COL - Colistin

CTX – Cefotaxime

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

EN - Norme Européenne /Europäische Norm (European Standard)

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

EU – European Union

EUCAST - European Committee on Antimicrobial Susceptibility Testing

FSA – Food Standards Agency

HCCA - α-Cyano-4-hydroxycinnamic acid

ISO - International Organisation for Standardisation

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

MCA – MacConkey agar

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

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

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

Sampling criteria

The 2018 poultry sampling plan used “proportionate stratified sampling” to allocate samples to NUTS-3 areas and the samples were distributed in proportion to population size. Eighty NUTS-3 locations were selected representing England, Scotland, Wales and Northern Ireland and covering at least 80% of the total population. Samples were taken from all but the smallest NUTS-3 regions in the UK. The smallest areas were disregarded when together they comprised less than 20% of the national population. Any selected remote areas were replaced with geographically adjacent NUTS-3 regions, or another NUTS-3 area in the same NUTS-2 region.

Work performed at APHA Weybridge

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

These SOPs are:-

- Isolation of background (indicator commensal) and antibiotic resistant *Enterobacteriaceae* from meats and caecal contents according to CRL, EU and / or APHA protocols (CBU 0278).
- Microbank -70ºC Bacterial Storage System (CBU 0155).
- Identification of Bacteria by Oxidase (BA 050) and Indole Spot Test – a Rapid Method for Bacteria (BA0130) and by MALDI ToF (BAC 0334).
- Minimum Inhibitory Concentration (MIC) – The Sensititre Method (BA0604).
- Oxidase (BA 050)
- Indole Spot Test – a Rapid Method for Bacteria (BA 0130)
- Identification of bacteria by MALDI ToF (BAC0334)

- Real Time PCR for plasmid mediated colistin resistance genes *mcr-1* 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 in EU documents, and the SOP CBU 0278 is based on these EU methods as below for the work outlined in this report:-

- **EU method -** Isolation of ESBL, AmpC and carbapenemase producing *E. coli* from fresh meat – December 2017
- **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 2017
- **EU method** - Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli* January 2015

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.

In brief, 27 grams of retail meat sample collected, transported and stored under conditions as stipulated by the EU protocols, was homogenised in ~ 100 ml (from 243 ml sterile chilled BPW) of sterile chilled BPW, before adding this homogenate to the remaining BPW and gently mixing, providing 270 ml of BPW homogenate. From this 270 ml BPW homogenate, 20 mls was taken for the viable bacterial counts. The remaining 250 mls of BPW homogenate (e.g. 25 grams of meat and 225 mls of BPW as per EU protocols) was incubated at 37 ± 1ºC for 18-22 hours.

The incubated BPW / meat homogenate was used to inoculate (10µl) MacConkey agar containing 1 mg/L cefotaxime (MCA-CTX), chromID® CARBA (CARBA) and chromID® OXA-48 (OXA-48).

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

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

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

Lactose fermenters from MCA-CTX were assumed to be presumptive AmpC / EBSL *E. coli,* red/purple colonies from CA-ESBL were assumed to be presumptive ESBL-producing *E. coli* and pink to burgundy colour colonies from CARBA and OXA-48 agars were assumed to be presumptive carbapenem resistant *E. coli.* Three single presumptive *E. coli* from each of these agars was plated again to the agar of origin to ensure purity prior to confirming one of the isolates as *E. coli,* and then storing this isolate pending further tests.

Overall, this method has the theoretical potential to detect one *E. coli* of interest per 25 grams of meat.

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

Storage of purified 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 “beads” (frozen in cryogenic material at -70ºC).

For “beads,” purified bacterial culture was aseptically transferred using a 10 µl loop from the pure culture on agar to a commercial “beads” tube. The cryogenic liquid

and bacterial growth 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 isolates as *E. coli.* Presumptive *E. coli* from other agars, such as CA-ESBL, CARBA and OXA-48, were first streaked to MCA and incubated for 18-22 hours at 44 ± 0.5 °C to confirm isolates as lactose fermenters. If isolates were lactose fermenters, they were then identified as *E. coli* by combined use of oxidase and indole tests as described by in-house SOPs.

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

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

MALDI ToF was used for identification of problem isolates giving equivocal results by other tests only if required, and was used as described by an in-house SOP and based on that previously described.[6] For MALDI ToF identifications if required, isolates were also grown on blood agar. A small amount of bacterial growth was applied to the metal target plate. Growth on the target plates was overlaid with 1 µl of 70% formic acid to perform a partial protein extraction, and allowed to dry. Each spot was then overlaid with 1 µl of HCCA matrix, and again this was allowed to dry before the target plate was loaded into the MALDI ToF machine. Using Biotyper software, resulting spectra from the MALDI ToF run were searched against the

Bruker database of spectra, and if the resulting score was ≥ 2.000, this was taken as reliable identification to the species level, dependant also on consistency score and caveats that might apply for some bacteria species.

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

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

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

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, mcr-2* and *mcr-3* genes

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

Statistics

All p-values and confidence intervals were calculated using a two sample test of proportions, using the *prtest* command in Stata 15 (Stata Corporation, College Station, TX, USA). The test to compare the proportion of positive on MCA-CTX agar between supermarkets was performed using a Fisher's exact test (Stata 15).

6. Results

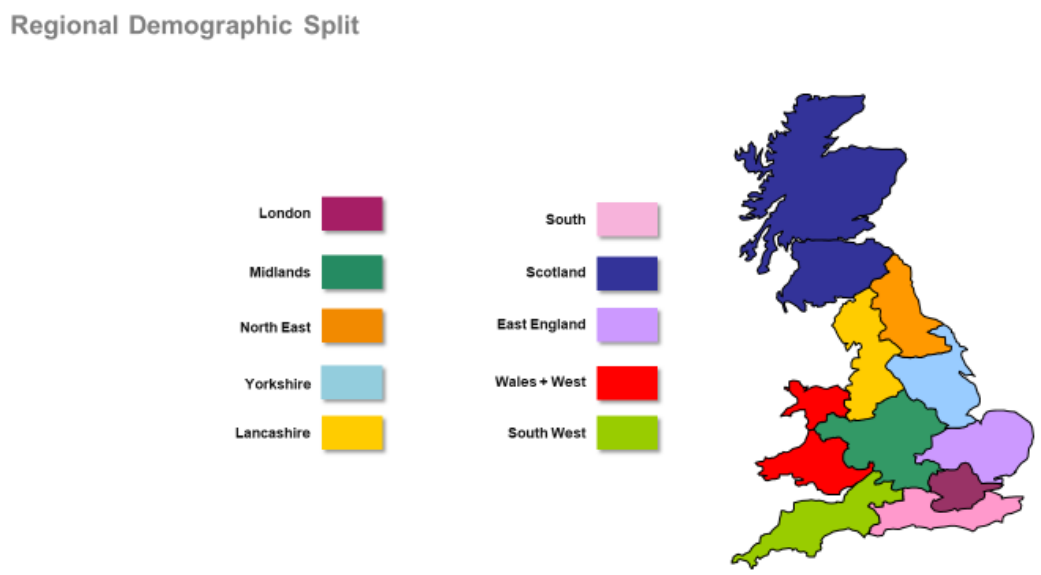
General considerations

An excellent working partnership continued with the company contracted by FSA to supply the meat samples (HallMark Veterinary and Compliance Services) in previous years. Communication between the two organisations and all other aspects of the partnership were highly satisfactory.

Sampling

The FSA looked into how the population data (NUTS-3) should map to the regions in the Kantar market share data. Kantar make use of ITV regions and have supplied a map (Figure 1), rather than a precise geographical location. From this map (assisted by other information about ITV regions available online), FSA allocated the most appropriate Kantar ITV region to each NUTS-3 area. It was not an exact match, but it was considered adequate assigning samples more-or-less in proportion to regional market share. The population data was updated to include the Kantar ITV regions.

Figure 1 – Regional demographic split



There were 109 NUTS-3 regions which cover 80% of the UK population. The number of samples in each NUTS-3 region was proportional to the population size of these 109 regions combined. Kantar's region codes were used to determine the market share % among these 109 regions. For example, the first 19 samples were from seven NUTS-3 regions and they all belonged to the same Kantar region (E. England); the retailer (shop) and cuts % from the Kantar E. England data were used. The number of samples planned and collected per NUT-1 region (Table 1).

Table 1 – Number of samples per NUTS-1 area

NUTS-1	Location Name	No. samples planned	No. samples collected	Difference
UKF	East Midlands (England)	24	24	0
UKH	East of England	26	26	0
UKI	London	49	44	-5
UKN	Northern Ireland	10	10	0
UKC	North East (England)	10	10	0
UKD	North West (England)	33	33	0
UKM	Scotland	19	19	0
UKJ	South East (England)	47	47	0
UKK	South West (England)	30	30	0
UKL	Wales	11	11	0
UKG	West Midlands (England)	26	26	0
UKE	Yorkshire and The Humber	30	29	-1
Total		**315**	**309**	**-6**

The shops from which the samples were obtained in the UK are shown below (Table 2).

Table 2 – Collected samples* per shop, per UK region

Retailer code	England	Wales	Scotland	Northern Ireland	United Kingdom
A	8	1	1	0	10
B	26	1	2	1	30
C	73	3	4	5	85
D	6	1	1	0	8
E	31	1	3	0	35
F	16	0	2	0	18
G	27	1	3	0	31
H	13	0	0	0	13
I	32	2	2	2	38
J	37	1	1	2	41
Total	**269**	**11**	**19**	**10**	**309**

*** Above retailers supply at least 80% of the market share for chicken meat.**

In agreement with the FSA, the types of chicken to be sampled included both whole chicken carcasses and chicken joints/portions such as quarters, legs, thighs, drumsticks, breasts. 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.

Details of the meat samples tested

Of the 315 samples planned, ten samples were rejected because their temperatures on arrival at the laboratory were above that stated in the EU guidelines, but six of these were re-sampled giving 311 samples. A further two were not collected due to unavailability.

Only fresh chicken with skin on or off was collected. Samples were collected each month. A total of 309 samples were collected and tested between January and December 2018 from England (n=269), Scotland (n=19), Wales (n=11), and Northern Ireland (n=10). The types of chicken meat collected were whole chicken (n=125, all skin on), chicken breast (n=112, of which 19 were skin on) and other cuts, including quarters, legs, thighs & drumsticks (n=72, of which 61 were skin on). The samples collected by region and the number that were positive for AmpC / ESBL-phenotype *E. coli* on MCA-CTX agar are shown in Table 3. All but two of the chicken meat samples (which were stated to be from Poland) were stated to be of UK origin.

Samples positive for AmpC / ESBL or carbapenem resistant *E. coli* – EU harmonised test

Of the 309 samples tested, 42 (13.6%, 95% confidence interval 9.97% to 17.92%) grew on MacConkey agar + 1 mg/L cefotaxime (Table 4). Between 8 and 85 samples were tested from the 10 different supermarket chains, and between 2.6% and 30.8% samples from each supermarket gave rise to *E. coli* on MacConkey agar + 1 mg/L cefotaxime (Table 4), and these differences were not significantly different (p=0.08). None of the samples were positive on the two carbapenem agars (Table 3).

By MICs, 26/42 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 8.4% (95% confidence interval, 5.57% to 12.09%) of samples tested overall (Table 5); 16/42 were found to have an AmpC-phenotype (excluding the 3 isolates that also had an ESBL-phenotype), representing 5.2% (95% confidence interval, 2.99 % to 8.27%) of samples tested overall (Table 6). If including the AmpC + ESBL-phenotype isolates, 6.1% (95% confidence interval, 3.74% to 9.44%) of the samples tested had an AmpC-phenotype (Tables 6).

Between 2.6% and 15.4%, and between 0% and 15.4% of the samples tested per supermarket had an ESBL or AmpC-phenotype respectively (Table 4).

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

None of the 42 isolates from MacConkey agar + 1 mg/L cefotaxime were microbiologically resistant (using EUCAST ECOFFS) to the last resort carbapenem antimicrobials meropenem or to colistin (Tables 7 and 8). The MICs against one AmpC isolate were just above the EUCAST ECOFFS for the carbapenem antimicrobials ertapenem and imipenem, with MICs of 0.12 and 1 mg/L respectively. As such this isolate was microbiologically resistant (Tables 7 and 8) to ertapenem and imipenem, but it was not clinically resistant (using EUCAST clinical breakpoints) and was not positive for any carbapenem resistance genes based on whole genome sequencing results. The isolate on retest was microbiologically sensitive with ertapenem and imipenem MICs of 0.06 and 0.25 mg/L respectively. Given the inherent test variation usually expected in MIC determination, coupled with the lack of carbapenem resistance genes, then it is probable that none of the isolates were carbapenem- resistant.

None of the isolates were resistant to the antimicrobials azithromycin, temocillin or tigecycline, as was also observed for the isolates in 2016 (Tables 7 and 8).

Only two and three isolates were resistant to chloramphenicol and gentamicin respectively, but as would be expected, all isolates were resistant to the β-lactam antimicrobial ampicillin and cefotaxime (Tables 7 and 8). All of the isolates designated as ESBL-phenotype were also resistant to the cephalosporin antimicrobials cefepime and ceftazidime, and all of the isolates designated as AmpC were also resistant to cefoxitin (Tables 7 and 8). Only 50% of the AmpC-phenotype isolates were resistant to cefepime (Tables 7 and 8).

Most of the ESBL-phenotype isolates were resistant to the antimicrobials sulfamethoxazole and tetracycline, although a lower proportion of the AmpC-phenotype antimicrobials were resistant to these two antimicrobials (Tables 7 and 8). About 50% of the ESBL-phenotype isolates were resistant to the quinolone antimicrobials ciprofloxacin and nalidixic acid, but only 25% of the AmpC-phenotype

isolates were resistant to these two antimicrobials (Tables 7 and 8). Overall 19.0% of isolates were resistant to trimethoprim (Tables 7 and 8).

The isolates were tested to a total of 19 antimicrobials for which EUCAST ECOFFS were applied (excludes cefotaxime or ceftazidime with clavulanic acid). Isolates with an AmpC, ESBL or ESBL + AmpC-phenotype were resistant to an average of 6.3, 7.0 or 8.8 of these 19 antimicrobials respectively (Tables 7 and 8), and as such the isolates with a combined ESBL + AmpC-phenotype were resistant to more antimicrobials on average.

Counts of presumptive ESBL-producing *E. coli* from MCA-CTX and CA-ESBL - UK non-harmonised additional test

Only 2 samples (0.65%, 95% confidence interval 0.08% to 2.32%) from two different supermarkets gave rise to presumptive *E. coli* counts on MacConkey agar + 1 mg/L cefotaxime (Table 9). These counts were 20 cfu/gram (detection limit). None of the samples gave rise to counts on CHROMagar™ ESBL.

Presumptive ESBL-producing *E. coli* from CA-ESBL and PCR results ESBL - UK non-harmonised additional test

A total of 31 samples, representing 10.0% (95% confidence interval 6.92% to 13.94%) of samples tested overall, gave rise to growth of presumptive ESBL-producing *E. coli* on CA-ESBL (Table 10). For these 31 isolates from CA-ESBL, 87.1% were positive for the *bla*$_{\text{CTX-M}}$ gene by PCR, whilst 3 / 4 of the remaining isolates were positive for the *bla*$_{\text{SHV}}$ gene (two also with *bla*$_{\text{TEM}}$) and one was positive for *bla*$_{\text{TEM}}$ only (Table 10).[3] All of the *bla*$_{\text{CTX-M}}$ positive isolates had the sequence of the *bla*$_{\text{CTX-M}}$ gene determined by sequencing of PCR products. Isolates were all *bla*$_{\text{CTX-M 1}}$ (n=26), except one isolate that was *bla*$_{\text{CTX-M 55}}$ (Table 10). The sequence types of the *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes were not determined.

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

Whilst thirteen 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, mcr-2* or *mcr-3.* As such “sweeps” of isolates were not kept for further tests.

Comparison of results for skin on/off and for 2016 and 2018

Summary results for samples positive for *E. coli* on MCA-CTX and CA-ESBL and samples positive for AmpC or ESBL-phenotype *E. coli* in 2016 and 2018 are shown in Table 11 and Figures 2 and 3. Results are also shown for all samples and AmpC or ESBL phenotype isolates for skin on and skin off samples, with statistical differences shown between years and between skin on and skin off samples.

In 2016, results showed a significant decrease (odds ratio 0.45 p-value < 0.001) in the proportion of samples positive for ESBL-producing *E. coli* compared to a previous (2013/14) UK study, which reported that 65.4% of 159 retail chicken samples were positive for ESBL-producing *E. coli.*[1] 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.

Significant differences were also observed between 2016 and 2018 using identical EU methods (Table 11). During this time period, the numbers of chicken meat samples positive on MCA-CTX dropped from 45.1% to 13.6% (significant, p-value < 0.0001) and from 30.4% to 10.0% on CA-ESBL (significant, p-value < 0.0001) as shown in Table 11 and Figure 2. Additionally, the number of ESBL and AmpC-phenotype positive isolates were significantly lower (p-value < 0.0001) in 2018 compared to 2016 (Table 11 and Figure 3).

It was also interesting to note the higher numbers of skin off compared to skin on samples positive on both MCA-CTX and CA-ESBL for both 2016 and 2018 samples (Table 11 and Figure 2). On MCA-CTX the percentage of skin on / off samples positive were 11.2% / 18.3% (not significant) and 38.8% / 60.7% (significant, p-value = 0.0004) for 2018 and 2016 samples respectively (Table 11 and Figure 2). On CA-ESBL the percentage of skin on / off samples positive were 6.8% / 16.4% (significant, p = 0.008) and 25.0% / 43.8% (significant, p-value = 0.0004) for 2018 and 2016 samples respectively (Table 11 and Figure 2).

Results for isolates with the AmpC or ESBL phenotype were also lower from skin on samples for both 2016 and 2018, but these differences were not significant between years or within years (Table 11).

Table 3. 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 samples) 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	0 (0)	0	0
Barking & Dagenham and Havering	3	1 (33.3)	0	1
Barnet	2	1 (50)	0	1
Barnsley, Doncaster and Rotherham	5	0 (0)	0	0
Bath and NE Somerset, N Somerset and S Gloucestershire	4	1 (25)	0	1

Berkshire	6	0 (0)	0	0
Bexley and Greenwich	3	1 (33.3)	0	1
Birmingham	7	1 (14.3)	0	1
Bournemouth and Poole	2	1 (50)	0	1
Bradford	3	0 (0)	0	0
Brent	2	0 (0)	0	0
Brighton and Hove	2	0 (0)	0	0
Bristol, City of	3	0 (0)	0	0
Bromley	2	0 (0)	0	0
Buckinghamshire CC	3	1 (33.3)	0	1
Calderdale and Kirklees	4	0 (0)	0	0
Cambridgeshire CC	4	2 (50)	0	2

Cardiff and Vale of Glamorgan	3	0 (0)	0	0
Central Hampshire	3	0 (0)	0	0
Central Valleys	2	1 (50)	0	1
Cheshire East	2	0 (0)	0	0
Cheshire West and Chester	2	0 (0)	0	0
Clackmannanshire and Fife	3	0 (0)	0	0
Cornwall and Isles of Scilly	3	0 (0)	0	0
Coventry	2	1 (50)	0	1
Croydon	2	0 (0)	0	0
Devon CC	5	0 (0)	0	0
Dorset CC	3	1 (33.3)	0	1
Dudley	2	1 (50)	0	1

Durham CC	3	0 (0)	0	0
Ealing	2	0 (0)	0	0
East Kent	3	0 (0)	0	0
East Lancashire	2	0 (0)	0	0
East Merseyside	3	0 (0)	0	0
East of Northern Ireland	3	0 (0)	0	0
East Riding of Yorkshire	2	0 (0)	0	0
East Surrey	2	0 (0)	0	0
East Sussex CC	3	0 (0)	0	0
Edinburgh, City of	3	0 (0)	0	0
Enfield	2	0 (0)	0	0
Essex Haven Gateway	3	0 (0)	0	0

Essex Thames Gateway	2	0 (0)	0	0
Flintshire and Wrexham	2	1 (50)	0	1
Glasgow City	4	1 (25)	0	1
Gloucestershire	4	1 (25)	0	1
Greater Manchester North East	4	0 (0)	0	0
Greater Manchester North West	4	0 (0)	0	0
Greater Manchester South East	3	0 (0)	0	0
Greater Manchester South West	3	0 (0)	0	0
Gwent Valleys	2	0 (0)	0	0
Hackney and Newham	4	0 (0)	0	0
Haringey and Islington	3	0 (0)	0	0
Harrow and Hillingdon	3	1 (33.3)	0	1

Heart of Essex	2	0 (0)	0	0
Hertfordshire	7	1 (14.3)	0	1
Hounslow and Richmond upon Thames	3	0 (0)	0	0
Inverclyde, East Renfrewshire and Renfrewshire	2	0 (0)	0	0
Kensington & Chelsea and Hammersmith & Fulham	2	0 (0)	0	0
Kent Thames Gateway	2	0 (0)	0	0
Leeds	5	0 (0)	0	0
Leicester	2	0 (0)	0	0
Leicestershire CC and Rutland	4	0 (0)	0	0
Lewisham and Southwark	4	0 (0)	0	0
Lincolnshire	4	0 (0)	0	0
Liverpool	3	2 (66.6)	0	2

Manchester	3	0 (0)	0	0
Mid Kent	2	0 (0)	0	0
Mid Lancashire	2	0 (0)	0	0
North and North East Lincolnshire	2	1 (50)	0	1
North Hampshire	2	1 (50)	0	1
North Lanarkshire	2	0 (0)	0	0
North Northamptonshire	2	0 (0)	0	0
North Nottinghamshire	3	2 (66.6)	0	2
North of Northern Ireland	2	0 (0))	0	0
North Yorkshire CC	3	1 (33.3)	0	1
Northumberland	2	0 (0)	0	0
Norwich and East Norfolk	2	1 (50)	0	1

Nottingham	2	1 (50)	0	1
Outer Belfast	2	0 (0)	0	0
Oxfordshire	4	1 (25)	0	1
Redbridge and Waltham Forest	3	1 (33.3)	0	1
Sandwell	2	1 (50)	0	1
Sheffield	3	1 (33.3)	0	1
Shropshire CC	2	0 (0)	0	0
Somerset	3	0 (0)	0	0
South and West Derbyshire	3	1 (33.3)	0	1
South Hampshire	3	0 (0)	0	0
South Lanarkshire	2	0 (0)	0	0
South Nottinghamshire	2	1 (33.3)	0	1

South West Wales	2	0 (0)	0	0
Staffordshire CC	5	1 (0)	0	1
Suffolk	4	2 (50)	0	2
Tower Hamlets	2	0 (0)	0	0
Tyneside	5	0 (0)	0	0
Wakefield	2	1 (50)	0	1
Wandsworth	2	1 (50)	0	1
Warwickshire	3	1 (33.3)	0	1
West and South of Northern Ireland	3	0 (0)	0	0
West Essex	2	0 (0)	0	0
West Kent	2	0 (0)	0	0
West Northamptonshire	2	1 (50)	0	1

West Surrey	5	0 (0)	0	0
West Sussex (North East)	2	0 (0)	0	0
West Sussex (South West)	3	1 (33.3)	0	1
Wiltshire	3	1 (33.3)	0	1
Wirral	2	0 (0)	0	0
Worcestershire	3	0 (0)	0	0

Table 4. Number of samples per supermarket tested that gave rise to *E. coli* on MCA-CTX with resistance phenotypes

Supermarket Code	Total number of samples tested	No. positive on MCA-CTX agar (%)*	ESBL-phenotype confirmed by MICs (%)*	AmpC-phenotype confirmed by MICs (%)*	AmpC/ESBL-phenotype confirmed by MICs (%)*
A	10	1 (10.0)	1 (10.0)	0 (0)	0 (0)
B	30	3 (10.0)	1 (3.3) **	1 (3.3) **	1 (3.3)
C	85	11 (12.9)	8 (9.4) **	4 (4.7) **	1 (1.2)
D	8	2 (25)	1 (12.5)	1 (12.5)	0 (0)
E	35	7 (20)	4 (11.4) **	4 (11.4) **	1 (2.9)
F	18	1 (5.6)	1 (5.6)	0 (0)	0 (0)
G	31	3 (9.7)	2 (6.4)	1 (3.2)	0 (0)
H	13	4 (30.7)	2 (15.4)	2 (15.4)	0 (0)
I	38	1 (2.6)	1 (2.6)	0 (0)	0 (0)

Supermarket Code	Total number of samples tested	No. positive on MCA-CTX agar (%)*	ESBL-phenotype confirmed by MICs (%)*	AmpC-phenotype confirmed by MICs (%)*	AmpC/ESBL-phenotype confirmed by MICs (%)*
J	41	9 (21.9)	5 (12.2)	4 (9.7)	0 (0)

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

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

Figure 2. Percentages of chicken meat samples (skin on or off) positive on MacConkey agar + 1 mg/L cefotaxime and CHROMagar ESBL for 2016 and 2018

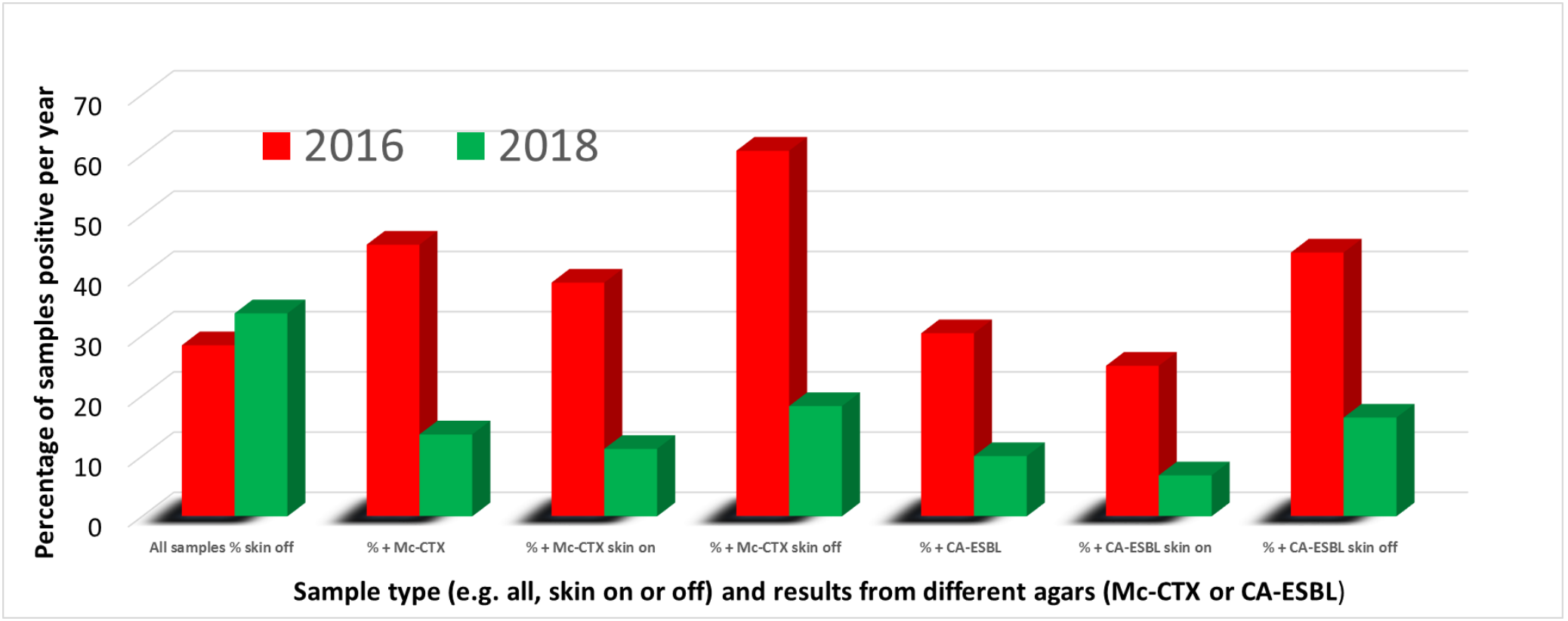


Figure 3. Percentages of chicken meat samples (skin on or off) with ESBLs (including AmpC+ESBL) or AmpC-phenotype (excluding AmpC+ESBL) for 2016 and 2018

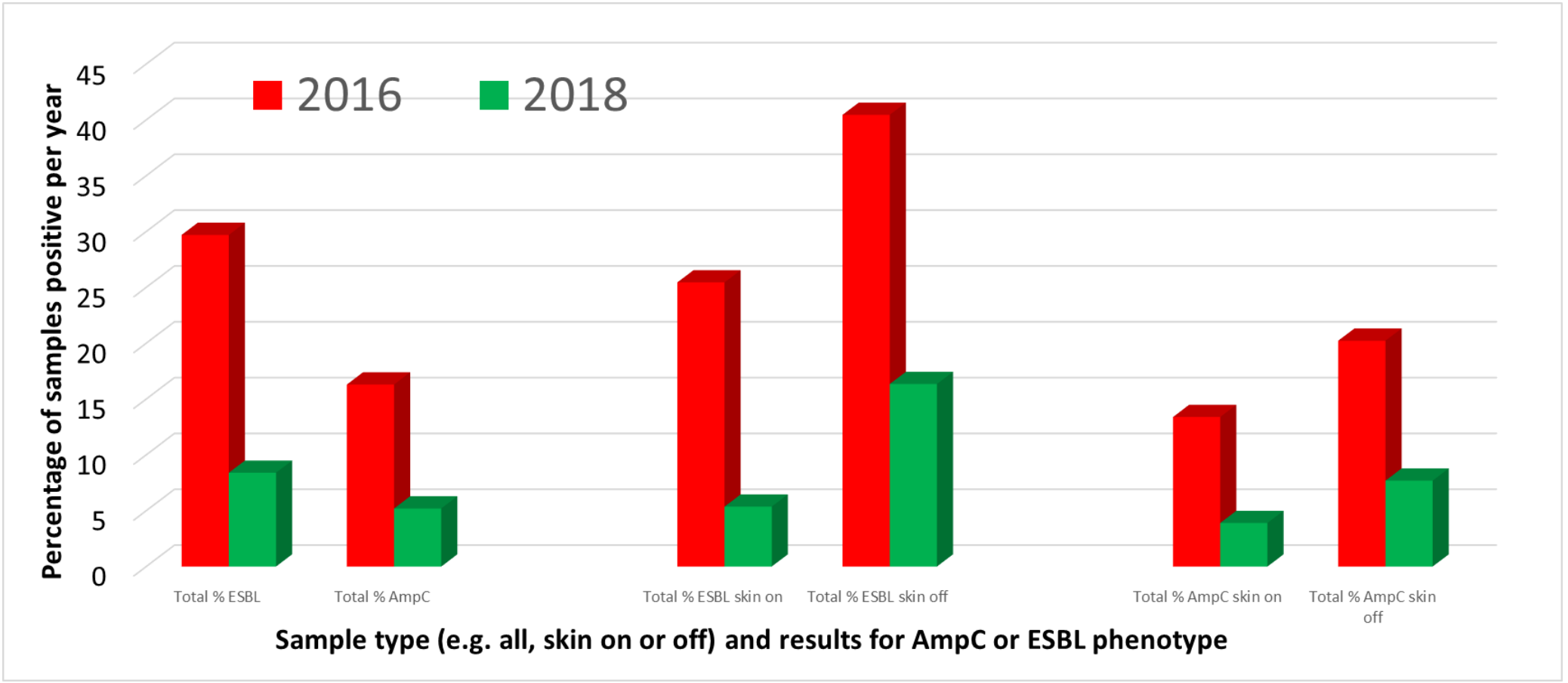


Table 5. Samples positive for ESBL-phenotype *E. coli* from MCA-CTX (Grey cells indicates isolates have an AmpC+ESBL-phenotype)

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)
00007609	15/11/2018	C	On	Other cuts	North Hampshire
00364585	13/11/2018	G	On	Other cuts	Glasgow City
00364920	15/08/2018	C	On	Other cuts	Norwich and East Norfolk
00364925	15/08/2018	C	On	Other cuts	Suffolk
00364928	14/08/2018	C	On	Other cuts	Liverpool
00364930	14/08/2018	D	Off	Chicken breast	Liverpool
00364958	25/06/2018	E	On	Other cuts	South Nottinghamshire
00364959	25/06/2018	J	On	Whole chicken	South and West Derbyshire
00364978	21/05/2018	J	Off	Other cuts	Bexley and Greenwich

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)
00365001	20/04/2018	E	On	Whole chicken	Coventry
01614392	16/01/2018	A	On	Whole chicken	Warwickshire
01614401	16/01/2018	B	Off	Other cuts	Redbridge and Waltham Forest
01614403	22/01/2018	E	On	Whole chicken	Staffordshire CC
02447919	17/09/2018	H	Off	Chicken breast	Harrow and Hillingdon
02447920	11/09/2018	J	Off	Chicken breast	West Northamptonshire
02447930	16/08/2018	E	Off	Chicken breast	Suffolk
02447943	20/07/2018	C	Off	Chicken breast	North Yorkshire CC
02447958	14/06/2018	C	Off	Chicken breast	Wiltshire
02447971	30/04/2018	C	Off	Chicken breast	Greater Manchester North East
02447979	20/04/2018	J	Off	Chicken breast	Dudley

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)
02447984	14/03/2018	J	Off	Chicken breast	North Nottinghamshire
02448050	17/01/2018	H	Off	Chicken breast	Bournemouth and Poole
02448247	12/02/2018	B	On	Other cuts	Wakefield
02448250	17/05/2018	I	Off	Chicken breast	Flintshire and Wrexham
02558653	17/08/2018	G	Off	Chicken breast	Cambridgeshire CC
02558654	17/08/2018	C	Off	Chicken breast	Cambridgeshire CC

Table 6. 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	Skin on or off	Food Category	Sampling Location (NUTS3) (S#05)
00364638	18/10/2018	C	On	Whole chicken	Buckinghamshire CC
00364664	06/12/2018	J	On	Whole chicken	Hertfordshire
00364957	25/06/2018	F	On	Whole chicken	Nottingham
00364958	25/06/2018	E	On	Other cuts	South Nottinghamshire
00364982	14/06/2018	H	On	Whole chicken	Bath & NE Somerset, N Somerset & Gloucestershire
00364998	20/04/2018	C	On	Whole chicken	Birmingham
00365004	20/04/2018	J	On	Whole chicken	Sandwell
01614401	16/01/2018	B	Off	Other cuts	Redbridge and Waltham Forest
02447893	17/09/2018	H	Off	Chicken breast	Barnet

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3) (S#05)
02447915	18/10/2018	B	Off	Chicken breast	North and North East Lincolnshire
02447938	17/08/2018	J	Off	Chicken breast	Central Valleys
02447947	18/10/2018	C	Off	Chicken breast	Oxfordshire
02447964	17/05/2018	D	Off	Chicken breast	Barking & Dagenham and Havering
02447967	17/05/2018	J	Off	Chicken breast	West Sussex (South West)
02447971	30/04/2018	C	Off	Chicken breast	Greater Manchester North East
02447985	14/03/2018	E	Off	Chicken breast	North Nottinghamshire
02447996	09/02/2018	E	Off	Other cuts	Wandsworth
02447999	06/02/2018	G	On	Whole chicken	Sheffield
02448014	17/01/2018	E	On	Chicken breast	Dorset CC

Table 7. MIC results of 19 antimicrobials against all isolates from MCA-CTX with AmpC (A) ESBL (E) or AmpC + ESBL (A+E) phenotype

Pheno-type	Sample No.	Resistant (R) or Sensitive (S)																		No. of antimicrobials strain resistant to
		AMP	AZM	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ETP	IPM	MEM	GEN	TMC	TET	TCG	SUL	TMP	
A	00364638	R	S	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	R	S	7
A	00364664	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	5
A	00364957	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	7
A	00364982	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	5
A	00364998	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
A	00365004	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	5
A	02447893	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	R	S	R	S	9
A	02447915	R	S	S	R	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	6
A	02447938	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	4
A	0244794	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	6

Pheno-type	Sample No.	Resistant (R) or Sensitive (S)																		No. of antimicrobials strain resistant to
		AMP	AZM	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ETP	IPM	MEM	GEN	TMC	TET	TCG	SUL	TMP	
	7																				
A	02447964	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	R	S	R	R	10
A	02447967	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	5
A	02447985	R	S	R	R	R	R	S	S	S	S	R/S	R/S	S	R	S	R	S	R	S	10/8 retest*
A	02447996	R	S	R	R	R	R	S	S	S	S	S	S	S	R	S	R	S	R	S	8
A	02447999	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	5
A	02448014	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	5
A+E	00364958	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	R	S	7
A+E	01614401	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	R	S	R	R	10
A+E	02447971	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S	R	R	9
E	0007609	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
E	0036458	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6

Pheno-type	Sample No.	Resistant (R) or Sensitive (S)																		No. of antimicrobials strain resistant to
		AMP	AZM	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ETP	IPM	MEM	GEN	TMC	TET	TCG	SUL	TMP	
	5																				
E	00364920	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	S	8
E	00364925	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	S	8
E	00364928	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R	10
E	00364930	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	R	9
E	00364959	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
E	00364978	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
E	00365001	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	4
E	01614392	R	S	R	R	S	R	R	R	S	S	S	S	S	S	S	R	S	R	R	9
E	01614403	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	S	S	R	R	8
E	2447919	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
E	0244792	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6

Pheno-type	Sample No.	Resistant (R) or Sensitive (S)																		No. of antimicrobials strain resistant to
		AMP	AZM	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ETP	IPM	MEM	GEN	TMC	TET	TCG	SUL	TMP	
	0																				
E	02447930	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	S	8
E	02447943	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
E	02447958	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	S	8
E	02447979	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
E	02447984	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S	6
E	02448050	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	6
E	02448247	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	S	8
E	02448250	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	S	8
E	02558653	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	S	8

***For sample 02447985 isolate was microbiologically resistant to ertapenem and imipenem, but was sensitive on retest.**

Green, amber or red – resistant to < 6, 6 or 7 antimicrobials and > 8 antimicrobials respectively.

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 8. Summary of resistance phenotypes for all isolates from MCA-CTX

Antimicrobial	No. resistant[a] / No. tested	
	ESBL*	AmpC**
Ampicillin	26/26	16/16
Azithromycin	0/26	0/16
Cefepime	26/26	13/16
Cefotaxime	26/26	16/16
Cefoxitin	3/26*	16/16
Ceftazidime	26/26	16/16
Chloramphenicol	2/26	0/16
Ciprofloxacin	13/26	4/16
Colistin	0/26	0/16
Ertapenem	0/26	0[b]/16
Gentamicin	0/26	3/16
Imipenem	0/26	0 [b]/16
Meropenem	0/26	0/16
Nalidixic Acid	12/26	4/16
Sulfamethoxazole	25/26	6/16
Temocillin	0/26	0/16
Tetracycline	22/26	6/16

Antimicrobial	No. resistant[a] / No. tested	
	ESBL*	**AmpC****
Tigecycline	0/26	0/16
Trimethoprim	7/26	1/16

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

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

Green highlight denotes a lower proportion of ESBL *versus* AmpC or AmpC versus ESBL isolates resistant for stated antimicrobial.

a Microbiologically resistant using EUCAST ECOFFS

b One AmpC isolate was microbiologically but not clinically resistant to ertapenem and imipenem but lacked any carbapenem resistance genes by WGS and was microbiologically sensitive on retest.

*** 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 9. Viable count of presumptive *E. coli* above the detection limit[a]

Sample number	Supermarket code	Product text description	Skin on or off	Viable counts [a] (cfu/g) on agars	
				MCA-CTX	CA-ESBL
00365004	J	Whole Chicken	On	20	< 20
00365027	C	Whole Chicken	On	20	< 20

***a* – Limit of detection = 20 cfu/gram of meat**

Table 10. 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
7609	C	Chicken Thighs	CTX	CTXM-1
233860	E	Small Whole Chicken without giblets	CTX	CTXM-1
364585	G	British Chicken Thighs	CTX	CTXM-1
364664	J	British Fresh Medium Whole Chicken	CTX	CTXM-1
364920	C	Chicken Drumsticks & Thighs	CTX	CTXM-1
364925	C	Chicken Wings	CTX	CTXM-1
364928	C	Chicken drumsticks and thighs	OXA, CTX, TEM	CTXM-55
364930	D	Class A Fresh Chicken Breast Fillets	CTX	CTXM-1
364959	J	Small whole chicken	CTX	CTXM-1
364978	J	British fresh chicken thigh fillets	CTX	CTXM-1
365001	E	Medium whole chicken	SHV	NA
1563554	B	2 Chicken Breast Fillets	CTX	CTXM-1
1614392	A	British Chicken Medium	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
1614395	G	British Chicken Wings	TEM, SHV	ND
1614403	E	Small whole chicken	CTX,T	CTXM-1
2447919	H	British chicken breast chunks	CTX	CTXM-1
2447920	J	2 whole chicken breast fillets	CTX	CTXM-1
2447930	E	2 Chicken Breast Fillets	CTX	CTXM-1
2447943	C	Chicken breast portions	CTX, TEM	CTXM-1
2447958	C	Diced Chicken Breast	CTX	CTXM-1
2447964	D	Class A fresh chicken breast fillets	TEM, SHV	NA
2447968	C	Chicken Thighs	CTX	CTXM-1
2447971	C	Chicken Breast Portions	CTX	CTXM-1
2447979	J	Diced chicken breasts	CTX	CTXM-1
2447984	J	2 whole chicken breast fillets	CTX	CTXM-1
2448011	B	Free Range British Chicken Breast Fillets	TEM	NA
2448050	H	British Chicken Mini Breast Fillets	CTX, TEM	CTXM-1
2448247	B	British Chicken Thighs	CTX	CTXM-1
2448250	I	British Chicken Breast Fillets	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
2558653	G	British Chicken Breast Fillets	CTX	CTXM-1
2558654	C	British Chicken Breast Fillets	CTX	CTXM-1

NA – Not applicable.

Table 11. Summary results for 2016 and 2018 skin on and skin off samples

Test criteria	Skin on or off	Year samples tested		Significance comparison	p-value
		2016	2018		
No. Tested	All	313	309		
	On	224	205		
	Off	89	104		
% + MCA-CTX	All	45.1	13.6	Less + 2018 vs 2016	< 0.0001
	On	38.8	11.2	Less + skin within 2016	0.0004
	Off	60.7	18.3	Less + skin within 2018	NS
% + CA-ESBL	All	30.4	10.0	Less + 2018 vs 2016	< 0.0001
	On	25.0	6.8	Less + skin within 2016	0.0004
	Off	43.8	16.4	Less + skin within 2018	0.008
% ESBL	All	29.7	8.4	Less + 2018 vs 2016	< 0.0001

Test criteria	Skin on or off	Year samples tested		Significance comparison	p-value
Phenotype*	On	25.4	5.4	Less + skin within 2016	NS
	Off	40.4	16.3	Less + skin within 2018	NS
	On	25.4	5.4	Less 2018 vs 2016	NS
	Off	40.4	16.3	Less 2018 vs 2016	NS
% AmpC	All	16.3	5.2	Less + 2018 vs 2016	< 0.0001
phenotype†	On	13.4	3.9	Less + skin within 2016	NS
	Off	20.2	7.7	Less + skin within 2018	NS
	On	13.4	3.9	Less 2018 vs 2016	NS
	Off	20.2	7.7	Less 2018 vs 2016	NS

NS – Not significant, p > 0.05.

*** ESBL-phenotype positives include those with and ESBL + AmpC-phenotype.**

† AmpC-phenotype positives don't include those with and ESBL + AmpC-phenotype.

7. Discussion

The discussion from the 2016 report on "EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in E. coli from Retail Meats (Year 2 - Chicken)" is given in the appendix of this report.[5] Much of the discussion relating to previous studies of ESBL-producing *E. coli* in raw poultry meat in the UK and other countries is of relevance for the 2018 survey. It is recommended to read this previous discussion if a wider background overview is required. The discussion below focuses on results from the 2018 survey.

For the 2016 survey, results showed a significant decrease in the proportion of retail chicken meat samples positive for ESBL-producing *E. coli* compared to a previous (2013/14) UK study, albeit sampling strategies and isolation methodology were similar, but not identical between these two studies.[1, 5] A major finding from the testing of the 2018 chicken meat samples was a significant reduction in the proportion of samples positive for ESBL and AmpC-producing *E. coli* compared with the 2016 survey,[5] using identical methods.

Overall, the proportion of UK retail chicken meat samples positive for ESBL-producing *E. coli* fell from 65.4% in 2013/14 to 29.7% in 2016 and to 8.4% in 2018. Whilst the proportion of chicken samples positive for AmpC-producing *E. coli* was not determined for the 2013/14 study, the samples positive for AmpC-producing *E. coli* fell from 16.3% in 2016 to 5.2% in 2018.

In the 2016 report, it was discussed (see appendix) that in Quebec Canada, ceftiofur resistance in *Salmonella* Heidelberg isolates from chicken meat and humans prompted broiler chicken hatcheries to voluntarily interrupt the extra-label in-ovo use of ceftiofur during 2005-2006.[7] 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%; $p<0.0001$) *S.* Heidelberg isolates and retail chicken *E. coli* isolates (34% to 6%; $p<0.0001$), which was reversed when ceftiofur use was reintroduced.[7]

Whilst it is unknown why the marked decrease in ESBL and AmpC-producing *E. coli* has occurred in retail chicken meat in the UK since 2013/14, it seems reasonable to assume that this decrease was linked to the banning in the UK in 2012 of

cephalosporin use in poultry meat flocks by the British Poultry Council.[2] All meat samples since 2013, excluding two samples from 2018, were stated to be of UK origin, although for the earlier published study for 2013/14 samples, the origin of 7.5% of the chicken meat samples was not stated.

In 2016 the proportions of skin on versus skin off chicken meat samples positive on different agars and for AmpC and ESBL-phenotype *E. coli* was not determined. It was interesting to note that higher proportions of skin off compared to skin on samples were positive on both MCA-CTX and CA-ESBL for both 2016 and 2018 samples, and these results were significant for CA-ESBL for both years and for MCA-CTX for 2016.

In a recent study using high resolution molecular data the authors found "evidence for the cross-contamination of carcasses with ESBL-producing Enterobacteriaceae during scalding and de-feathering in the slaughterhouse."[8] The authors suggested that the evidence "clearly shows the need not only for intervention measures on farm level, but also for effective interventions against cross contamination with ESBL-producing Enterobacteriaceae in the slaughterhouse."[8] It is possible that the process of skin removal from chicken meat might cause cross contamination with ESBL-producing Enterobacteriaceae which may explain for the higher levels of ESBL-producing *E. coli* in skin off compared to skin on chicken meat.

With respect to viable counts on agars, in 2016, based on the 141 samples positive on MCA-CTX agar after enrichment, 7.8% had counts on either MCA-CTX or CA-ESBL agars, using a method with a detection rate on 40 cfu/gram. These counts ranged from 40 to 400 cfu/gram. In 2018 a slightly more sensitive method for viable counts was used, with a detection limit of 20 cfu/gram. Despite a more sensitive method being used in 2018, based on 42 samples positive after enrichment on MCA-CTX agar, only two samples (4.8%) had counts on either MCA-CTX only and both counts were only 20 cfu/gram indicating a low burden of ESBL *E. coli.*

Whilst in China a recent publication reports the "rapid rise of the ESBL and *mcr-1* genes in *Escherichia coli* of chicken origin, 2008-2014,"[9] none of the isolates from agar with colistin in this study were positive for *mcr1, 2* or *3* genes. The China study reports the co-existence of CTX-M genes and *mcr-1* and the Inc type of the majority of *mcr-1* carrying plasmids was IncI2.[9] The authors also comment that the

prevalence of *mcr-1* was higher in the ESBL *E. coli* than in the non-ESBL-producing *E. coli* ($p < 0.001$, 77.3% vs 22.7%).[9] The China study mentions that *bla*$_{\text{CTX-M-55}}$ has become the dominant *bla*$_{\text{CTX-M}}$ type in the ESBL-producing *E. coli* of animal origin in the last decade, but was still very rare in the ESBL-producing *E. coli* of human origin.[9] The authors suggest that this finding might suggest that *bla*$_{\text{CTX-M-55}}$ and *mcr-1* emerged and rose under the heavy selective pressure of antimicrobial usage in the animal husbandry in the last decade.[9] For the 2016 and 2018 UK retail chicken samples, most of the ESBL-producing isolates tested were *bla*$_{\text{CTX-M1}}$, but in both survey years one of the isolates was *bla*$_{\text{CTX-M55}}$. In addition to the British Poultry Council banning the use of third and fourth generation cephalosporins for use in poultry meat flocks in 2012, the UK poultry meat sector has stopped using polymixins (colistin) in 2016.[2]

None of the samples gave rise to isolates on the two agars that selected for carbapenem=resistant *E. coli,* although one isolate from MCA-CTX agar with an AmpC-phenotype was microbiologically resistant using EUCAST ECOFFS to the carbapenem antimicrobials ertapenem and imipenem. This isolate was not clinically resistant to these two antimicrobials when EUCAST clinical breakpoints were applied, and additionally no carbapenemase resistance genes were detected in this isolate by WGS. On retest, the isolate was sensitive using microbiological breakpoints to both ertapenem and imipenem. MIC results can vary between tests, and this is illustrated by the fact that the acceptable range of MIC results for *E. coli* control strain ATCC 25922 according to EUCAST is 0.004 to 0.016 mg/L for ertapenem and 0.06 to 0.25 mg/L for imipenem, both representing one doubling dilution either side of a mean value, or three doubling dilutions. The retest MIC results for this isolate reflect the inherent test variation expected for MIC determination.

Isolates from MCA-CTX agar were MIC tested and classified as resistant or sensitive to 19 of the different antimicrobials using EUCAST ECOFFS. Isolates with an AmpC, ESBL or ESBL+AmpC-phenotype were resistant to an average of 6.3, 7.0 or 8.8 of these 19 antimicrobials respectively, and as such the isolates with a combined ESBL + AmpC-phenotype were on average resistant to more antimicrobials. None of the isolates tested were resistant to the antimicrobials Azithromycin, Temocillin, Meropenem or Tigercycline.

With respect to recent studies of ESBL-producing *E. coli* in poultry and poultry meat, in one study from the Netherlands the authors concluded that "most likely it is a combination of vertical transmission to a low proportion of birds and contamination from the environment followed by rapid transmission between birds. This implies that preventing contamination with ESBL-producing *E. coli* in a broiler flock needs a multidisciplinary approach."[10] Based on the results of surveys in the UK since 2013/14, results would suggest that banning the use of cephalosporins in poultry destined for meat production is perhaps the most effective method of reducing ESBL-producing *E. coli* in chicken meat. Additionally, for broilers in particular in the UK, overall antimicrobial use is recorded to have reduced from 49 mg/kg in 2014 to 10 mg/kg in 2017.[11]

According to the EFSA report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016, "member states tested 6,241 retail meat samples and, following culture on selective media, 32.5% yielded presumptive ESBL-producing *E. coli*, while 26.8% yielded presumptive AmpC-producing *E. coli* and 2% yielded *E. coli* with an ESBL+AmpC-phenotype."[12]

The report went on to state that "among the reporting countries, marked variations were observed in the prevalence of presumptive ESBL-producing *E. coli* isolates, which ranged from none in Norway, 4.9% and 13.4% in Finland and Hungary, up to 60.3% in Italy, 71% in Spain, nearly 75% in Latvia and 78.3% in Belgium. The levels of presumptive AmpC-producing *E. coli* were very high (50–70%) in member states from eastern Europe (Slovenia, Croatia, and Hungary) and moderate to high in most of the other member states. Low prevalence of AmpC-producers was only reported by Portugal and Denmark (6.1–9.5%)."[12] Based on 2016 results, the proportion of retail chicken samples in the UK positive for ESBL-producing *E. coli* was similar to the EU average for samples from all member states (29.7% UK, versus 32.5% EU average). In 2016, the proportion of retail chicken samples in the UK positive for AmpC-producing *E. coli* was 16.3% compared to an EU average of 26.8% in all member states.

8. Conclusions

- None of the UK retail chicken samples tested in 2018 were positive for *E. coli* on the two carbapenemase agars, although one AmpC-phenotype isolate from MCA-CTX agar was initially microbiologically resistant but clinically sensitive to the carbapenem antimicrobials ertapenem and imipenem. This isolate was sensitive on retest, and was shown to be negative of any carbapenem resistance genes using whole genome sequencing.

- None of the UK retail chicken samples tested in 2018 were positive for plasmid- mediated colistin resistance encoded for by *mcr1, 2* and *3* genes in *E. coli*.

- Of the 309 UK retail chicken samples tested in 2018, 26 (8.4%) were positive for ESBL *E. coli* (including AmpC+ESBL phenotype isolates) and 16 (5.2%) were positive for AmpC phenotype *E. coli* (excluding AmpC+ESBL phenotype isolates), based on results from MCA-CTX agar and MICs to determine AmpC or ESBL phenotype.

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

- Only two retail chicken meat samples (0.65%) had viable counts (without enrichment) of presumptive AmpC or ESBL-producing *E. coli* above the detection limit (20 cfu/gram), and these counts were both just 20 cfu/gram.

- The proportion of retail chicken samples positive for ESBL-producing and AmpC-producing *E. coli* dropped from 29.7% to 8.4% and from 16.3% to 5.2% respectively between 2016 and 2018 and this drop was significant for both phenotypes (p-value < 0.0001).

- This marked reduction in the proportions of chicken meat samples positive for AmpC and ESBL phenotype *E. coli* might be linked to the banning of cephalosporins for use in poultry meat flocks in the UK by the British Poultry Council in 2012, as all meat samples since 2014, excluding two samples from 2018, were stated to be of UK origin.

9. References

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10. Appendix

Discussion from 2016 report.

Previous 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*,[13] in Germany in 2012, where 60% of 120 chicken meat samples purchased were positive for mainly CTX-M-1 ESBL-producing Enterobacteriaceae,[14] in Portugal,[15] and in the UK[15, 16].

In the UK study carried out by Dhanji et al,[15] 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.[15] 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.[17] 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.[18]

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 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.[1] For the EU survey reported here, samples were also enriched in BPW, and then

plated to MCA-CTX and CA-ESBL.[1] 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.[1] Comparing the findings of the 2013-2014 study[1] 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), 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.[19] 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.[20] 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 resistance 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.[7] 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.[7] 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.[7] 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.[7]

In this study and the UK study of 2013-2014, the predominant CTX-M sequence type was CTX-M1.[1] This therefore differs from the CTX-M group 2 and 8 *E. coli* isolates found on imported chicken in an earlier study.[15]

The predominant *E. coli* strain associated with human infections is the pandemic O25-ST131 CTX-M-15-producing clone.[21, 22] 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,[23] 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.[23] 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.[23]

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.[17] 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.