FINAL REPORT

FS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in *E. coli* from Retail Meats in UK (2020 - Year 6, chicken)

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1. 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 2020.

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 UK continued to be subject to EU rules during the transition period up to the end of December 2020. Further testing of retail beef, chicken and pork is being considered based on surveillance priorities.

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. Third generation cephalosporins are 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. Carbapeems are 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. Additionally, each meat sample is tested for counts of the number of

background and AMR (AmpC and ESBL type resistance only) *E. coli* in each meat sample according to an EU protocol.

At the request of the FSA, other agar culture media 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. Colistin resistance in *E. coli* isolates may involve 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 known as plasmids. As plasmids are “mobile” (can pass from one bacterium to another), the resistance genes located on them can potentially be shared with other bacteria within the gut.

In total during 2020, 327 samples of fresh chicken were collected of which 315 were eligible for testing. The 315 eligible retail chicken meat samples were collected from England (n=274), Scotland (n=20), Wales (n=11), and Northern Ireland (n=10) from ten different supermarket chains.

Sample collection was impacted by the coronavirus pandemic. Monthly sampling was suspended for 3 months from April to June 2020, resuming in July. Sample numbers were adjusted in subsequent months to reach the target of 300 samples.

The types of chicken meat collected were whole chicken (n=127), chicken breast (n=113) and other cuts, including quarters, legs, thighs & drumsticks (n=75). Of the samples collected, 58.7% and 41.2% had skin on or off respectively. Breast samples were the main sample type from which skin was removed. Of the 315 samples, 309 were stated as originating from the UK, five from Poland and one from Ireland.

No growth was observed from any of the samples (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.*

Forty-one (13.0%) of the samples gave rise to *E. coli* on MacConkey agar + 1 mg/L cefotaxime. These positive results imply the *E. coli* were resistant to cefotaxime. MIC analysis of these 41 isolates found that 39 of the total number of samples tested (12.4%) expressed an ESBL-phenotype resistance (including two isolates additionally expressed the AmpC phenotype resistance). The remaining two of

these 41 *E. coli* isolates (0.63%) expressed an AmpC-phenotype resistance but not ESBL-phenotype resistance.

The observed frequencies of recovery of ESBL-phenotype *E. coli* from samples from individual supermarkets ranged from 0% to and 22.1% of the samples tested per supermarket, including those with an AmpC+ESBL-phenotype.

A total of 54 of all the samples tested, representing 17.1%, gave rise to growth on the ESBL-only specific agar and a total of 3 (0.95%) of all the samples tested were positive for the *mcr-1* transferable colistin resistance gene. These three samples all originated from Poland.

A further two samples were also originally positive for *mcr-3* when multiple suspect colonies was tested. However, it was not possible to isolate individual *mcr-3* positive *E. coli* from the mix, so these results must be considered equivocal.

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.

None of the 41 isolates from the AmpC/ESBL specific agar were microbiologically resistant to the ‘last resort’ carbapenem antimicrobials imipenem and meropenem or to colistin. The MIC of ertapenem against one AmpC+ESBL-phenotype isolate was just above the previous EUCAST ECOFF (currently there is only a tentative ECOFF for ertapenem), and as such was microbiologically resistant. This isolate was not clinically resistant though, using EUCAST clinical breakpoint..

None of the *E. coli* were resistant to the antibiotics temocillin or tigecycline.

Only one isolate was resistant to the antibiotic’s azithromycin or gentamicin, whilst about 60% of isolates were resistant to the quinolone antibiotics (ciprofloxacin or nalidixic acid) or to chloramphenicol.

Isolates obtained from agar with 1 mg/L cefotaxime were all resistant to cefotaxime and to antibiotics of a similar type, such as ampicillin and ceftazidime and most were also resistant to cefepime.

Most of the isolates were resistant to the older antibiotics’ sulfamethoxazole and tetracyclines, and approximately 50% were resistant to trimethoprim.

Genetic tests (whole genome sequencing) showed that most of the *E. coli* isolates from the ESBL agar carried the *bla*$_{CTX-M}$ 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 and 2018 surveys.

None of the meat samples had bacterial counts of background *E. coli* (isolates obtained from agar without antibiotics) or presumptive AmpC/ESBL-producing *E. coli* above the detection limit (when using the EU method) of 3,000 *E. coli* colony forming units (cfu) per gram of meat.

In summary, the results in 2020 showed that 12.4% and 1.6% 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 in both the ESBL or AmpC-phenotype groups) on the AmpC/ESBL specific agar.

Whilst there was an increase in the percentage of isolates with an ESBL-phenotype there was a decrease in the percentage of isolates with an AmpC-phenotype between 2018 and 2020. Overall, between 2018 and 2020, the percentages of samples positive on the AmpC/ESBL specific agar remained almost identical at 13.6% and 13.0%, respectively.

None of the samples were positive for carbapenem-resistant *E. coli* on either of the two carbapenem selective agars.

Between the 2016 and 2018 EU surveys[1] there was a significant reduction in the proportion of chicken samples positive on the AmpC/ESBL specific agar and the ESBL agar. Comparison with a paper on UK samples tested in 2013/14[2] also showed a significant reduction in samples positive for ESBL-producing *E. coli* between 2013/14 and the 2016 EU survey (65.4% to 29.7%), albeit sampling and isolation methods were similar, but not identical for the two studies.

The 2018 report for chicken meat samples[1] suggests that these drops in the level of antimicrobial-resistant *E. coli* on retail chicken meat since 2013/14 may be linked to the restriction by the British Poultry Council to 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.[3]

The 2020 survey results suggest that the proportions of AmpC or ESBL-phenotype *E. coli* in retail chicken have not changed since 2018. There was a slight increase in the proportions of samples that were positive on the ESBL-specific agar compared

to the 2018. This was the first year that retail chicken samples were found to be positive for *mcr* plasmid- mediated colistin resistant *E. coli.*

It should be considered that 2020 was an unusual year due to the impact of the coronavirus pandemic, although there is no reason to suppose this affected the proportions of retail chicken meat positive for AMR *E. coli.*

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 UK retail chicken meat in 2020 for AmpC, ESBL and carbapenem-resistant *E. coli*, following European Union (EU) guidelines and methods. Further testing of retail beef, chicken and pork is being considered based on surveillance priorities.

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 and viable counts pre-enrichment were performed on MacConkey agar containing ± 1 mg/L cefotaxime, according to an EU protocol.

At the request of the FSA (non-harmonised testing outside the remit of Decision 2013/652/EU) samples were also plated to CHROMagar™ ESBL for specific detection of ESBL-producing *E. coli. E. coli* from this agar were tested for the presence and sequence type of $bla_{\text{CTX-M}}$, bla_{OXA}, bla_{SHV} and bla_{TEM} genes by WGS.

Additionally, samples were plated MacConkey agar containing 2 mg/L colistin, for

detection of colistin-resistant *E. coli* that may harbour *mcr-1,2,3* transferable colistin resistance genes.

As in previous survey 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.

In total, 327 samples of fresh chicken meat were collected of which 315 were eligible for testing. The 315 eligible chicken samples were collected across four UK 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 2020 poultry sampling plan used "proportionate stratified sampling" to allocate samples to NUTS-3 (NUTS - Nomenclature des Unités territoriales statistiques) areas and the samples were collected 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. Whilst free range and organic meats were not specifically selected as part of sampling, they were included as part of sampling.

The 315 eligible retail chicken meat samples were collected from England (n=274), Scotland (n=20), Wales (n=11), and Northern Ireland (n=10) from ten different supermarket chains. Sample collection was impacted by the coronavirus pandemic 'lockdown' restrictions, with monthly sampling being suspended for 3 months from April to June 2020, resuming in July. Sample numbers were adjusted in subsequent months to reach the target of 300 samples.

The types of chicken meat collected were whole chicken (n=127), chicken breast (n=113) and other cuts, including quarters, legs, thighs & drumsticks (n=75). Of the samples collected, 58.7% and 41.2% had skin on or off, respectively, and breast samples were the main sample type from which skin was removed. Of the 315 samples, 309 were stated as originating from the UK, five from Poland and one from Ireland.

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 homogenisation of 27 grams of meat in 243 mls Buffered Peptone Water (BPW) yielding 270 mls of BPW:meat homogenate. In line with EFSA guidance as outlined in the APHA internal SOP, the 27 grams of meat was taken as skin if possible. If less than 27 grams of skin was available, then this was supplemented with surface muscle, and surface muscle was used entirely for skinless samples.

From this homogenate, a 20 ml aliquot was taken prior to incubation for viable counts and the remaining 250 mls was incubated at 37 ± 1ºC for 18-22 hours, before plating to the selective agars. The method has the theoretical potential to detect one target (e.g. AmpC/ESBL-producing, *mcr,* carbapenem-resistant) *E. coli* in 25 grams of meat.

Between 6 and 86 samples were tested from the 10 different supermarket chains. Of the 315 samples tested, 41 (13.0%, 95% confidence interval 9.5% to 17.2%) grew on MacConkey agar + 1 mg/L cefotaxime.

Between 2.8% and 23.3% of samples from eight of the ten supermarkets gave rise to *E. coli* on MacConkey agar + 1 mg/L cefotaxime. A total of 18 samples, all of which were taken from the other two supermarkets, were negative.

A total of 54 samples, representing 17.1% of all the samples tested (95% confidence interval 13.1% to 21.8%) of samples tested gave rise to growth of presumptive ESBL-producing *E. coli* on CHROMagar™ ESBL. For these 54 isolates from CHROMagar™ ESBL, most were positive for the *bla*$_{\text{CTX-M}}$ gene and most of the CTX-M sequence types were *bla*$_{\text{CTX-M 55}}$, then *bla*$_{\text{CTX-M 1,}}$ then *bla*$_{\text{CTX-M 27.}}$ Single isolates were positive for *bla*$_{\text{CTX-9}}$ or *bla*$_{\text{CTX-M 14}}$. Two isolates were positive for *bla*$_{\text{SHV-134}}$ and four isolates were only positive for *bla*$_{\text{TEM-1b.}}$

None of the samples were positive on the two carbapenem-containing agars.

A total of 3 out of 315 or 0.95% (95% confidence interval 0.2% to 2.8%) samples that all originated from Poland were positive for the *mcr-1* transferable colistin resistance gene. A further two samples were also originally positive for *mcr-3* when a “sweep” of multiple suspect colonies was tested, but it was not possible to isolate individual *mcr-3* positive *E. coli* from the “sweep” of colonies, despite sub-culture of multiple different colonies from primary culture plates, so these results must be considered equivocal.

None of the samples pre-enrichment gave rise to presumptive AmpC/ESBL *E. coli*-producing counts on MacConkey agar ± 1 mg/L cefotaxime above the detection level of 3,000 cfu/gram of meat, based on the EU method for performing counts. Determination of MICs of isolates to a panel of relevant antimicrobials, coupled with interpreting strains as sensitive or resistant using ECOFFs (as published by EUCAST[4]), 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.

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

By MICs, 39/41 of the isolates from MacConkey agar + 1 mg/L cefotaxime were found to have ESBL-phenotype *E. coli*, including 2 isolates that had an AmpC+ESBL-phenotype, representing 12.4% (95% confidence interval, 9.0% to 16.5%) of all the samples tested..

Also by MICs, 2/41 of the isolates from MacConkey agar + 1 mg/L cefotaxime were found to have AmpC-phenotype *E. coli*, excluding the 2 isolates that also had an ESBL-phenotype, representing 0.63% (95% confidence interval, 0.1% to 2.3%) of all the samples tested.

If including the AmpC+ESBL-phenotype isolates, 1.3% (95% confidence interval, 0.3% to 3.2%) of the samples tested had an AmpC-phenotype *E. coli*. Between 0% and 22.1% of the samples tested per supermarket had an ESBL-phenotype *E. coli*, including those with an AmpC+ESBL-phenotype.

None of the 41 isolates from the AmpC/ESBL-specific agar were microbiologically resistant to the ‘last resort’ carbapenem antimicrobials imipenem and meropenem or to colistin. The MIC of ertapenem against one AmpC+ESBL-phenotype isolate was just above the previous EUCAST ECOFF (currently there is only a tentative ECOFF for ertapenem), and as such was microbiologically resistant. This isolate was not

clinically resistant, if assessed using the EUCAST clinical breakpoint of > 0.5 mg/L to denote resistance.
None of the isolates were resistant to the antibiotics temocillin or tigecycline.
Only one isolate was resistant to azithromycin or gentamicin, whilst about 60% of isolates were resistant to the quinolone antibiotics (ciprofloxacin or nalidixic acid) or to chloramphenicol.
As would be expected, as the isolates were obtained from agar with 1 mg/L cefotaxime, all were resistant to the beta-lactam antimicrobials ampicillin, cefotaxime and ceftazidime and most were also resistant to cefepime. All AmpC or AmpC+ESBL-phenotype isolates were resistant to cefoxitin as resistance to this antibiotic is what defines isolates as AmpC phenotype.
Most of the isolates were resistant to the older antibiotics sulfamethoxazole and tetracycline, and about 50% were resistant to trimethoprim.

In summary, none of the samples were positive for carbapenem-resistant *E. coli* on the two carbapenem-selective agars.
Results for 2020 showed that 12.4% and 1.6% of retail chicken meat samples from MacConkey agar + 1 mg/L cefotaxime were positive for ESBL or AmpC-phenotype *E. coli* respectively (including the three isolates with the combined AmpC+ESBL-phenotype in both the ESBL- or AmpC-phenotype groups).
Whilst there was an increase in the percentage of isolates with an ESBL-phenotype, there was a decrease in the percentage of isolates with an AmpC-phenotype between 2018 and 2020. Overall, between 2018 and 2020, the percentages of samples positive on MacConkey agar + 1 mg/L cefotaxime remained almost identical at 13.6% and 13.0%, respectively.
Using CHROMagar™ ESBL, 54 of all the samples tested representing 17.1% were positive for presumptive ESBL-phenotype *E. coli.* Most of these were positive for *bla*$_{\text{CTX-M}}$. In 2018 the predominate *bla*$_{\text{CTX-M}}$ type was *bla*$_{\text{CTX-M 1}}$, whilst for 2020 samples the *bla*$_{\text{CTX-M}}$ types were more varied, but predominantly *bla*$_{\text{CTX-M 55}}$, then *bla*$_{\text{CTX-M 1}}$ and then *bla*$_{\text{CTX-M 27,}}$ but also *bla*$_{\text{CTX-M 1.}}$
Between the 2016 and 2018 EU surveys[1] there was a significant 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 being confirmed as positive for AmpC or ESBL-phenotype *E. coli* (p-value, < 0.0001 in both cases).

Comparison with a paper on UK samples tested in 2013/14[2] also showed a significant reduction in samples positive for ESBL-producing *E. coli* between 2013/14 and the 2016 EU survey (65.4% to 29.7%), albeit sampling and isolation methods were similar, but not identical for the two studies.

The 2018 report for chicken meat samples[1] suggests that these drops in the level of antimicrobial-resistant *E. coli* on retail chicken meat since 2013/14 may be linked to the British Poultry Council restriction 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.[3]

The results for 2020 suggest that the proportions of AmpC or ESBL-phenotype *E. coli* in retail chicken has not changed since 2018. There was a slight increase in the proportions of the samples positive on CHROMagar™ ESBL compared to the 2018 results and this was the first year that retail chicken samples were found to be positive for *mcr* plasmid- mediated, colistin-resistant *E. coli.*

It should be considered that 2020 was an unusual year due to the impact of the coronavirus pandemic, although there is no reason to suppose this affected the proportions of retail chicken meat positive for AMR *E. coli.*

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

3. Glossary

- **AmpC phenotype** – A phenotype of resistance to cephalosporin antimicrobials such as cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor-β-lactam combinations.
- **AmpC enzyme** – Enzyme conferring AmpC type resistance
- **AMR** – Antimicrobial resistance
- **APHA** – Animal and Plant Health Agency
- **BPW** – Buffered Peptone broth, a liquid media widely used to grow bacteria
- **CRL** – Community Reference Laboratory
- **CTX-M** – 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

4. Materials and Methods

Sampling criteria – Taken from the HallMark report with permission

The 2020 AMR sampling plan was designed by the Food Standards Agency (FSA). It included the collection of 315 chicken samples, comprising three food groups. For Year 6 of the EU Harmonised Survey (chicken), FSA required to keep the sampling plan the same as for 2018, using the Kantar market share data in order to make the results from 2020 more directly comparable to the 2018 results.
As a brief, there are 109 NUTS3 regions which covered 80% of the UK population. The number of samples in each NUTS3 region was proportional to the population size of these 109 regions combined. Kantar's regions codes were used to determine the market share % among these 109 regions.
For example, the first 19 samples were from 7 NUTS3 regions and they all belonged to the same Kantar region (East of England); the retailer (shop) and cuts % from the Kantar East of England data were used. These were all based on the instructions FSA prescribed.
For further details, refer to FSA Proposals for the sampling plan (2018).
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, 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.

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, held at APHA).

These SOPs are:-

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

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

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

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

- **EU method -** Isolation of ESBL, AmpC and carbapenemase producing *E. coli* from fresh meat – Version 7, December 2019.
- **EU method -** Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AmpC-producing *E. coli* in meat and animals – Version 3, November 2017.
- **EU method** – Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli* – Version 2, January 2015.
- **EU method -** Quantification of ESBL/AmpC-producing *E. coli* in caecal content and fresh meat samples – Version 1, December 2017.

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

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

From this 270 ml BPW homogenate, 20 mls was taken for the viable bacterial counts. Viable counts were performed according to the EU protocol with slight variation. This variation was homogenisation of one meat portion per sample in chilled BPW only, compared to one portion for counts in chilled saline and another portion for enrichment in chilled BPW. The full rationale and validation of this variation, which was approved by the FSA and the Danish Technical University (DTU) is outlined in Appendix 1.

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

The remaining 250 mls of BPW homogenate (e.g. 25 grams of meat and 225 mls of BPW as per EU protocols) was incubated aerobically 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 incubated aerobically and 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 were plated again to the agar of origin to ensure purity prior to confirming one of the isolates as *E. coli,* and then storing this isolate pending further tests.
Overall, this method post enrichment in BPW has the theoretical potential to detect one *E. coli* of interest per 25 grams of meat.
From MCA-COL plates, a sweep of ~ 10 to 20 lactose fermenters (based on SOP BAC 0415) was used to prepare a crude DNA sample for detection of *mcr-1, mcr-2* and *mcr-3* plasmid mediated colistin resistance genes by real time PCR. A sweep was taken to increase the sensitivity of detection of the *mcr* genes.

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

Isolates from MCA-CTX agar and if present from CARBA and OXA-48 agars will be stored for up to five years to comply 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 were mixed in the tube, before removing most of the supernatant cryogenic liquid, and then storing the tube at - 70ºC.

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

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

isolates as lactose fermenters. If isolates were lactose fermenters, they were identified as *E. coli* by combined use of oxidase and indole tests as described by in-house SOPs.

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

For the oxidase test, in-brief, a portion of bacterial colony to be tested was taken with a sterile plastic loop and rubbed onto filter paper impregnated with oxidase reagent. A deep purple colour developing within 10 seconds was taken to be “oxidase positive". The indole test was performed in the same way but using filter paper impregnated with James reagent (BioMerieux). Within 10 seconds, a positive reaction was indicated by the presence of a colour change to pink/red. Lactose fermenter colonies from MCA-CTX that grew 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.[5] 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. Following

incubation aerobically at 37^{o}C for 18 hours, the plates were examined, and growth end points established for each antimicrobial to provide MIC's. Microbiologically resistant and susceptible interpretation for the MIC's were obtained by comparison with ECOFF's published by EUCAST based Decision 2013/652/EU.

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

For *E. coli*, the presence of carbapenemase producing strains, Extended Spectrum Beta Lactamase producers (ESBL) or AmpC enzyme producers were determined initially by assessing isolate MIC's against the microbiological breakpoints for meropenem, cefotaxime and ceftazidime.

Any isolates showing meropenem MIC's greater than 0.125mg/l, cefotaxime MIC's greater than 0.25mg/l or ceftazidime MIC's greater than 0.5mg/l were tested against a further panel of antimicrobials. This further panel of antibiotics included cefotaxime, ceftazidime, cefotaxime / clavulanate, ceftazidime / clavulanate, imipenem, ertapenem, temocillin, cefoxitin, cefepime and meropenem.

Consequently, isolates have MICs for all of these confirmatory antimicrobials where an MIC greater than the cut off values stated was observed for any of the screening compounds (cefotaxime, ceftazidime or meropenem) included in the first panel of antimicrobials.

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

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

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

Detection and sequencing of *bla*$_{CTX-M}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$

Presence of *bla*$_{CTX-M}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ from CA-ESBL and subsequent sequencing was performed by Illumina whole genome sequencing (WGS).
Resulting FASTQ files were assembled using "SPAdes - St Petersburg aligner"[6] and analysed using DTU pipelines "ResFinder 4.1."[7]

Plasmid mediated colistin resistance genes *mcr-1, mcr-2* and *mcr-3*

Samples that gave rise to lactose fermenting colonies on MCA-COL were tested for the presence of plasmid-mediated colistin resistance genes *mcr-1, mcr-2* and *mcr-3* by real time (RT) PCR, according to an in-house SOP (BAC0415). To make detection more sensitive, a "sweep" of ~ 10 to 20 colonies was taken to prepare the crude DNA for RT-PCR.
If the initial "sweep" was PCR positive, multiple individual suspect *E. coli* colonies (up to 10 as available) were further examined by PCR for *mcr-1, 2* and *3* genes.
It should be noted that only lactose fermenters with an *E. coli* phenotype were investigated. As such it is possible that *mcr* if detected in the original "sweep," but not in isolated colonies, it could be present in other bacterial genera. This might include non-target lactose fermenters such as *Klebsiella* and *Citrobacter*[8] as well as non-lactose fermenters.
Individual suspected *mcr E. coli* colonies were, at the request of the FSA, subjected to illumina whole genome sequencing (WGS).
Resulting FASTQ files were assembled using "SPAdes - St Petersburg aligner"[6] and analysed using DTU pipelines "MLST,"[9] "SpeciesFinder,"[10] "ResFinder 4.1,"[7] "VirulenceFinder,"[11] and "PlasmidFinder."[12]

5. 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 number of samples planned and collected per NUTS-1 region is shown in Table 1.

Table 1 – Number of samples per NUTS-1 area†

NUTS-1	**Location Name**	**No. samples planned**	**No. samples completed**	**Difference**
UKF/UKH	E. England	19	19	0
UKD	Lancashire	33	32	-1
UKH/UKI	London	65	65	0
UKF/UKG/UKJ/UKK	Midlands	53	53	0
UKC	North East	10	10	0
UKN	Northern Ireland	10	10	0
UKM	Scotland	19	20	1
UKJ/UKK	South	37	37	0
UKK	South West	21	21	0
UKL	Wales+W	11	11	0
UKE/UKF	Yorkshire	37	37	0
Total	**-**	**315**	**315**	**0**

† Sourced from the HallMark report with permission

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

Table 2 – Collected samples* completed per retail chain, per UK region†

Retailer code	England	Wales	Scotland	Northern Ireland	United Kingdom
A	16	0	2	0	18
B	6	0	0	0	6
C	73	4	5	4	86
D	30	1	3	0	34
E	27	2	3	2	34
F	12	0	0	0	12
G	37	1	2	2	42
H	8	0	0	0	8
I	33	1	2	0	36
J	32	2	3	2	39
Total	**274**	**11**	**20**	**10**	**315**

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

† Taken from HallMark report with permission

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

In total, 327 samples of fresh chicken were collected and tested of which 315 were eligible for testing (Table 3).

Only fresh chicken with skin on or off was collected. Samples were collected each month with the exceptions of April, May and June 2020, when sampling was temporarily suspended in view of the COVID-19 pandemic. Samples were from England (n=274), Scotland (n=20), Wales (n=11), and Northern Ireland (n=10). The types of chicken meat collected were whole chicken (n=127, all skin on), chicken breast (n=113, of which 1 was skin on) and other cuts, including quarters, legs,

thighs & drumsticks (n=75, of which 57 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. The stated origin of the chicken samples was UK (n=309), Poland (n=5) and Ireland (n=1).

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

Of the 315 samples tested, between 6 and 86 samples were tested from the 10 different supermarket chains (Tables 4 and 5). Of these 315 samples, 41 (13.0%, 95% confidence interval 9.5% to 17.2%) were positive for *E. coli* on MCA-CTX.. Between 2.8% and 23.3% of samples from eight of the supermarkets gave rise to *E. coli* on MCA-CTX (Table 4). A total of 18 samples, all of which were taken from the other two supermarkets, were negative (Table 4). None of the samples were positive on the two carbapenem agars (Table 3).

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

By MICs (Tables 6 and 7), 39/41 of the *E. coli* from MCA-CTX were found to have an ESBL-phenotype *E. coli* (including 2 isolates that had an AmpC+ESBL-phenotype), representing 12.4% (95% confidence interval, 9.0% to 16.5%) of all the 315 eligible samples tested..

Also, by MICs, 2/41 of the *E. coli* from MCA-CTX were found to have an AmpC-phenotype *E. coli (*excluding the 2 isolates that also had an ESBL-phenotype), representing 0.63% (95% confidence interval, 0.1% to 2.3%) of all the 315 eligible samples tested.

If including the AmpC+ESBL-phenotype isolates, 1.3% (95% confidence interval, 0.3% to 3.2%) of the 315 samples tested had an AmpC-phenotype *E. coli*. Between 0% and 22.1% of the samples tested per supermarket had an ESBL-phenotype *E. coli*, including those with an AmpC+ESBL-phenotype.

None of the 41 *E. coli* from MCA-CTX were microbiologically resistant (when ECOFFs were applied to the MIC results) to the ‘last resort’ carbapenem antimicrobials imipenem and meropenem or to colistin (Tables 6 and 7 and Figure 2).

One AmpC+ESBL-phenotype isolate was just above the EUCAST ECOFF for the carbapenem antimicrobial ertapenem with an MIC of 0.12 mg/L (ECOFF > 0.06

mg/L), and as such was microbiologically resistant. This isolate was not clinically resistant (using EUCAST clinical breakpoint of > 0.5 mg/L to denote resistance). Additionally, none of the isolates were resistant to the antibiotics temocillin or tigecycline.

Only 1/41 of the *E. coli* from MCA-CTX were resistant to azithromycin or gentamicin, whilst about 60% of these *E. coli* were resistant to the quinolone antibiotics (ciprofloxacin or nalidixic acid) or to chloramphenicol (Table 7).

Isolates obtained from agar with 1 mg/L cefotaxime, all were resistant to the beta β-lactam antimicrobials ampicillin, cefotaxime and ceftazidime and most were also resistant to cefepime. All AmpC or AmpC+ESBL-phenotype isolates were resistant to cefoxitin as resistance to this antibiotic is what defines isolates as AmpC phenotype.

Most of the isolates were resistant to the older antibiotics sulfamethoxazole and tetracycline, and about 50% were resistant to trimethoprim.

A summary of 2018 and 2020 MICs results were compared for isolates obtained from MCA-CTX (Table 7 and Figure 2). This showed a decrease in the numbers of AmpC phenotypes isolated for 2020 compared to 2018 and conversely an increase in ESBL phenotypes isolated. Also, of interest was the increase in the numbers of ESBL phenotype isolates resistant to chloramphenicol in 2020 compared to 2018.

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

Using the EU method "*Quantification of ESBL/AmpC-producing Escherichia coli in caecal content and fresh meat samples*" none of the chicken meat samples gave rise to background *E. coli* on MCA (e.g. *E. coli* obtained from media without antibiotics) or to presumptive ESBL/AmpC-producing *E. coli* on MCA-CTX.

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

A total of 54 samples, representing 17.1% (95% confidence interval 13.1% to 21.8%) of samples tested overall, gave rise to growth of presumptive ESBL-producing *E. coli* on CA-ESBL (Table 8). Most of these were positive for *bla*$_{\text{CTX-M}}$. In 2018 the predominate *bla*$_{\text{CTX-M}}$ type was *bla*$_{\text{CTX-M 1}}$, whilst for 2020 samples the *bla*$_{\text{CTX-M}}$ types were more varied. Most of the CTX-M sequence types were *bla*$_{\text{CTX-M}}$

$_{55}$, then *bla*$_{\text{CTX-M 1,}}$ then *bla*$_{\text{CTX-M 27.}}$ Single isolates were positive for *bla*$_{\text{CTX-9}}$ or *bla*$_{\text{CTX-M 14}}$. Two isolates were positive for *bla*$_{\text{SHV-134,}}$ four isolates were only positive for *bla*-$_{\text{TEM-1b}}$ and for some isolates (n=6) no ESBL type genes were detected. The samples that were positive on both CA-ESBL and MCA-CTX are shown in Table 9.

Comparison of samples positive between 2016 and 2020 from MCA-CTX and CA-ESBL

The results for all, skin on and skin off samples positive on MCA-CTX and CA-ESBL for surveys in 2016, 2018 and 2020 can be seen in Figure 1.
It was interesting to note that the proportion of retail chicken samples collected that were skinless has risen from 28.4% of samples in 2016 to 41.3% of samples in 2020.
A reduction in the proportion of samples positive on MCA-CTX can clearly be seen between 2016 and 2018, although this plateaus out between 2018 and 2020. There was a slight increase in the proportion of samples positives on MCA-CTX for skin on samples between 2018 and 2020, but a decrease for skin off samples.
The graph also illustrates the increase in percentage of samples positive on CA-ESBL between 2018 and 2020 overall, particularly for skin on samples, although there was a reduction in the percentage of skin off samples positive.

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

Three of the 315 retail chicken meat samples tested were confirmed as positive for *mcr-1*-carrying *E. coli* (Tables 10, 11, 13, 1 and 15). These three meat samples were obtained from one retail supermarket chain from three different parts of the UK on 5-8-20, 6-10-20 and 8-10-20. The origin of all three samples was stated to be Poland.
For the three samples from which *mcr-1*-positive *E. coli* were recovered, multiple colonies were characterised by PCR, short read WGS and MIC determination, with bacterial identity confirmed by MALDI ToF (Tables 10-14).
In two further chicken retail meat samples where the PCR of the initial “sweep” of ~ 10 to 20 suspect *E. coli* colonies was positive for *mcr-3* (Tables 11 and 13), it was not possible to isolate individual *mcr-3* positive *E. coli* despite sub-culture of multiple different colonies from primary culture plates. These two chicken meat samples were

obtained from a different supermarket chain to the *mcr-1* positive samples and originated in the UK.

Only lactose fermenters with an *E. coli* phenotype were investigated. As such it is possible that the *mcr-3* gene could be present in other bacterial genera. This might include non-target lactose fermenters such as *Klebsiella* and *Citrobacter*[8] as well as non-lactose fermenters.

For sample 2798047 only one of the colonies was *mcr-1*-positive by WGS (Tables 10 and 11), and the other two isolates were identified as *Hafnia alvei. Hafnia alvei* has been described as naturally resistant to colistin and can be readily isolated from mammalian guts and food.[13]

The colony that was *mcr-1* positive was *E. coli* with a predicted serotype O153:H21 and ST162. *E. coli* O153:H21 has previously been described as an EPEC strain,[14], but neither *eae* or shiga toxin genes were detected in this isolate from the WGS data. This isolate was positive for the resistance genes *aadA5, blaTEM-1B, catA1, dhfrA17, qnrB19, mcr-1.1* and *tetB* conferring resistance to older antibiotics such as streptomycin, ampicillin, chloramphenicol, quinolones, colistin and tetracycline respectively.

For sample 563345 one of the colonies was also identified as *Hafnia alvei* and the other two as *E. coli,* but this sample did not give rise to *mcr*-positive single colonies.

One of the *E. coli* colonies (Table 12) was predicted serotype O25 which can be associated with the human pandemic clone O25:H4 ST131.[15] This isolate was *E. coli* O25:H5 and although there were multiple MLST matches, none were ST131 (Table 12).

Samples 2798073 and 2672451 each gave rise to 3 and 4 colonies respectively that were all confirmed as *E. coli* carrying the *mcr-1* gene (Tables 13 and 14). Of these seven isolates, two from each sample were ST744. As such at least four of the seven isolates from these two meat samples were similar, and there was at least some commonality in the resistance genes and plasmids seen in all seven isolates.

MICs of antibiotics against the eight *E. coli* confirmed to be carrying the *mcr-1* gene by WGS are shown in tables 15-18.

All *mcr-1* postive *E. coli* were resistant to colistin, as would be expected, and also resistant to the quinolone antibiotics ciprofloxacin and nalidixic acid (Table 15). Resistance to the quinolone antibiotics correlated with the WGS data as all isolates

had mutations in *gyrA* and all but one of the isolates also had mutations in *parC* (tables 11, 13, 14).

All of the *mcr-1* postive *E. coli* were resistant to ampicillin, but susceptible to cefotaxime, ceftazidime and meropenem (Table 16). Again, this correlates with the WGS data in that all isolates had a bla_{TEM} gene that confers resistance to ampicillin, but none had genes likely to confer resistance to cefotaxime, ceftazidime or meropenem.

Most of the *mcr-1* postive *E. coli* were resistant to the older antibiotics sulfamethoxazole, tetracycline, and trimethoprim (Table 17) and such resistance in general correlated with the presence of *sul, tet* and *dhfr* resistance genes (Tables 11, 13, 14).

Finally, all *mcr-1* postive *E. coli* were sensitive to azithromycin and tigecycline, but all but two were resistant to chloramphenicol (Table 18). All of the *mcr-1* postive *E. coli* resistant to chloramphenicol were positive for the *catA* gene (Tables 11, 13, 14).

At the request of the FSA, further work was performed on the *mcr-1 E. coli* to resolve the plasmids as reported in Appendix 2.

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	4	0 (0)	0	0
Barking & Dagenham and Havering	3	1 (33.3)	0	1
Barnet	1	0 (0)	0	0
Barnsley, Doncaster and Rotherham	5	1 (20)	0	1
Bath & North East Somerset, North Somerset and South Gloucestershire	4	2 (50)	0	2
Berkshire	6	1 (16.7)	0	1
Bexley and Greenwich	3	0 (0)	0	0
Birmingham	7	1 (14.3)	0	1
Bournemouth and Poole	2	0 (0)	0	0
Bradford	3	1 (33.3)	0	1
Brent	3	1 (33.3)	0	1
Brighton and Hove	2	0 (0)	0	0
BRol, City of	3	0 (0)	0	0

Bromley	2	0 (0)	0	0
Buckinghamshire CC	3	0 (0)	0	0
Calderdale and Kirklees	4	0 (0)	0	0
Cambridgeshire CC	4	0 (0)	0	0
Cardiff and Vale of Glamorgan	3	1 (33.3)	0	1
Central Hampshire	3	0 (0)	0	0
Central Valleys	2	0 (0)	0	0
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	1 (33.3)	0	1
Coventry	2	1 (50)	0	1
Croydon	2	0 (0)	0	0
Devon CC	5	0 (0)	0	0
Dorset CC	3	0 (0)	0	0
Dudley	2	0 (0)	0	0
Durham CC	3	1 (33.3)	0	1
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	1 (33.3)	0	1
Enfield	2	0 (0)	0	0
Essex Haven Gateway	3	0 (0)	0	0
Essex Thames Gateway	3	1 (33.3)	0	1
Flintshire and Wrexham	2	0 (0)	0	0
Glasgow City	4	0 (0)	0	0
Gloucestershire	4	0 (0)	0	0
Greater Manchester North East	4	1 (25)	0	1
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	1 (50)	0	1
Hackney and Newham	4	0 (0)	0	0
Haringey and Islington	4	1 (25)	0	1
Harrow and Hillingdon	3	0 (0)	0	0
Heart of Essex	2	0 (0)	0	0
Hertfordshire	7	0 (0)	0	0

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	1	0 (0)	0	0
Kent Thames Gateway	2	1 (50)	0	1
Lambeth	2	0 (0)	0	0
Leeds	5	1 (20)	0	1
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	0 (0)	0	0
Manchester	2	0 (0)	0	0
Merton, Kingston upon Thames and Sutton	3	1 (33.3)	0	1
Mid Kent	2	1 (50)	0	1
Mid Lancashire	1	0 (0)	0	0
North and North East Lincolnshire	2	1 (50)	0	1
North Hampshire	2	0 (0)	0	0
North Lanarkshire	2	1 (50)	0	1
North Northamptonshire	2	1 (50)	0	1
North Nottinghamshire	3	1 (33.3)	0	1

North of Northern Ireland	2	0 (0)	0	0
North Yorkshire CC	4	1 (25)	0	1
Northumberland	2	0 (0)	0	0
Norwich and East Norfolk	2	0 (0)	0	0
Nottingham	2	0 (0)	0	0
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	0 (0)	0	0
Sheffield	3	0 (0)	0	0
Shropshire CC	2	0 (0)	0	0
Somerset	3	0 (0)	0	0
South and West Derbyshire	3	0 (0)	0	0
South Hampshire	3	1 (33.3)	0	1
South Lanarkshire	2	0 (0)	0	0
South Nottinghamshire	2	0 (0)	0	0
South West Wales	2	0 (0)	0	0
Staffordshire CC	5	1 (20)	0	1
Suffolk	4	0 (0)	0	0
Tower Hamlets	1	1 (100)	0	1
Tyneside	5	2 (40)	0	2

Wakefield	2	0 (0)	0	0
Wandsworth	3	0 (0)	0	0
Warwickshire	3	1 (33.3)	0	1
West and South of Northern Ireland	3	0 (0)	0	0
West Essex	2	1 (50)	0	1
West Kent	2	0 (0)	0	0
West Northamptonshire	2	1 (50)	0	1
West Surrey	5	3 (60)	0	3
West Sussex (North East)	2	1 (50)	0	1
West Sussex (South West)	3	0 (0)	0	0
Wiltshire	3	0 (0)	0	0
Wirral	2	1 (50)	0	1
Worcestershire	3	0 (0)	0	0
Total	315	41	0	41

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	18	3 (16.7)	3 (16.7)	0 (0)	0 (0)
B	6	0 (0)	0 (0)	0 (0)	0 (0)
C	86	20 (23.3)	19 (22.1) **	3 (3.5) **	2 (2.3)
D	34	5 (14.7)	5 (14.7)	0 (0)	0 (0)
E	34	1 (2.9)	1 (2.9)	0 (0)	0 (0)
F	12	0 (0)	0 (0)	0 (0)	0 (0)
G	42	2 (4.8)	2 (4.8)	0 (0)	0 (0)
H	8	1 (12.5)	1 (12.5)	0 (0)	0 (0)
I	36	1 (2.8)	1 (2.8)	0 (0)	0 (0)
J	39	8 (20.5)	7 (17.9)	1 (2.6)	0 (0)
Total	**315**	**41**	**39**	**4**	**2**

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

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

Table 5 - Samples positive on for *E. coli* on MCA-CTX with MIC phenotype.

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)	Phenotype from MICs
462539	03/11/2020	C	On	Other cuts	Bath and North East Somerset, North Somerset & South Gloucestershire	ESBL
462541	09/11/2020	C	On	Other cuts	West Surrey	ESBL
462543	09/11/2020	C	On	Whole chicken	West Surrey	ESBL
462597	09/11/2020	E	On	Whole chicken	Oxfordshire	ESBL
462684	03/11/2020	J	On	Other cuts	Edinburgh, City of	ESBL
462700	04/11/2020	C	On	Other cuts	Tower Hamlets	ESBL
462703	04/11/2020	J	On	Other cuts	Barking & Dagenham and Havering	ESBL
462815	04/12/2020	C	Off	Other cuts	Redbridge and Waltham Forest	ESBL
462816	04/12/2020	J	On	Whole chicken	Brent	ESBL
512143	07/12/2020	A	Off	Chicken breast	Tyneside	ESBL
512147	04/12/2020	C	On	Other cuts	Merton, Kingston upon Thames and Sutton	ESBL
540894	04/03/2020	J	On	Whole chicken	Greater Manchester North East	AmpC
560648	05/08/2020	C	On	Whole chicken	North Yorkshire CC	AmpC
560654	06/08/2020	J	On	Whole chicken	Tyneside	ESBL
560848	10/07/2020	G	On	Whole chicken	Kent Thames Gateway	ESBL

560906	10/02/2020	C	On	Whole chicken	West Sussex (North East)	ESBL
560966	13/01/2020	I	On	Other cuts	Coventry	ESBL
560971	08/01/2020	D	On	Whole chicken	North Nottinghamshire	ESBL
563132	07/10/2020	C	On	Other cuts	Gwent Valleys	ESBL
563133	07/10/2020	J	On	Other cuts	Cardiff and Vale of Glamorgan	ESBL
563165	08/09/2020	C	On	Whole chicken	Staffordshire CC	ESBL+Amp C
563320	04/11/2020	A	On	Other cuts	Bradford	ESBL
563384	11/09/2020	C	On	Other cuts	Birmingham	ESBL
563600	10/07/2020	C	On	Whole chicken	Wirral	ESBL+Amp C
2664374	10/02/2020	A	On	Other cuts	Cornwall and Isles of Scilly	ESBL
2664383	08/01/2020	D	Off	Chicken breast	North Lanarkshire	ESBL
2664391	13/01/2020	D	Off	Chicken breast	Warwickshire	ESBL
2664433	10/07/2020	D	Off	Chicken breast	South Hampshire	ESBL
2672451	09/10/2020	H	On	Other cuts	Barnsley, Doncaster and Rotherham	ESBL
2797770	07/12/2020	C	Off	Chicken breast	North Northamptonshire	ESBL
2797771	07/12/2020	C	Off	Chicken breast	West Northamptonshire	ESBL
2797783	04/12/2020	J	Off	Chicken breast	Essex Thames Gateway	ESBL
2797849	04/12/2020	C	Off	Other cuts	West Essex	ESBL
2797865	07/12/2020	J	On	Other cuts	Durham CC	ESBL

2797896	09/11/2020	C	Off	Chicken breast	West Surrey	ESBL
2797898	03/11/2020	C	Off	Chicken breast	Bath and North East Somerset, North Somerset & South Gloucestershire	ESBL
2797973	04/11/2020	C	Off	Chicken breast	Leeds	ESBL
2797979	04/11/2020	C	Off	Other cuts	Haringey and Islington	ESBL
2798033	11/09/2020	C	Off	Chicken breast	Berkshire	ESBL
2798055	10/07/2020	G	On	Other cuts	Mid Kent	ESBL
2978071	05/08/2020	D	Off	Chicken breast	North and North East Lincolnshire	ESBL

Table 6 - MIC results of 19 antimicrobials against all *E. coli* from MacConkey agar + 1 mg/L cefotaxime with AmpC (A) ESBL (E) or AmpC + ESBL (A+E) phenotype. Resistant (R) or Sensitve (S) to differet antimicrobials

Sample Number	Phenotype	AMP	AZI	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ETP	IMP	MEM	GEN	TMC	TET	TGC	SUL	TMP
462539	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
462541	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
462543	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	S
462597	E	R	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	R	S
462684	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
462700	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
462703	E	R	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	R	S
462815	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S
462816	E	R	R	S	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	R
512143	E	R	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	R	R
512147	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
540894	A	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S	R	S	S	S
560648	A	R	S	S	R	R	R	S	R	S	S	S	S	S	S	S	R	S	R	S
560654	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
560848	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S
560906	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
560966	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	R	R
560971	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S
563132	E	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	R	S	S	S
563133	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
563165	A+E	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S
563320	E	R	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	R	S
563384	E	R	S	S	R	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S
563600	A+E	R	S	R	R	R	R	R	R	R	S	R	S	S	S	S	R	S	R	R

Sample Number	Phenotype	AMP	AZI	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ETP	IMP	MEM	GEN	TMC	TET	TGC	SUL	TMP
2664374	E	R	S	R	R	S	R	R	R	R	S	S	S	S	R	S	R	S	R	R
2664383	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	S
2664391	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S
2664433	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
2672451	E	R	S	R	R	S	R	S	R	R	S	S	S	S	S	S	R	S	R	R
2797770	E	R	S	R	R	S	R	R	S	S	S	S	S	S	S	S	R	S	R	R
2797771	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
2797783	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
2797849	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R
2797865	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
2797896	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
2797898	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
2797973	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
2797979	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R

Sample Number	Phenotype	AMP	AZI	FEP	CTX	FOX	CAZ	CHL	CIP	NAL	CST	ETP	IMP	MEM	GEN	TMC	TET	TGC	SUL	TMP
2798033	E	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	S	R	R
2798055	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S
2978071	E	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	R	S

R – Resistant; S – Sensitive.

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

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

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

Table 7 - Summary of resistance phenotypes for *E. coli* from MacConkey agar + 1 mg/L cefotaxime - Comparison between 2018 and 2020 isolates.

Number resistant 2018 in brackets.

Antimicrobial	**ESBL***	**ESBL***	**AmpC****	**AmpC****
Ampicillin	39/39	(26/26)	2/2	(16/16)
Azithromycin	1/39	(0/26)	0/2	(0/16)
Cefepime	37/39	(26/26)	1/2	(13/16)
Cefotaxime	39/39	(26/26)	2/2	(16/16)
Cefoxitin	2/39*	(3/26*)	2/2	(16/16)
Ceftazidime	39/39	(26/26)	2/2	(16/16)
Chloramphenicol	26/39	(2/26)	0/2	(0/16)
Ciprofloxacin	25/39	(13/26)	2/2	(4/16)
Colistin	0/39	(0/26)	0/2	(0/16)
Ertapenem	1/39[b]	(0/26)	0/2	(0/16)
Gentamicin	1/39	(0/26)	0/2	(3/16)
Imipenem	0/39	(0/26)	0 /2	(0/16)
Meropenem	0/39	(0/26)	0/2	(0/16)
Nalidixic Acid	24/39	(12/26)	1/2	(4/16)
Sulfamethoxazole	35/39	(25/26)	1/2	(6/16)
Temocillin	0/39	(0/26)	0/2	(0/16)
Tetracycline	35/39	(22/26)	2/2	(6/16)
Tigecycline	0/39	(0/26)	0/2	(0/16)
Trimethoprim	24/39	(7/26)	0/2	(1/16)

Orange highlight denotes the four different cephalosporin antimicrobials which were tested; Cefepime, Cefotaxime, Cefoxitin and Ceftazidime.

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

a Microbiologically resistant using EUCAST ECOFFS
b One AmpC+ESBL 2020 isolate was microbiologically but not clinically resistant to ertapenem.

* Includes isolates with an AmpC/ESBL-phenotype which show resistance to cefoxitin

** Does not include isolates with an AmpC/ESBL-phenotype

Table 8 - Samples positive for *E. coli* on CHROMagar™ ESBL.

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)	Presence of *bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes from WGS data
462331	08/10/2020	C	On	Whole chicken	Hertfordshire	CTX-M-55
462539	03/11/2020	C	On	Other cuts	Bath and North East Somerset, North Somerset & South Gloucestershire	CTX-M-55
462541	09/11/2020	C	On	Other cuts	West Surrey	CTX-M-55
462543	09/11/2020	C	On	Whole chicken	West Surrey	CTX-M-55
462597	09/11/2020	E	On	Whole chicken	Oxfordshire	None
462684	03/11/2020	J	On	Other cuts	Edinburgh, City of	CTX-M-55
462700	04/11/2020	C	On	Other cuts	Tower Hamlets	TEM-1b
462703	04/11/2020	J	On	Other cuts	Barking & Dagenham and Havering	None
462726	04/12/2020	C	On	Whole chicken	Croydon	CTX-M14, TEM-1b
462815	04/12/2020	C	Off	Other cuts	Redbridge and Waltham Forest	CTX-M-55
512143	07/12/2020	A	Off	Chicken breast	Tyneside	SHV-134
560645	05/08/2020	J	On	Other cuts	East Riding of Yorkshire	CTX-M-55
560648	05/08/2020	C	On	Whole chicken	North Yorkshire CC	CTX-M-55
560654	06/08/2020	J	On	Whole chicken	Tyneside	CTX-M-55

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)	Presence of *bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes from WGS data
560848	10/07/2020	G	On	Whole chicken	Kent Thames Gateway	CTX-M-1
560899	04/02/2020	C	On	Whole chicken	Cheshire East	CTX-M-27
560906	10/02/2020	C	On	Whole chicken	West Sussex (North East)	CTX-M-55
560917	10/07/2020	I	On	Whole chicken	North Hampshire	CTX-M-1
560929	09/07/2020	C	On	Other cuts	Gloucestershire	CTX-M-55
560930	09/07/2020	I	On	Whole chicken	Gloucestershire	CTX-M-1
560966	13/01/2020	I	On	Other cuts	Coventry	TEM-1b
563132	07/10/2020	C	On	Other cuts	Gwent Valleys	None
563133	07/10/2020	J	On	Other cuts	Cardiff and Vale of Glamorgan	CTX-M-55
563165	08/09/2020	C	On	Whole chicken	Staffordshire CC	TEM-1b
563181	11/09/2020	D	On	Whole chicken	Buckinghamshire CC	CTX-M-1
563197	09/10/2020	J	On	Other cuts	Mid Lancashire	CTX-M-55
563320	04/11/2020	A	On	Other cuts	Bradford	None
563384	11/09/2020	C	On	Other cuts	Birmingham	None
563442	05/08/2020	I	On	Other cuts	South and West Derbyshire	None
563444	05/08/2020	J	On	Other cuts	Leicester	CTX-M-55

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)	Presence of *bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes from WGS data
563551	13/07/2020	G	On	Other cuts	Hounslow and Richmond upon Thames	CTX-M-1
563569	04/08/2020	J	On	Whole chicken	East Sussex CC	CTX-M-55
563600	10/07/2020	C	On	Whole chicken	Wirral	CTX-M-55
2664374	10/02/2020	A	On	Other cuts	Cornwall and Isles of Scilly	CTX-M-27
2664383	08/01/2020	D	Off	Chicken breast	North Lanarkshire	TEM-1b
2664391	13/01/2020	D	Off	Chicken breast	Warwickshire	CTX-M-1
2664433	10/07/2020	D	Off	Chicken breast	South Hampshire	CTX-M-27
2672451	09/10/2020	H	On	Other cuts	Barnsley, Doncaster and Rotherham	CTX-M-1
2797770	07/12/2020	C	Off	Chicken breast	North Northamptonshire	SHV-134
2797771	07/12/2020	C	Off	Chicken breast	West Northamptonshire	CTX-M-55
2797783	04/12/2020	J	Off	Chicken breast	Essex Thames Gateway	CTX-M-55
2797849	04/12/2020	C	Off	Other cuts	West Essex	CTX-M-55
2797865	07/12/2020	J	On	Other cuts	Durham CC	ND
2797896	09/11/2020	C	Off	Chicken breast	West Surrey	CTX-M-55
2797898	03/11/2020	C	Off	Chicken breast	Bath and North East Somerset, North Somerset & South Gloucestershire	CTX-M-55

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)	Presence of *bla*$_{CTX}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes from WGS data
2797973	04/11/2020	C	Off	Chicken breast	Leeds	CTX-M-55
2797979	04/11/2020	C	Off	Other cuts	Haringey and Islington	CTX-M-55
2798033	11/09/2020	C	Off	Chicken breast	Berkshire	CTX-M-55
2798034	11/09/2020	C	Off	Chicken breast	Berkshire	CTX-M-55
2798037	11/09/2020	J	Off	Chicken breast	Berkshire	CTX-M-55
2798055	10/07/2020	G	On	Other cuts	Mid Kent	CTX-M-1
2798056	13/07/2020	C	Off	Chicken breast	Ealing	CTX-M-9
2798058	13/07/2020	C	Off	Chicken breast	Hounslow and Richmond upon Thames	CTX-M-1
2798067	10/07/2020	F	Off	Chicken breast	North Hampshire	CTX-M-1, TEM-1d

ND – Not determined

Table 9 - Samples positive for *E. coli* on both MacConkey agar + 1 mg/L cefotaxime and CHROMagar™ ESBL.

Sample number	Date tested	Super-market code	Skin on or off	Food Category	Sampling Location (NUTS3)
462539	03/11/2020	C	On	Other cuts	Bath & North East Somerset, North Somerset and South Gloucestershire
462541	09/11/2020	C	On	Other cuts	West Surrey
462543	09/11/2020	C	On	Whole chicken	West Surrey
462597	09/11/2020	E	On	Whole chicken	Oxfordshire
462684	03/11/2020	J	On	Other cuts	Edinburgh, City of
462700	04/11/2020	C	On	Other cuts	Tower Hamlets
462703	04/11/2020	J	On	Other cuts	Barking & Dagenham and Havering
462815	04/12/2020	C	Off	Other cuts	Redbridge and Waltham Forest
512143	07/12/2020	A	Off	Chicken breast	Tyneside
560648	05/08/2020	C	On	Whole chicken	North Yorkshire CC
560654	06/08/2020	J	On	Whole chicken	Tyneside
560848	10/07/2020	G	On	Whole chicken	Kent Thames Gateway
560906	10/02/2020	C	On	Whole chicken	West Sussex (North East)
560966	13/01/2020	I	On	Other cuts	Coventry
563132	07/10/2020	C	On	Other cuts	Gwent Valleys
563133	07/10/2020	J	On	Other cuts	Cardiff and Vale of Glamorgan

563165	08/09/2020	C	On	Whole chicken	Staffordshire CC
563320	04/11/2020	A	On	Other cuts	Bradford
563384	11/09/2020	C	On	Other cuts	Birmingham
563600	10/07/2020	C	On	Whole chicken	Wirral
2664374	10/02/2020	A	On	Other cuts	Cornwall and Isles of Scilly
2664383	08/01/2020	D	Off	Chicken breast	North Lanarkshire
2664391	13/01/2020	D	Off	Chicken breast	Warwickshire
2664433	10/07/2020	D	Off	Chicken breast	South Hampshire
2672451	09/10/2020	H	On	Other cuts	Barnsley, Doncaster and Rotherham
2797770	07/12/2020	C	Off	Chicken breast	North Northamptonshire
2797771	07/12/2020	C	Off	Chicken breast	West Northamptonshire
2797783	04/12/2020	J	Off	Chicken breast	Essex Thames Gateway
2797849	04/12/2020	C	Off	Other cuts	West Essex
2797865	07/12/2020	J	On	Other cuts	Durham CC
2797896	09/11/2020	C	Off	Chicken breast	West Surrey
2797898	03/11/2020	C	Off	Chicken breast	Bath & North East Somerset, North Somerset and South Gloucestershire
2797973	04/11/2020	C	Off	Chicken breast	Leeds
2797979	04/11/2020	C	Off	Other cuts	Haringey and Islington
2798033	11/09/2020	C	Off	Chicken breast	Berkshire
2798055	10/07/2020	G	On	Other cuts	Mid Kent

Figure 1 - Percentages of chicken meat samples (skin on or off) positive for *E. coli* on MacConkey agar + 1 mg/L cefotaxime and CHROMagar™ ESBL for 2016, 2018 and 2020.

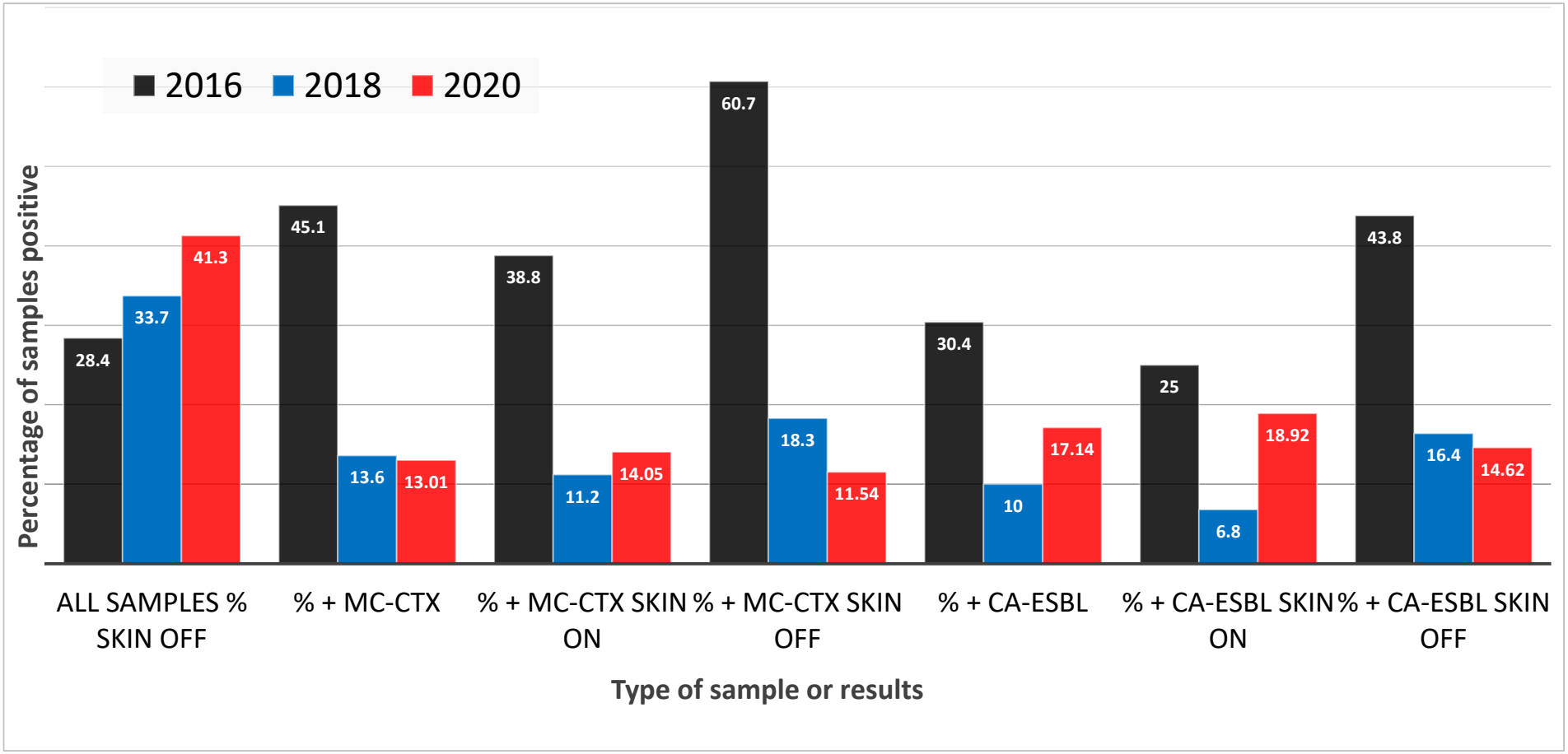
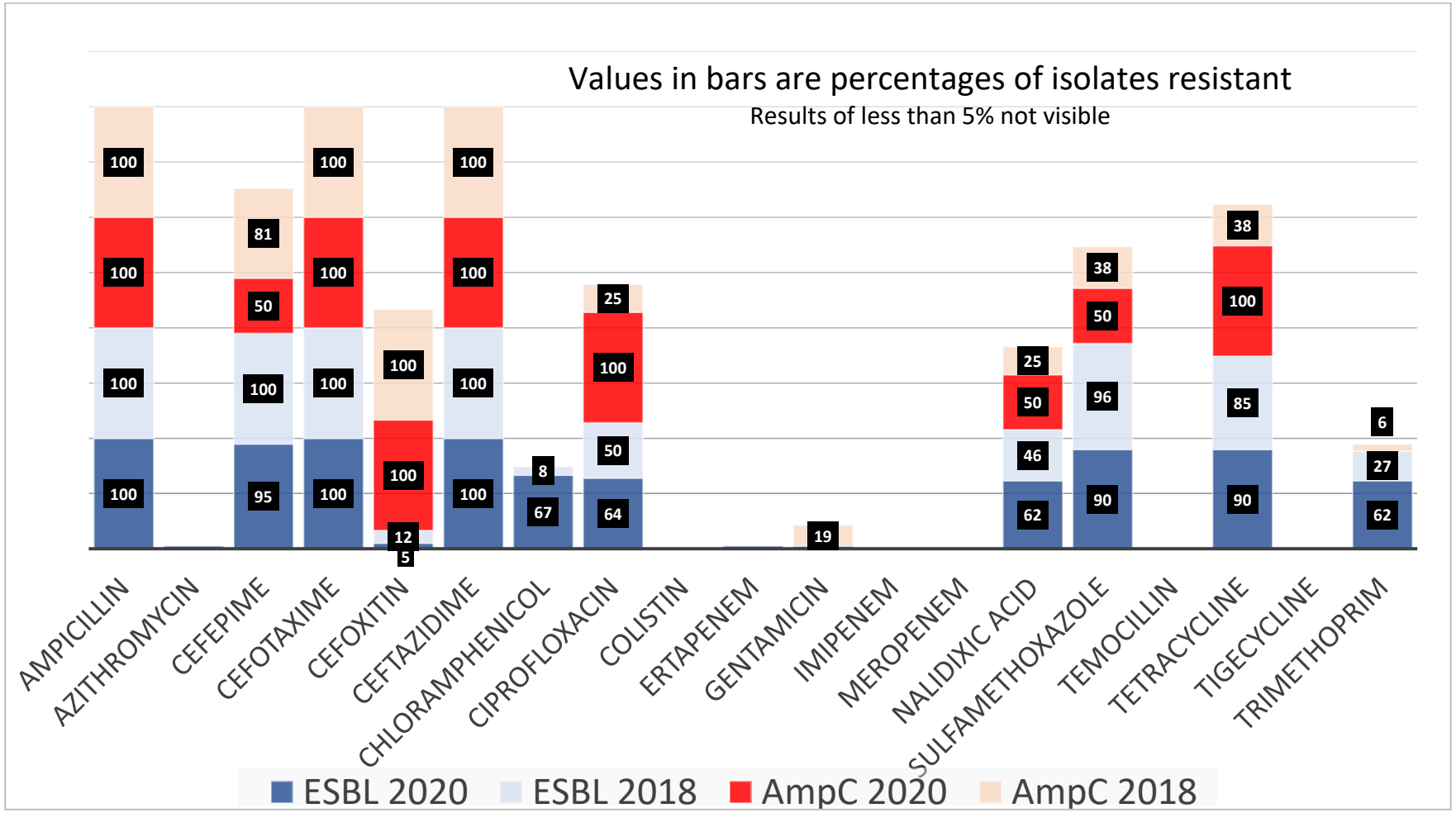


Figure 2 - Breakdown of resistance to antibiotics for AmpC- and ESBL-phenotype *E. coli* from isolated on MacConkey agar + 1 mg/L cefotaxime from retail chicken meat in 2018 and 2020.



Less than 5% but greater than zero – MIC results for azithromycin, ertapenem and gentamicin against 2020 ESBLs.

Table 10 – Details of *mcr* positive meat samples and overall *mcr* results by PCR and WGS.

Meat sample ID Meat type Sampling date	**Stated sources**	**Initial "sweep" RT PCR result**	**Colony Reference [a]**	**Repeat *mcr* RT PCR result on purified colony**	***mcr* by WGS analysis**
2798047 Chicken breast 5-8-20	Supermarket H Lewisham and Southwark Poland	*mcr-1*	**3**	**Negative ***	**Negative ***
		mcr-1	**7**	**Negative ***	**Negative ***
		mcr-1	10	Not tested	*mcr-1*
563345 Whole chicken 3-8-20	Supermarket G Inverclyde, East Renfrewshire and Renfrewshire UK	*mcr-3***	**1**	**Negative ***	**Negative ***
		*mcr-3***	5	Negative *	Negative *
		*mcr-3***	10	Negative *	Negative *
2798073 Chicken breast 6-10-20	Supermarket H Greater Manchester North West Poland	*mcr-1*	a	*mcr-1*	*mcr-1*
		mcr-1	b	*mcr-1*	*mcr-1*
		mcr-1	c	*mcr-1*	*mcr-1*
		mcr-1	d	*mcr-1*	Not tested
		mcr-1	e	*mcr-1*	Not tested
2672451 Other cuts 8-10-20	Supermarket H Barnsley, Doncaster and Rotherham Poland	*mcr-1*	a	*mcr-1*	*mcr-1*
		mcr-1	b	*mcr-1*	*mcr-1*
		mcr-1	c	*mcr-1*	*mcr-1*
		mcr-1	d	*mcr-1*	*mcr-1*

Please note: Red/Bold text – These isolates were confirmed by WGS to be *Hafnia alvei* – see tables 11 and 12. All other isolates were *E. coli.*

a – As available, up to 10 colonies may be selected for further investigation. Only

those with the correct phenotype are subject to further tests such as PCR and WGS. Negative results are also shown in order to provide information on the different colonies of interest that were progressed to repeat PCR and / or WGS.

* Initial “sweeps” positive, but PCR and WGS on sub-cultured bacterial isolates was negative for *mcr* genes.

** A further meat sample (whole chicken) 462641 sampled on 2-11-20 was also initially positive for *mcr-3* based on “sweep” results only. No single *mcr-3*-positive *E. coli* colonies could be isolated from this meat sample.

Table 11 – WGS results for isolates from meat sample 2798047.

Meat sample ID Meat type Sampling date	**Colony replicate (*E. coli* serotype)**	**All AMR genes detected**	**Mutations in QRDRs‡**	**MLST† (% ID)**	**Plasmid type**	**Bacterial ID***
2798047 Chicken breast 5-8-20	3 (NA)	*blaACC-5* *dfrA17* *tet(B)*	ND	ND	Col(Ye4449) IncFIB(AP001918)	*Hafnia alvei*
2798047 Chicken breast 5-8-20	7 (NA)	*aadA5* *blaACC-1c* *dfrA17*	ND	ND	Col(Ye4449) Col(pHAD28 IncFIB(AP001918)	*Hafnia alvei*
2798047 Chicken breast 5-8-20	10 (O153:H21)	*aadA5* *blaTEM-1B* *catA1,* *dhfrA17* *qnrB19* *mcr-1.1*, *tetB*	gyrA	162 (100%)	Col(pHAD28) IncFIB(AP001918) IncFIC(FII) IncHI2 IncHI2A IncI2(Delta) IncX1	*E. coli*

† - MLST compared to DTU *Escherichia coli#1* - Achtman scheme. ND – No match for MLST. * Bacterial ID was determined by MALDI-ToF or from the WGS data. ‡ - QRDR – quinolone resistance determining region. NA – Not applicable.

Table 12 – WGS results for isolates from meat sample 563345.

Meat sample ID Meat type Sampling date	**Colony replicate (*E. coli* serotype)**	**All AMR genes detected**	**Mutations in QRDRs‡**	**MLST† (% ID)**	**Plasmid type**	**Bacterial ID***
563345 Whole chicken 3-8-20	1 (NA)	*blaACC-1a*	ND	ND	Col(MG828) Col(pHAD28) Col440I ColpVC IncFIC(FII)	*Hafnia alvei*
563345 Whole chicken 3-8-20	5 (O?:H4/45)	*aph(6)-Id* *aph(3'')-Ib* *blaACC-1a*	ND	1011 (100%)	Col(BS512) Col(Ye4449) IncFIB(AP001918) IncFIC(FII)	*E. coli*
563345 Whole chicken 3-8-20	10 (O25:H5)	*aadA1* *aph(3'')-Ib* *aph(6)-Id* *blaTEM-1B* *dhfrA1, sul2*	ND	10, 1585, 5720	Col(BS512) ColpVC IncFIB(AP001918) IncFIC(FII) IncI1-I(Gamma) p0111	*E. coli*

† - MLST compared to DTU *Escherichia coli#1* - Achtman scheme. ND – No match for MLST. * Bacterial ID was determined by MALDI-ToF or from the WGS data. ‡ - QRDR – quinolone resistance determining region. NA – Not applicable.

Table 13 – WGS results for isolates from meat sample 2798073.

Meat sample ID Meat type Sampling date	**Colony replicate (*E. coli* serotype)**	**All AMR genes detected**	**Mutations in QRDRs‡**	**MLST† (% ID)**	**Plasmid type**	**Bacterial ID***
2798073 Chicken breast 6-10-20	a (O101:H9)	*aph(3")-la/Ib* *aph(6)-Id* *blaTEM-1B* *catA1* *mcr-1.1*, *sul2* *tetB*	gyrA parC	744 (100%)	IncFIA IncFIB(AP001918) IncFIC(FII) IncQ1 IncX4	*E. coli*
2798073 Chicken breast 6-10-20	B (O5/101:H9/10)	*aadA1* *aph(3")-Ib* *aph(6)-Id* *blaTEM-1B* *catA1,* *dhfrA1/8* *mcr-1.1,* *sul2* *tetA/B*	gyrA parC	93 (100%)	IncFIA IncFIB(AP001918) IncFIC(FII) IncI1-I(Gamma) IncQ1 IncX4	*E. coli*
2798073 Chicken breast 6-10-20	C (O101:H9)	*aadA1* *aph(3")-la/Ib* *aph(6)-Id* *blaTEM-1B* *catA1,* *dhfrA1* *mcr-1.1,* *sul2* *tetB*	gyrA parC	744 (100%)	ColpVC IncFIA IncFIB(AP001918) IncFIC(FII) IncI1-I(Gamma) IncQ1 IncX4	*E. coli*

† - MLST compared to DTU *Escherichia coli#1* - Achtman scheme. ND – No match for MLST. * Bacterial ID was determined by MALDI-ToF or from the WGS data. ‡ - QRDR – quinolone resistance determining region.

Table 14 – WGS results for isolates from meat sample 2672451.

Meat sample ID Meat type Sampling date	**Colony replicate (*E. coli* serotype)**	**All AMR genes detected**	**Mutations in QRDRs‡**	**MLST† (% ID)**	**Plasmid type**	**Bacterial ID***
2672451 Other cuts 8-10-20	A (O?:H27)	*aph(6)-Id* *aadA24* *aph(3")-Ib* *blaTEM-1B* *dhfrA1/14* *lnu(G)* *mcr-1.1*, *sitABCD,* *sul2 tetA*	gyrA parC	7941 (100%)	Col(MG828) IncFIA IncFIB(AP001918) IncFIC(FII) IncI1-I(Gamma) IncX4	*E. coli*
2672451 Other cuts 8-10-20	B (O101:H9/27)	*aadA1* *aph(6)-Id* *aph(3")-Ib* *blaTEM-1B* *catA1* *dhfrA1/14* *mcr-1.1,* *sul2* *tetA/B*	gyrA parC	7941 (100%)	IncFIA IncFIB(AP001918) IncFIC(FII) IncI1-I(Gamma) IncX4	*E. coli*
2672451 Other cuts 8-10-20	C (O101:H9/27)	*aph(6)-Id* *aph(3")-Ib* *blaTEM-1B* *catA1* *dhfrA1/14* *lnu(G)* *mcr-1.1,* *sul2* *tetA/B*	gyrA parC	744 (100%)	IncFIA IncFIB(AP001918) IncFIC(FII) IncI1-I(Gamma) IncX4	*E. coli*
2672451 Other cuts 8-10-20	D (ND)	*aadA1* *blaTEM-1B/102/182* *catA1* *dhfrA1* *lnu(G)* *mcr-1.1* *sitABCD,* *sul2* *tetB*	gyrA parC	744 (100%)	IncFIA IncFIB(AP001918) IncFIC(FII) IncFII(pSE11) IncI1-I(Gamma) IncX4	*E. coli*

† - MLST compared to DTU *Escherichia coli#1* - Achtman scheme. ND – No match for MLST or Not determined. * Bacterial ID was determined by MALDI-ToF or from the WGS data. ‡ - QRDR – quinolone resistance determining region.

Table 15 – MICs of colistin and quinolone antibiotics against *E. coli* carrying *mcr-1.*

Isolate ID	Ant*	MIC	R or S	Ant*	MIC	R or S	Ant*	MIC	R or S
2798047-COL-10	CIP	= 8	R	NAL	> 128	R	COL	= 8	R
2798073-COLa	CIP	> 8	R	NAL	> 128	R	COL	= 4	R
2798073-COLb	CIP	> 8	R	NAL	> 128	R	COL	= 4	R
2798073-COLc	CIP	> 8	R	NAL	> 128	R	COL	= 4	R
2672451-COLa	CIP	> 8	R	NAL	> 128	R	COL	= 4	R
2672451-COLb	CIP	> 8	R	NAL	> 128	R	COL	= 8	R
2672451-COLc	CIP	> 8	R	NAL	> 128	R	COL	= 8	R
2672451-COLd	CIP	> 8	R	NAL	> 128	R	COL	= 4	R

Ant* - Antibiotic

R – Resistant

CIP – Ciprofloxacin, NAL – Nalidixic acid, COL – Colistin.

Microbiologically resistant and susceptible interpretation for the MIC's were obtained by comparison with ECOFF's published by EUCAST.

Table 16 – MICs of beta-lactam antibiotics including meropenem against *E. coli* carrying *mcr-1.*

Isolate ID	**Ant***	**MIC**	**R or S**	**Ant***	**MIC**	**R or S**	**Ant***	**MIC**	**R or S**	**Ant***	**MIC**	**R or S**
2798047-COL-10	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S
2798073-COLa	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S
2798073-COLb	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S
2798073-COLc	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S
2672451-COLa	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S
2672451-COLb	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S
2672451-COLc	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S
2672451-COLd	AMP	> 64	R	CTX	<= 0.25	S	CAZ	<= 0.5	S	MER	<= 0.03	S

Ant* - Antibiotic

R – Resistant, S – Sensitive.

AMP – Ampicillin, CTX – Cefotaxime, CAZ - ceftazidime, MER – Meropenem.

Microbiologically resistant and susceptible interpretation for the MIC's were obtained by comparison with ECOFF's published by EUCAST.

Table 17 – MICs of sulfamethoxazole, tetracycline and trimethoprim against *E. coli* carrying *mcr-1.*

Antibiotic	**Ant***	**MIC**	**R or S**	**Ant***	**MIC**	**R or S**	**Ant***	**MIC**	**R or S**
2798047-COL-10	SUL	<= 8	S	TET	<= 2	S	TRIM	<= 0.25	S
2798073-COLa	SUL	> 1024	R	TET	> 64	R	TRIM	> 32	R
2798073-COLb	SUL	> 1024	R	TET	> 64	R	TRIM	<= 0.25	S
2798073-COLc	SUL	> 1024	R	TET	> 64	R	TRIM	> 32	R
2672451-COLa	SUL	> 1024	R	TET	> 64	R	TRIM	> 32	R
2672451-COLb	SUL	> 1024	R	TET	= 64	R	TRIM	> 32	R
2672451-COLc	SUL	> 1024	R	TET	> 64	R	TRIM	> 32	R
2672451-COLd	SUL	> 1024	R	TET	> 64	R	TRIM	> 32	R

Ant* - Antibiotic

R – Resistant, S – Sensitive.

SUL – sulfamethoxazole, TET – tetracycline, TRIM – trimethoprim.

Microbiologically resistant and susceptible interpretation for the MIC's were obtained by comparison with ECOFF's published by EUCAST.

Table 18 – MICs of azithromycin, chloramphenicol, gentamicin and tigecycline against *E. coli* carrying *mcr-1.*

Isolate ID	Ant*	MIC	R or S	Ant*	MIC	R or S	Ant*	MIC	R or S	Ant*	MIC	R or S
2798047-COL-10	AZI	<= 2	S	CHL	> 128	R	GENT	<= 0.5	S	TIG	<= 0.25	NOINTP
2798073-COLa	AZI	= 8	S	CHL	= 128	R	GENT	<= 0.5	S	TIG	<= 0.25	NOINTP
2798073-COLb	AZI	= 8	S	CHL	= 128	R	GENT	<= 0.5	S	TIG	<= 0.25	NOINTP
2798073-COLc	AZI	= 8	S	CHL	= 128	R	GENT	<= 0.5	S	TIG	<= 0.25	NOINTP
2672451-COLa	AZI	= 8	S	CHL	<= 8	S	GENT	<= 0.5	S	TIG	<= 0.25	NOINTP
2672451-COLb	AZI	= 4	S	CHL	<= 8	S	GENT	<= 0.5	S	TIG	<= 0.25	NOINTP
2672451-COLc	AZI	= 8	S	CHL	= 128	R	GENT	<= 0.5	S	TIG	= 0.5	NOINTP
2672451-COLd	AZI	= 8	S	CHL	> 128	R	GENT	<= 0.5	S	TIG	<= 0.25	NOINTP

Ant* – Antibiotic

R – Resistant, S – Sensitive.

R – Resistant, S – Sensitive. NOINT – no interpretation for resistant or sensitive.

AZI – azithromycin, CHL – Chloramphenicol, GENT – gentamycin, TIG – tigecycline.

Microbiologically resistant and susceptible interpretation for the MIC's were obtained by comparison with ECOFF's published by EUCAST.

6. Discussion

Comparisons with previous years

The results for the 2016 EU/FSA survey for presumptive ESBL- and/or AmpC-producing *E. coli* from retail chicken meat showed a significant decrease in the proportion of samples positive for ESBL-producing *E. coli* compared to a previous (2013/14) UK study. It should be noted however, that the sampling strategies and isolation methods were similar, but not identical between these two studies.[2, 16] A major finding for the testing of the 2018[1] chicken meat samples was a further significant reduction in the proportion of samples positive for presumptive ESBL- and/or AmpC-producing *E. coli* compared with the 2016 survey,[16] which did use identical methods. These results were published in 2020 in a paper entitled "*A decline in the occurrence of extended-spectrum β-lactamase-producing Escherichia coli in retail chicken meat in the UK between 2013 and 2018.*"[17] It was considered that *"significant reductions in antimicrobials used in the UK poultry meat sector between 2012 and 2016 may be linked to significant reductions in AmpC/ESBL-phenotype E. coli in retail chicken between 2013/14 and 2018*".[17]

Of interest, the EFSA report for 2017/18 samples also showed a reduction in the prevalence of presumptive ESBL- and/or AmpC-producing *E. coli* in broiler meat from several MS between 2016 and 2018.[18] Overall EFSA reported that the prevalence of presumptive ESBL- and/or AmpC-producing *E. coli* in meat from broilers in 2018 was 39.8%, which is markedly lower compared to 57.4% in 2016.[18]

UK results from 2020 suggest that the proportions of retail chicken samples positive for presumptive ESBL- and/or AmpC-producing *E. coli* have not changed since 2018, since in 2018/20 13.6% and 13.0% of samples respectively were positive for ESBL and/or AmpC-producing *E. coli* on MCA-CTX. The drop from 65.4% in the 2013/14 study to 29.7% in the 2016 study being positive for ESBL-producing *E. coli* was statistically significant, as mention previously.

Examining the results in more detail, for 2020 (compared to 2016/2018 surveys), 12.4% (29.7%/8.4%) and 1.6% (16.3%/6.1%) of retail chicken meat samples were positive for presumptive ESBL- or AmpC-phenotype *E. coli* respectively (including the three isolates with the combined AmpC+ESBL-phenotype in both the ESBL or AmpC-phenotype groups) on MCA-CTX.

Between 2018 and 2020 on MCA-CTX the proportions of samples positive have remained almost identical, although there has been an increase in the percentage of isolates with an ESBL-phenotype, and a decrease in the percentage with an AmpC-phenotype. This was also illustrated by the use of CA-ESBL which selects more specifically for ESBL-phenotype *E. coli.* In 2020, 54 samples representing 17.1% of samples were positive for ESBL-phenotype *E. coli*, compared to 30.4% positive in 2016 and 10% positive in 2018. An increase between 2018 and 2020 from 10% to 17.1% positive for ESBL-phenotype *E. coli* on CA-ESBL.

There was also an observed change in the types of CTX-M genes isolated in *E. coli* from CA-ESBL. In 2018 the predominate *bla*$_{CTX-M}$ type for *E. coli* from CA-ESBL was *bla*$_{CTX-M\ 1}$, whilst for 2020 samples the bla$_{CTX-M}$ types were more varied. Most of the CTX-M sequence types were *bla*$_{CTX-M\ 55}$, then *bla*$_{CTX-M\ 1,}$ then *bla*$_{CTX-M\ 27.}$ Single isolates were positive for *bla*$_{CTX-9}$ or *bla*$_{CTX-M\ 14}$. Two isolates were positive for *bla*$_{SHV-134,}$ four isolates were only positive for *bla*$_{TEM-1b}$ and some isolate (n=6) were not positive for any specific ESBL gene. *bla*$_{SHV-134}$ was first described in 2012 in *Klebsiella pneumoniae* from a patient in Spain.[19] *bla*$_{SHV-134}$ has also recently been described in a paper reviewing SHV ESBLs, where it has been reported to be an ESBL type enzyme detected in *Klebsiella pneumoniae* and associated with an IS26 mobile genetic element and plasmid borne.[20]

Resistance to last resort antibiotics

As in previous years, none of the samples gave rise to isolates on the two agars that selected for carbapenem- resistant *E. coli,* suggesting that in the UK retail chicken meat samples are not contaminated with carbapenem resistant *E. coli.*

With respect to the last resort antibiotic colistin though, in China, there was reported to be a rapid rise of the ESBL and *mcr-1* genes in *E. coli* of chicken origin between 2008 and 2014.[21] In a recent study in the Netherlands, *mcr-1* was detected from 24.8% of 214 retail chicken meat samples.[22] *E. coli* carrying the plasmid-mediated colistin resistance gene *mcr-1* have also been reported from retail chicken in other countries such as South Korea[23] and Latin America.[24] A German study examining over 10,600 *E. coli* isolates from the national monitoring on zoonotic agents from the years 2010–2015 for phenotypic colistin resistance found that the highest prevalence of *mcr-1* was detected in the turkey food chain (10.7%), followed by broilers (5.6%).[25] In a 2019 study of retail meat, bacteria of the Enterobacteriaceae

family carrying the *mcr-1* gene were detected in 21% (18/86) of the examined samples, especially in turkey meat and liver (16/24 positive for *mcr-1* or 66.7%) originating from EU and non-EU countries.[8]

In this 2020 UK survey, 3/315 retail chicken samples were found to be positive for *mcr-1*-bearing *E. coli.* To our knowledge this is the first detection of *mcr-1-positive E. coli* in retail chicken meat in the UK. All three chicken products came from Poland. Eight *mcr-1* positive *E. coli* isolated from the three positive meat samples were characterised by WGS.

The authors of the Chinese study suggested that *mcr-1* emerged and rose under the heavy selective pressure of antimicrobial usage in the animal husbandry in the last decade.[21] Of note is that the UK poultry meat sector stopped using polymixins (colistin) in 2016.[3]

Performing MICs against the *mcr-1* positive isolates confirmed that all were colistin resistant, but none were resistant to cefotaxime. Whilst the Chinese study reported the co-existence of CTX-M genes and *mcr-1*, WGS did not detect any ESBL genes in the eight *mcr-1* positive isolates detected in the UK survey and this correlated with the sensitivity to cefotaxime. Other MIC results correlated well with the WGS results.

Further context of whole genome sequencing studies

Apart from resistance genes, WGS was able to identify the ST, plasmids and give a predicted serotype. This information allowed for some comparison between these eight *mcr-1-positive* isolates and *mcr-1-positive* isolates from poultry meat in other countries.

Four of the isolates from two of the meat samples were positive for ST744 *mcr-1 E. coli* and ST744 *mcr-1 E. coli* has previously been detected in turkey meat from Brazil, Germany and Poland,[8] which could possible suggest some shared origins of such *E. coli*, but further work such as comparative analysis of core genomes would be needed to elucidate this further.

A further two isolates from two of the meat samples were positive for ST93 or ST162 *mcr-1 E. coli.* These STs have both been detected in turkey meat from Poland and also from Germany for ST162.[8]

A Swiss study that analysed *mcr-1 E. coli* from poultry and turkey meat and from humans revealed that each of the six isolates tested in their study had a distinct ST, suggesting a high degree of clonal diversity amongst these isolates of different origins.[26] In the Swiss study the *mcr-1* plasmids were transferred by transformation and transferrable *IncI2* and *IncX4 mcr-1* plasmids were found associated with human and food isolates. The authors suggested that “epidemic” plasmids rather than specific *E. coli* clones might be responsible for the spread of the *mcr-1* gene along the food chain.[26]

For the *mcr-1 E. coli* in the UK survey, WGS results showed seven of the isolates from two of the samples harboured *IncX4* plasmids and the remaining sample was positive for an *mcr-1 E. coli* harbouring an *IncI2(Delta)* plasmid. These plasmids were not verified as encoding the *mcr-1* gene in the eight positive isolates.

Traceback of *mcr-1* positive samples

All the *mcr-1* positive samples had their stated country of origin as being Poland. FSA trace back investigations found that the three chicken meat samples positive for *mcr*-1 *E. coli* originated from two approved premises in Poland. The products were distributed straight from Poland to the UK retail market, with no additional cutting/processing or repackaging of product in the UK. It is therefore likely that the contamination occurred in Poland rather than in the UK. Additionally, the UK retailer followed up with the Polish supplier regarding the use of colistin and confirmed that colistin was used on the flock of chickens under investigation.

Samples initially positive for *mcr-3*

The lack of confirmation of the *mcr-3* status of a further five meat samples from purified *E. coli* may be because other lactose fermenters such as *Klebsiella* and *Citrobacter*[8] or non-lactose fermenters carried *mcr-3*. The current APHA protocol used to detect *mcr* genes focuses on *E. coli.*

Another explanation may be that *mcr-3* positive *E. coli* were outnumbered by isolates that were chromosomally resistant to colistin. Previous studies have detected chromosome-mediated *mcr-3* variants in *Aeromonas veronii* from chicken meat.[27] As *Aeromonas* are non-lactose fermenters, if such isolates that were *mcr-3*

positive were contaminating the *mcr-3* positive samples in this study, they would have only been detected in the initial “sweep” of multiple colonies, and not when lactose fermenters were purified and subsequently tested.

MIC results

MIC results of *E. coll* from MCA-CTX were in general similar to results from the 2018 study, when *E. coli* were recovered from this agar.

None of the 41 isolates from the AmpC/ESBL specific agar were microbiologically resistant to the ‘last resort’ carbapenem antimicrobials imipenem and meropenem or to colistin. The MIC of ertapenem against one AmpC+ESBL-phenotype isolate was just above the previous EUCAST ECOFF (currently there is only a tentative ECOFF for ertapenem), and as such was microbiologically resistant. This isolate was not clinically resistant though, using EUCAST clinical breakpoint of > 0.5 mg/L to denote resistance.

Studies have shown that ertapenem resistance can arise in *E. coli* in the absence of carbapenem resistance genes. This can be due to factors such as overproduction of AmpC β-lactamase and decreased expression of outer membrane porins such as OmpC/OmpF[28] and also for CTX-M positive isolates that have reduction of outer membrane proteins.[29]

There was a decrease in the numbers of presumptive AmpC-phenotype *E. coli* isolated in 2020 compared to the numbers detected in 2018. Conversely there was an increase in presumptive ESBL-phenotype *E. coli* isolated between 2018 and 2020. EFSA report that in animal populations/food matrices, isolates with a presumptive ESBL-phenotype were more common than isolates with a presumptive AmpC-phenotype in the majority of the countries, although the occurrence of the different phenotypes did vary considerably among the Member States (MSs) and in some countries the AmpC-phenotype dominated.[18]

Comparison with results from other countries

In 2018, EFSA report that AMR monitoring of broiler meat was performed by 28 MSs and four non-MSs.[18] As in 2016 there was marked variations between MSs for

the prevalence of samples positive for presumptive ESBL and/or AmpC-producing *E. coli*.[18] Overall EFSA reported that the prevalence of presumptive ESBL- and/or AmpC-producing *E. coli* in meat from broilers from all participating countries in 2018 was 39.8%, compared to 13.6% UK retail chicken samples positive in 2018.[18]

7. Conclusions

- The proportion of retail chicken samples positive for presumptive ESBL-producing and AmpC-producing *E. coli* using the EU recommended agar MCA-CTX was similar to 2018 results at 13.0%. This compares to 45.1% of chicken meat samples being positive on this agar in 2016. There was a slight increase in the proportions of samples positive for presumptive ESBL-producing *E. coli* using the ESBL specific agar CA-ESBL.

- None of the UK retail chicken samples tested in 2020 were positive for *E. coli* on the two carbapenemase agars, although one presumptive AmpC+ESBL-phenotype isolate from MCA-CTX agar was microbiologically resistant, but clinically sensitive to the carbapenem antimicrobials ertapenem. It is possible that such resistance might be attributed to porin loss[29] or increased expression of AmpC,[28] although this was not investigated.

- Three (0.95%) of the UK retail chicken samples tested in 2020 were positive for plasmid-mediated colistin resistance encoded for by *mcr-1.* This is the first time the authors are aware that *mcr-1 E. coli* have been detected from retail chicken meat in the UK. The country of origin of these *mcr-1*- positive chicken samples was Poland. These isolates showed similarity (based on ST and plasmids) to *mcr-1 E. coli* isolated from poultry meat in Europe.

- Long read sequencing showed the *mcr-1* gene in *E. coli* from two samples were present on an Inc-X4 plasmid and highly conserved. These two plasmids also showed high sequence identity with the *mcr-1* IncX-4 bearing plasmid from pig faeces (RB5) that has been reported previously from the UK. There was however little similarity in *E. coli* from the third chicken meat sample with other *mcr-1* plasmids.

- The predominant CTX M types recovered from retail chicken meat (mainly CTX-M-1 and CTX-M-55) differ to those causing mainly human disease in the UK, since the major type causing disease in humans is the pandemic O25-ST131 CTX-M-15-producing clone.[30] This suggests, as stated in previous years and by a Public Health England publication,[30] that chicken meat is not a major source of ESBLs in humans,

since none of the samples were positive for CTX-M-15. There was an increase in samples positive for CTX-M-55 rather than CTX-M-1 *E. coli* compared to previous years.

- Using the EU method with a detection limit of 3,000 cfu/g, none of the retail chicken meat samples gave rise to counts of background *E. coli* on MCA or to presumptive ESBL/AmpC-producing *E. coli* on MCA-CTX media.

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

8. Appendix 1 - Viable counts of *E. coli* from chicken meat samples homogenised in Buffered Peptone water (BPW) or saline.

Aim

The EU method for performing viable bacterial counts states to homogenise meat samples in chilled saline. To comply with this method would require two lots of homogenisations for each meat sample tested. One sample in saline for the *E. coli* counts, and one sample in BPW for enrichment prior to plating on selective agars. This not only increases work, but also means that two different aliquots per sample would be processed potentially giving different results for counts and post enrichment, since two different aliquots of the original chicken sample would be tested.

With the agreement of the DTU and the FSA, it was agreed to compare different methods of homogenisation (in chilled BPW or chilled saline) of chicken meat samples for recovery of *E. coli* to determine if counts were comparable using both methods of homogenisation.

Comparison was performed using routine chicken meat samples, and spiked chicken meat samples.

Caveat

Low spike levels of chicken meat were chosen to be representative of low levels of organisms in actual meat samples.

The EU method states that “To ensure an accurate estimate of the number of *E. coli* cells (both ESBL/AmpC and total *E. coli*) in the original sample, plates with > 300 colonies and < 30 colonies should not be used for CFU counts” [31].

The APHA limit of detection of 100 cfu/gram and not the EU detection limit of 3,000 cfu/gram is given in this report.

It should be considered that at the lower two spike levels the colonies were less than 30 per agar plate and as such would be below the EU detection limit. These low counts tend to lead to the variation seen between replicates.

Conclusions

Performing viable bacteria counts on both test and spiked retail meat samples following homogenisation in saline and BPW, provided very similar results.

All 51 routine test meat samples were found to containcounts below the detection limit of 3,000 cfu/gram (EU method), regardless of homogenisation method.

As such it is considered appropriate to homogenise meat samples in chilled BPW only, and not chilled BPW and additionally chilled saline.

Abstract

For a total of routine 51 meat samples, although there was limited variation in viable bacterial counts following homogenisation in saline and BPW, the variation seen was only between zero, one, two or three detected colonies on agars. Such a level of variation would likely be seen when plating technical replicates.

As most of these routine retail meat samples were negative (below the detection limit of 100 cfu/gram) for *E. coli,* an experiment where meats were spiked with ESBL-producing *E. coli* was also performed.

Spike levels were chosen to represent just below, about 5x and about 50x the APHA limit of detection. It should be considered that no data were available as to the percentages of isolates that would be recovered from such spiking experiments, as it would not be 100%. In all, 120 spiked samples were tested, with 5 replicates per spike condition per experiment. Two experiments were performed.

At the lowest spike level just below the limit of detection, *E. coli* were not detected following homogenisation in saline for five replicates, but was detected following homogenisation in BPW, but only one colony was detected per sample, and thus very close to no detection as for homogenisation in saline.

At the mid-spike level, which was five times the limit of detection, mean results for the five replicates in duplicate experiments were very similar (about two logs) on MacConkey agar following homogenisation in saline or BPW.

At the mid-spike level, mean results for the five replicates in duplicate experiments were lower on MacConkey + 1 mg/L cefotaxime agar than MacConkey agar alone, following homogenisation in saline and BPW. These lower counts may represent a recovery issue of the spike strain on antibiotic-containing MacConkey + 1 mg/L cefotaxime agar.

On MacConkey + 1 mg/L cefotaxime agar mean results for the five replicates in duplicate experiments were very similar following homogenisation in saline and BPW, in particular when the variation between replicates is considered.

As for other spike levels, mean results at the higher spike level of ~ 5000 cfu/gram were very similar following homogenisation in saline and BPW, in view of the standard deviations of replicates.

Method

Routine samples over a two month period were homogenised either in chilled saline (25 grams + 225 mls) or BPW (27 grams + 243 mls), prior to performing viable counts on MacConkey agar and MacConkey agar + 1 mg/L cefotaxime, as per EU protocols.

As numbers of *E. coli* from routine samples are often below the detection limit, retail chicken samples were also spiked with ESBL-producing *E. coli.*

For meat spiking, sufficient quantities of diced chicken (two different experiments, each with a different batch of meat) were purchased from a retail store. Samples were weighed out into 15 x 27 gram portion (for homogenisation with 243 mls of BPW) and 15 x 25 gram portions (for homogenisation with 225 mls of saline).

Five replicate 25 gram and five replicate 27 gram portions of meat were spiked with two different levels of ESBL-producing *E. coli* per experiment, prior to homogenisation and counts as above.

Results and discussion

Routine meats

Fifty-one routine meat samples were tested for viable counts following homogenisation in saline and BPW.

Most of the counts for there 51 routine samples were below the detection limit, including all counts on MacConkey agar + 1 mg/L cefotaxime.

A total of three of the 51 routine meat samples homogenised in BPW gave rise to counts of 100 to 200 cfu/gram on MacConkey agar. It should be considered this represents only one or two colonies and as such only just above the minimum detectable number of colonies.

Six meat samples homogenised in saline gave rise to counts of 100 to 300 cfu/gram on MacConkey agar. It should be considered this represents only one to three colonies.

The results suggest equivalence between homogenisation in saline or BPW for test retail meat samples.

Spiked meat samples

Retail diced chicken was spiked at ~ 50 cfu/gram (just below the limit of detection), ~ 500 cfu/gram in duplicate experiments (close to limit of detection) and ~ 5000 cfu/gram with an ESBL-producing *E. coli* (Table 1).

On MacConkey agar, in view of the standard deviation between replicates, mean results were very similar for samples homogenised in saline or BWP. Additionally, mean counts were within one log of the spike, although generally below the spike level, which may represent some binding of the spike bacteria to the chicken meat.

Counts on MacConkey agar + 1 mg/L cefotaxime were below those on MacConkey agar despite the spike strain being an ESBL-producing *E. coli.* This may represent a recovery problem for the isolate plated direct to antibiotic containing medium without enrichment. For this agar, taking into account the standard deviation between replicates, counts were similar following homogenisation of meats in saline or BPW.

The results suggest equivalence between homogenisation in saline or BPW for test spiked retail meat samples, bearing in mind variation between replicates that may represent different levels of binding of spike strain to meat samples.

Table 1. Viable counts of *E. coli* from spiked chicken meat samples homogenised in saline or BPW – Results on McConkey agar**.**

Approximate **Spike level cfu/gram (Experiment number**	**Mean (standard deviation) of cfu/gram on different agar for replicate (n=5) spiked chicken meat : Saline**	**Mean (standard deviation) of cfu/gram on different agar for replicate (n=5) spiked chicken meat : BPW**
50 (1)	0 (0)	100* (0)
500 (1)	260 (134)	480 (238)
500 (2)	180 (303)	220 (204)
5000 (2)	2040 (862)	1040 (611)

Limit of detection is 100 cfu/gram: This detection limit increases to 3,000 cfu/gram if at least 30 colonies need to be counted as per EU method.[31]

* For the purpose of this study any colonies observed were counted, so 100 cfu/gram represents one colony only on an agar plate.

Table 2. Viable counts of *E. coli* from spiked chicken meat samples homogenised in saline or BPW – Results on McConkey CTX agar.

Approximate **Spike level cfu/gram (Experiment number)**	**Mean (standard deviation) of cfu/gram on different agar for replicate (n=5) spiked chicken meat: Saline**	**Mean (standard deviation) of cfu/gram on different agar for replicate (n=5) spiked chicken meat: BPW**
50 (1)	0 (0)	0 (0)
500 (1)	0 (0)	300 (212)
500 (2)	20 (45)	0 (0)
5000 (2)	340 (168)	460 (780)

Limit of detection is 100 cfu/gram: This detection limit increases to 3,000 cfu/gram if at least 30 colonies need to be counted as per EU method.[31]

* For the purpose of this study any colonies seen were counted, so 100 cfu/gram represents one colony only on an agar plate.

9. Appendix 2 – Further molecular characterisation of *mcr-1* plasmids

Background

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

Results and discussion

Long read sequencing was performed on selected isolates from all three of the *mcr-1* positive samples; hybrid assemblies produced from long- and short-read data was used to resolve the *mcr* bearing plasmid genome for further characterisation.

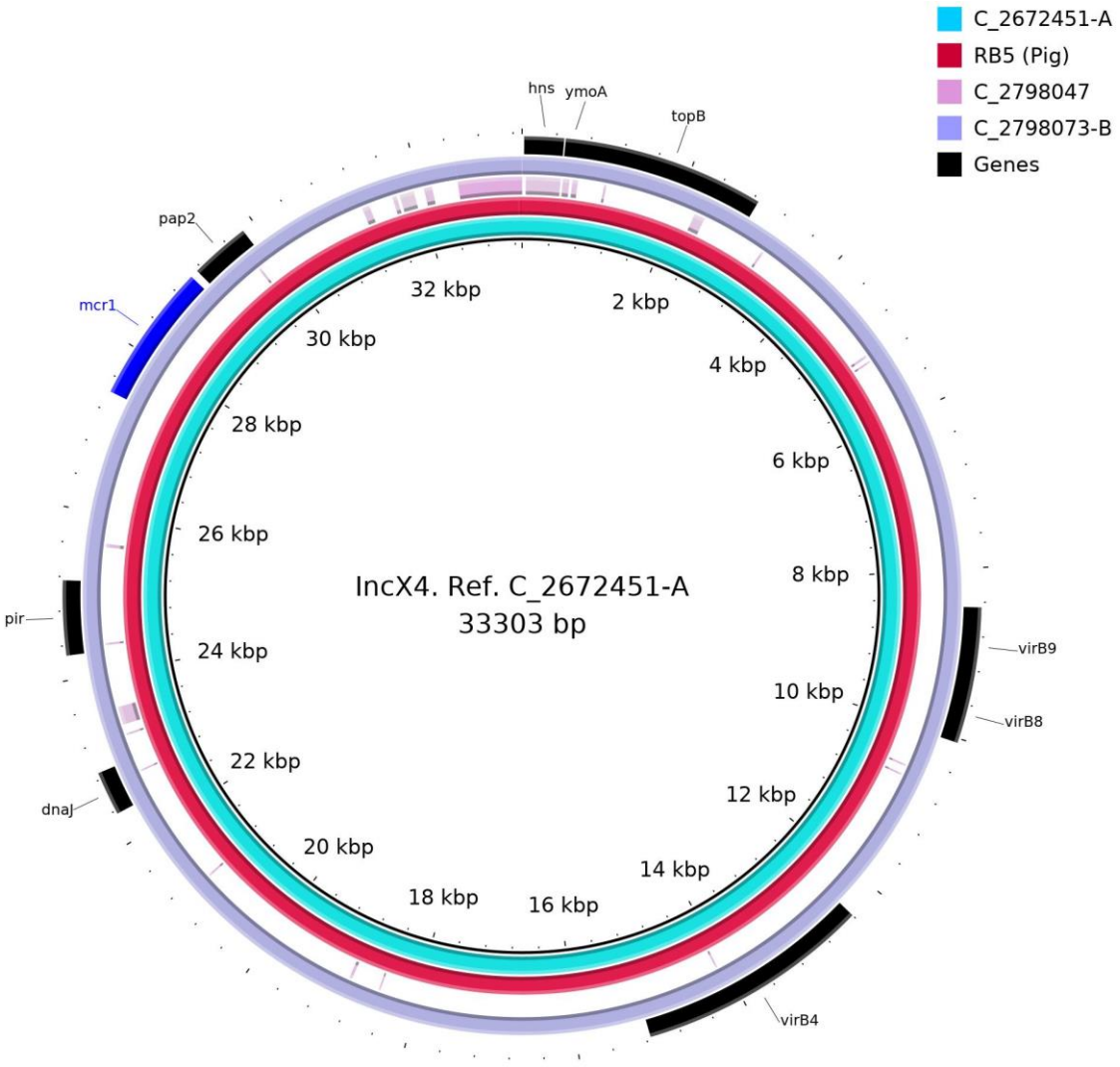
The results showed the *mcr-1* gene in *E. coli* from two samples (C_2672451 and C_2798073) were present on an Inc-X4 plasmid. Comparison of the resolved *mcr-1* Inc-X4 plasmid genomes indicated both to be highly conserved within *E. coli* isolated from these chicken meat samples (Figure 1). It also showed high sequence identity with the *mcr-1* IncX-4 bearing plasmid from pig faeces (RB5) that has been reported previously from the UK.[32] However, we noted little similarity in *E. coli* from the third chicken meat sample (C_2798047) with other *mcr-1* plasmids. The WGS data from this isolate was run through the APHA SeqFinder AMR pipeline to investigate this discrepancy. It showed that the *mcr-1* gene was present at very low copy number in this isolate, indicating the *mcr-1* plasmid was possibly unstable within the host *E. coli*. Further work is required in future to understand why the *mcr-1* IncX4 plasmid was unstable in this *E. coli* host background but not others.

Conclusions

In a recent study in the Netherlands, *mcr-1* was detected from 24.8% of 214 retail chicken meat samples, and the presence of *Enterobacteriaceae* carrying *mcr1* was confirmed from 34 of the positives.[22] *E. coli* carrying the plasmid mediated colistin resistance gene have also been reported from retail chicken in other countries such as South Korea[23] and Latin America.[24]

However, this is to our knowledge the first reported occurrence of *mcr E. coli* from retail chicken meat in the UK. The results from our study indicates that the *mcr-1* gene is carried in an Inc-X4 plasmid which was identical to that found in previously reported *E. coli* isolated from pigs in the UK. We are not clear why the *mcr- 1* Inc-X4 plasmid was unstable in one *E. coli* isolated but can speculate that it may be due to the genetic background of the host. Phylogenetic analysis performed in future may provide insight as to whether particular *E. coli* lineages are more likely to harbour the *mcr-1* plasmids more stably due to some fitness attribute associated with them.

Figure 1 - Comparison of the resolved genome of a *mcr-1* Inc-X4 plasmid from an *E. coli* isolated from a chicken meat sample with other *mcr-1 E. coli*.



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