FINAL REPORT

FS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) *in E. coli* Bacteria from Retail Meats (Year 5 - Beef and Pork, 2019)

10th August 2020

Animal and Plant Health Agency (APHA) Woodham Lane New Haw Surrey KT15 3NB





Table of contents

1.	Liability statement4					
2.	Lay person's summary					
3.	Project summary	8				
4.	Glossary1					
5.	Aaterials and Methods1	4				
55	.1 Sampling criteria - taken from the HallMark report 1 .2 Work performed at APHA Weybridge 1 .1 Isolation of background (indicator commensal) and antibiotic resistant Enterobacteriaceae from meats and caecal contents according to EU and / or APHA protocols 1 5.2.2 Storage of purified presumptive AMPC / EBSL <i>E. coli</i> prior to further tests 18 5.2.3 Identification of Bacteria by MALDI ToF or confirmation of lactose fermenters as <i>E. coli</i> using oxidase and indole tests 1 5.2.4 Determination of Minimum Inhibitory Concentrations (MICs) by broth micro dilution 2 5.2.5 Determination of isolates as AmpC- or ESBL-phenotype 2.2.6 Identification of <i>blacTX-M</i> , <i>blaOXA</i> , <i>blaSHV</i> and <i>blaTEM</i> and sequencing of <i>blacTX-M</i> genes 2 5.2.7 Real time PCR for plasmid mediated <i>mcr-1</i> , <i>mcr-2</i> and <i>mcr-3</i> genes 2 2.2	45 6 901 11				
6.	Results2	3				
6 6 h: 6 te	.1 General considerations .2 .2 Details of the meat samples tested. .2 .3 Samples positive on MacConkey agar + 1 mg/L cefotaxime – EU armonised method .2 .4 Samples positive on CHROMagar™ ESBL - UK non-harmonised additionatest .4 Samples positive on CHROMagar™ ESBL - UK non-harmonised additionatest .4 Samples positive for carbapenem resistance	3 3 4 1 5				
6 6 6	 .6 MIC results for isolates from MCA-CTX agar - EU harmonised method2 .7 Bacterial counts - EU harmonised method	5 6 6				
7.	Discussion2	8				
8.	Conclusions	1				
9.	Tables3	2				
Table 1. Beef samples (sorted by sample ID) tested at APHA (excludesDecember 2019 samples)32						
Table 2. Pork samples (sorted by sample ID) tested at APHA (excludesDecember 2019 samples)						
Tab	Table 3. Country of origin of samples (excludes December 2019 samples)54					

Table 4. Number of beef and pork samples tested per supermarket (excludesDecember 2019 samples)
Table 5. Number of beef and pork samples tested per brand if two or moresamples per brand (excludes December 2019 samples)
Table 6. Summary of samples positive for <i>E. coli</i> from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX) or CHROMagar™ ESBL (CA-ESBL)58
Table 7. Summary of resistances of <i>E. coli</i> isolated on MCA CTX agar60
Table 8a. MIC results for AmpC <i>E. coli</i> isolate from MacConkey agar + 1 mg/Lcefotaxime (MCA-CTX) from UK pork sample 38102961
Table 8b. MIC results for ESBL <i>E. coli</i> isolate from MacConkey agar + 1 mg/Lcefotaxime (MCA-CTX) from UK beef sample 2685696.62
Table 8c. MIC results for ESBL <i>E. coli</i> isolate from MacConkey agar + 1 mg/Lcefotaxime (MCA-CTX) from UK pork sample 268567663
Table 8d. MIC results for ESBL <i>E. coli</i> isolate from MacConkey agar + 1 mg/Lcefotaxime (MCA-CTX) from UK pork sample 266465564
Table 9. Samples positive on MacConkey agar + 2 mg/L colistin (excludesDecember 2019 samples) sorted by date.65
10. Figures
Figure 1. Decrease in the percentage of EU beef and pork samples testing positive for AmpC, ESBL and AmpC + ESBL <i>E. coli</i> between 2015 and 2017– EU EFSA data67
Figure 2. Decrease in the maximum country levels (for all countries submitting results) of AmpC-ESBL-producing <i>E. coli</i> reported for beef and pork between 2015 and 2017 – EU EFSA data67
Figure 3. Percentages of UK beef and pork samples positive for AmpC-/ESBL- phenotype <i>E. coli</i> on MCA-CTX agar from 2015 to 2019
11. References
12. Appendix – Discussion from the 2017 report73

1. Liability statement

This report has been produced by **The Animal and Plant Health Agency** under a contract placed by the Food Standards Agency (the FSA). The views expressed herein are not necessarily those of the FSA. **The Animal and Plant Health Agency** warrants that all reasonable skill and care has been used in performing tests and preparing this report. Notwithstanding this warranty, **The Animal and Plant Health Agency** shall not be under any liability for loss of profit, business, revenues or any special indirect or consequential damage of any nature whatsoever or loss of anticipated saving or for any increased costs sustained by the client or his or her servants or agents arising in any way whether directly or indirectly as a result of reliance on this report or of any error or defect in this report.

2. Lay person's summary

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

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

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

EU requirements also state that samples should be tested on two agars that will select for bacterial resistance to a group of antibiotics known as carbapenems. Carbapenem antibiotics are also really important in human medicine, and are termed "last resort" antibiotics, used to treat infections when all or almost all other treatment options are non-viable, due to the target bacteria being resistant to most / all other relevant antibiotics.

The numbers or counts of *E. coli* bacteria on meat samples are also tested using EU methods. These bacterial counts are determined using an agar to detect all *E. coli* as well as the EU specified agar to detect ESBL or AmpC type *E. coli*. These counts

are to determine what the levels of all *E. coli* and ESBL AmpC type *E. coli* are on the meat samples.

At the request of the FSA, other agars were used to test UK samples (UK nonharmonised additional test) for *E. coli* isolates with ESBL phenotype and / or colistin resistance. Colistin is another "last resort" antibiotic, so it is important to monitor if *E. coli* with resistance to this type of antibiotic is occurring in food samples.

Other additional work, outside of the scope of the EU survey (UK non-harmonised additional tests), also requested by the FSA, included genetic tests to determine what antibiotic resistance genes were associated with ESBL and colistin resistance in *E. coli* isolates. For colistin resistance, mobile colistin resistance genes referred to as *as mcr-1, mcr-2 and mcr-3* were discovered in the last few years so colistin resistant *E. coli* were tested for these three genes. The *mcr* genes are considered particularly important as they encode resistance to the "last resort" antibiotic colistin, and as they are mobile they have the potential to transfer resistance in the gut to other bacteria.

The survey required representative random colletion of a range of chilled, fresh beef and pork meats from retail premises across the UK, as outlined in some detail later in the report.

The aim was to collect 315 beef samples and 315 pork samples, 300 of each meat type with 5% extra. Between January and December 2019, 315 beef and 313 pork samples were collected as one pork sample was received by the laboratory after the 'use by date' whilst another pork sample was duplicated.

Due to a technical issue with selective agar affecting some of the samples tested in December 2019, it was decided, following discussion with the FSA, to exclude all meat samples tested in that month from analysis. As such, of the 315 and 313 beef and pork samples tested for the entire year, 289 and 285 beef and pork samples (collected from January to November 2019) were considered eligible for reporting.

Overall, results were very similar to the 2015 and 2017 surveys (no statistically significant differences), in that only 0.35% of beef and 1.05% of pork UK retail meats tested in 2019 were positive for AmpC- or ESBL-producing *E. coli* (third generation cephalosporin resistance) respectively using a sensitive detection method. Additionally, none of the samples gave rise to viable counts of *E. coli* above the detection limit of 100 bacteria per gram of meat on the two agars used, indicating numbers of total and resistant *E. coli* in these samples were either absent or very low (less that 100 *E. coli* per gram of meat). It is possible that a sample can be negative by counts but positive following enrichment, as enrichment is a more sensitive detection method, capable in theory of detecting one target *E. coli* from 25 grams of meat.

None of the samples in the survey were found to be contaminated with *E. coli* resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem, or with *E. coli* positive for the plasmid mediated colistin resistance genes *mcr1*, *mcr2* or *mcr-3*.

In 2017, EU monitoring of beef and pork for presumptive ESBL-/AmpC-/carbapenemase-producing *E. coli* was performed by 28 Member States (MSs) and three non-MSs on meat. Results in 2019 for UK beef and pork compared favourably with results for beef and pork from other countries in 2017. In 2017, of 6,621 and 6,803 beef and pork samples tested by all countries that took part in testing, 4.8% and 6% of samples respectively yielded AmpC or ESBL or Amp+ESBL phenotype *E. coli*, compared to 0.35% for beef and 1.05% for pork from the UK in 2019.

3. Project summary

In accordance with European Directive <u>2003/99/EC</u> 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 <u>2013/652/EU</u>, which came into force on **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 betalactamase (ESBL) *E. coli* and carbapenemase producing *E. coli*.

This report outlines the procedures put in place to fulfil these requirements for UK retail beef and pork in 2019 for AmpC, ESBL and carbapenem resistant *E. coli*, following European Union (EU) guidelines and methods. The requirements (with additional detailed guidance from the EU Reference Laboratory for Antimicrobial Resistance) state that 300 retail beef and 300 retail pork samples should be tested by culture for *E. coli* on MacConkey agar containing 1 mg/L of the cephalosporin antibiotic cefotaxime (MCA-CTX). *E. coli* isolates cultured from such media are expected to show resistance to third generation cephalosporin antibiotics. Samples were also tested for carbapenem resistant *E. coli* by plating to chromID[®] CARBA (CARBA) and chromID[®] OXA-48 (OXA-48) agars as recommended by the EU.

Finally, viable counts as colony forming units per gram of meat (cfu/g) of all *E. coli* and AmpC and ESBL-phenotype *E. coli* for all samples were also determined on MacConkey agar (MCA) and MCA-CTX agars, respectively.

Isolates from MCA-CTX were tested by performing Minimum Inhibitory Concentrations (MICs) to determine their susceptibility to a panel of antibiotics.

At the specific request of the FSA (outside of the remit of Decision 2013/652/EU), all samples were also plated to CHROMagarTM ESBL (CA-ESBL), for specific detection of Extended Spectrum β -lactamase-producing (ESBL) *E. coli* and to MacConkey

agar containing 2 mg/L colistin (MCA-COL), for detection of colistin resistant *E. coli*. Other additional work included a multiplex PCR to detect *bla*_{CTX-M}, *bla*_{OXA}, *bla*_{SHV} and *bla*_{TEM} genes [1] for *E. coli* isolated from CA-ESBL agar, and sequencing of the *bla*_{CTX-M} genes in CTX-M positive isolates from this agar.

Presumptive *E. coli* from MCA-COL were also tested for the presence of plasmid mediated colistin resistance genes *mcr1*, *mcr2* and *mcr3* [2].

For this study, the Animal and Plant Health Agency (APHA) worked in collaboration with Hallmark Veterinary Compliance Services, who arranged sampling, collection and posting of retail meat samples to APHA, and have reported separately on the sample details.

The survey required representative random collection across the UK of different chilled beef and pork fresh meat categories, outlined in some details later in the report.

A bespoke 'in-house' APHA Standard Operating Procedure (SOP) based on published EU test methods was used as per previous survey years. The method involved homogenisation of 27 grams of meat in 243 mls of Buffered Peptone Water (BPW), prior to removal of 20 mls of BPW homogenate for viable bacteria counts, and subsequent incubation of 250 mls BPW homogenate for enrichment prior to plating to selective agars.

The method has the theoretical potential to detect one target *E. coli* (e.g. AmpC or ESBL or carbapenem resistant or colistin resistant depending on final agar) in 25 grams of meat.

Homogenisation, in agreement with the FSA and EURL, was performed in BPW only, and not saline also, as per the EU method for counts [3]. This was done so that only one meat aliquot per meat sample required homogenisation and only after equivalent results were shown following homogenisation in saline and BPW for a subset of samples.

Due to a technical issue with MacConkey + 1 mg/L cefotaxime agar affecting some of the samples tested in December 2019, it was decided, following discussion with the FSA, to exclude all meat samples tested in that month from analysis. As such, of the 315 and 313 beef and pork samples tested for the entire year, 289 and 285 beef and pork samples (collected between January and November 2019) were considered eligible for reporting (96.3% and 95% of required beef and pork number respectively).

Overall, results were very similar to the 2015 and 2017 surveys (no statistically significant differences), in that only one beef sample (0.35%, 95% confidence interval 0.06% to 1.90%) and three pork samples (1.05%, 95% confidence interval 0.36% to 3.05%) tested were positive for AmpC-/ESBL-producing *E. coli* (third generation cephalosporin resistance) on MCA-CTX agar, using a sensitive detection method.

The beef sample and one of the pork samples that were positive on MCA-CTX agar were also positive on CA-ESBL agar (UK non-harmonised additional test). The resulting isolates tested were found to be positive for CTX-M 14 (beef) and CTX-M 24 (pork). Two additional pork samples not positive on MCA-CTX was also positive on CA-ESBL agar, and the resulting isolate tested were found to be positive for CTX-M 1 and CTX-14.

None of the beef and pork samples gave rise to viable counts of *E. coli* above the detection limit of 100 bacteria per gram of meat on the two agars used, indicating numbers of total and AmpC/ESBL-phenotype *E. coli* in these samples were either absent or very low (less that 100 *E. coli* per gram of meat).

None of the meat samples tested (n=574) were found to have carbapenem resistant *E. coli* on CARBA and OXA-48 agars by the EU harmonised method.

None of the meat samples (n=574) were found to be contaminated with *E. coli* resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem, or with *E. coli* positive for the plasmid mediated colistin resistance genes *mcr1*, *mcr2* or *mcr-3*.

Determination of the susceptibility of *E. coli* from MCA-CTX agar to a panel of relevant antibiotics allowed phenotypic characterisation of third generation cephalosporin resistance [4].

For an isolate to be designated as ESBL-phenotype it had to be resistant to cefotaxime and / or ceftazidime, but susceptible to cefoxitin. Additionally, the isolate needed to show increased sensitivity to cefotaxime and / or ceftazidime in the presence of the beta-lactamase inhibitor clavulanic acid [4].

For an isolate to be designated as AmpC-phenotype it had to be resistant to cefoxitin and also resistant to cefotaxime or ceftazidime. Additionally, the isolate needed to show no increased sensitivity to cefotaxime and / or ceftazidime in the present of the beta-lactamase inhibitor clavulanic acid [4].

One of the pork isolates from MCA-CTX agar had an AmpC-phenotype, whilst the remaining three isolates from MCA-CTX agar (one from beef and two from pork) had an ESBL-phenotype.

The percentages of 285 pork samples therefore that were positive for AmpCphenotype *E. coli* were 0.35% (95% confidence interval 0.06% to 1.96%). The percentages of 289 beef and 285 pork samples therefore that were positive for an ESBL-phenotype were 0.35% (95% confidence interval 0.06% to 1.90%) and 0.70% (95% confidence interval 0.19% to 2.52%) respectively.

As would be expected, all isolates from MCA-CTX agar were microbiologically resistant using EUCAST ECOFFs [5] to the beta-lactam antibiotic ampicillin, since they were isolated on agar with the beta-lactam antibiotic cefotaxime, and resistance to cefotaxime would also confer resistance to ampicillin. All of the isolates from MCA-CTX agar were also resistant to the cephalosporin antibiotics cefotaxime and ceftazidime, and for the AmpC isolate to cefoxitin also.

None of the isolates from MCA-CTX agar were resistant to the last resort antibiotics colistin, ertapenem, imipenem or meropenem.

Overall, results showed that less than 1% of retail beef and pork samples in the UK that were tested were positive for AmpC-/ESBL-phenotype *E. coli* using a sensitive detection method. These results were similar to previous UK surveys of 2015 and 2017.

In 2017, EU monitoring of beef and pork for presumptive AmpC-/ESBL-/carbapenemase-phenotype *E. coli* was performed on a mandatory basis by 28 member states (MSs) and three non-MSs [6]. Of 6,621 beef samples tested, 1.1% and 3.9% were positive for AmpC- and ESBL-phenotype *E. coli* respectively [6] 1.6% and 4.7% of the 6,803 pork samples were positive for AmpC- and ESBLphenotype *E. coli*, respectively [6].

Beef samples positive for AmpC- and ESBL-phenotype *E. coli* in 2017 ranged from 0% in Estonia, Finland, Iceland and Norway to low or moderate levels in most other countries, up to a maximum of 13.1% of samples in Malta [6].

For pork samples in 2017, samples from Finland, Luxembourg, Sweden and Iceland were negative, and in most other countries levels of samples positive were low to moderate, up to a maximum of 14.4% of samples positive for AmpC- and ESBL-phenotype *E. coli* in Romania [6].

The levels of beef and pork samples positive for AmpC-/ESBL-phenotype *E. coli* in the UK in 2019 compared favourably to the levels of samples positive other European countries that took part in testing in 2017.

4. Glossary

AmpC phenotype – Antimicrobial resistance profile type with resistance typically to cephalosporin antimicrobials including cefoxitin and also to 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.

ESBL phenotype – Antimicrobial resistance profile type with resistance typically to cephalosporin antimicrobials but excluding resistance to cefoxitin and β -lactamase inhibitor- β -lactam combinations

EU-European Union

EUCAST - European Committee on Antimicrobial Susceptibility Testing

EURL - European Union reference laboratories.

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

PHENOTYPE – In this context, antimicrobial resistance type

QC – Quality control

SOP – Standard Operating Procedure

5. Materials and Methods

5.1 Sampling criteria - taken from the HallMark report

The survey required representative random collection of a range of different chilled, fresh beef and pork retail meats across the UK; totalling 600 retail meat samples plus an additional 5% for contingency purposes [7].

The samples were allocated in proportion to market-share for outlet type and to population (NUTS-3) [7]. The sampling was split equally between the four quarters of the year, spreading out the collection days within each quarter to further increase the temporal coverage of the survey [7]. Due to the requirement to use the latest and most relevant data, the sampling plan for the 2017 red meat survey was updated; however, the same basic approach was used [7].

The 2019 red meat sampling plan used "proportionate stratified sampling" to allocate samples to NUTS3 areas and the samples were distributed almost in proportion to population size [7]. Eighty NUTS-3 locations with representation of England, Scotland, Wales and Northern Ireland that covers at least 80% of the total population were selected [7].

Samples were taken from all but the smallest NUTS 3 regions in the UK [7]. The population of GB NUTS3 regions was calculated from the latest MYE2 mid-year population estimates published by the Office of National Statistics [7]. For Northern Ireland, equivalent statistics produced by NISRA were used [7]. The number of samples allocated in each NUTS-3 area were proportional to the population size [7].

FSA suggested limiting sampling for 2019 to the same 11 supermarkets plus "Shops not on the list" [7]. This accounts for 94% of market share as based on the 2016/17 Family Food data which were provided by the FSA Statistics Branch [7].

The relevant fresh meat cut categories sampled repeated the cuts collected during the same study in 2017 [7]. Processed meat, minced meat, joints or meat with added herbs/spices were all excluded from sampling [7].

The pork samples categories were chops, fillets & steaks or other diced/sliced pork [7]. Each sample was randomly assigned to a cut category, according to the consumption data which maximise the power of detecting different AMR between these cut categories [7].

The beef samples categories were less expensive steaks, expensive steaks or other diced/sliced beef [7]. Steaks that cost under £2/100g were considered less expensive [7]. Each sample was randomly assigned to a cut category according to the consumption data [7]. The sampling of the beef and pork cuts were based on the proportions provided by FSA [7].

5.2 Work performed at APHA Weybridge

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

These SOPs are:

- Isolation of background (indicator commensal) and antibiotic resistant Enterobacteriaceae from meats and caecal contents according to CRL, EU and / or APHA protocols (CBU 0278).
- Microbank -70°C 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)
- Identification of bacteria by MALDI ToF (BAC0334)
- Real Time PCR for plasmid mediated colistin resistance genes *mcr-1*, *mcr-2* and *mcr3* (BAC0415).

The methodology is summarised briefly below.

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

The methodology follows that outlined in EU documents. The SOP CBU 0278 is based on these EU methods as below.

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

Pdf files of the most recent versions of the above EU methods can be found on-line at <u>EU protocols</u>.

In agreement with the EURL and the FSA, a minor modification of the EU method for the quantification of ESBL/AmpC-producing *E. coli* in caecal content and fresh meat samples was made. This modification was made so that only one aliquot per meat sample was homogenised in chilled BPW.

The rationale for this change was that:

- i. Counts should be identical for homogenisation in chilled BPW or chilled saline.
- ii. Homogenisation of two different meat sample samples could in theory give rise to slightly different counts, as such if possible, it is better to homogenise one sample.

iii. There would be savings in media costs and staff time.

The change was only implemented after a number of samples tested with homogenisation in both BPW and saline gave identical results.

Thus, rather than homogenisation of 25 grams of meat in 225 mls of saline (for counts) and homogenisation of a further 25 grams of meat in 225 mls BPW (for enrichment), one lot of 27 grams of meat was homogenised in 243 mls BPW and 20 mls of the meat:BPW homogenate was taken for counts. This leaves 250 mls meat: BPW homogenate as per the original method.

In brief, 27 grams of retail meat sample collected, transported and stored under conditions as stipulated by the EU protocols, was homogenised in ~ 100 ml (from 243 ml sterile chilled BPW) of sterile chilled BPW, before adding this homogenate to the remaining BPW and gently mixing, providing 270 ml of BPW homogenate.

From this 270 ml BPW homogenate, 20 mls was taken for the viable bacterial counts, which were performed as per the EU protocol on MacConkey agar (MCA) and MacConkey agar containing 1 mg/L cefotaxime (MCA-CTX). MCA and MCA-CTX plates for counts were incubated for 18-22 hours at 44 \pm 0.5 °C before checking for lactose fermenting colonies.

The remaining 250 mls of BPW homogenate (e.g. 25 grams of meat and 225 mls of BPW as per EU protocols) was incubated at 37 \pm 1°C 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 quality control (QC) tested prior to use and the week of use, according to EU or APHA methods as appropriate, as outlined in the SOP.

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

Lactose fermenters from MCA-CTX were assumed to be presumptive AmpC-/EBSLproducing *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*.

One single presumptive *E. coli* from each of these agars was plated again to the agar of origin and for a further two passages on blood agar, to ensure purity prior to confirmation as *E. coli* and storage pending further tests. If the first presumptive *E. coli* was found not to be *E. coli*, a further two colonies were passaged and tested as above. If neither of these colonies were *E. coli*, the sample was considered negative.

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

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

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

Isolates will be stored for up to five years to comply with EU requirements. Isolates were stored in duplicate, on "beads" (frozen in cryogenic material at -70°C).

For "beads", purified bacterial culture was aseptically transferred using a 10 μ l loop from the pure culture on agar to a commercial "beads" tube. The cryogenic liquid and bacterial growth was mixed in the tube, before removing most of the supernatant cryogenic liquid, and then storing the tube at - 70°C.

5.2.3 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 44°C, 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 \pm 0.5 °C to confirm isolates as lactose fermenters. If isolates were lactose fermenters, they were then identified as *E. coli* by combined use of oxidase and indole tests as described by inhouse SOPs.

For the oxidase test and indole tests, a single well isolated colony was taken from MCA or MCA-CTX agar, plated onto blood agar and incubated overnight at 37°C. 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 44°C 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, or for mucoid lactose fermenting colonies that can be *Klebsiella,* even if oxidase negative and indole positive. MALDI ToF was used as described by an in-house SOP and based on that previously described [8].

For MALDI ToF identifications, isolates were also grown on blood agar. A small amount of bacterial growth was applied to the metal target plate and overlaid with 1 μ I of 70% formic acid to perform a partial protein extraction and allowed to dry.

Each spot was then overlaid with 1 μ l of HCCA matrix and 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 \geq 2.000, this was taken as reliable identification to the species level, dependent also on consistency score and caveats that might apply for some bacteria species.

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

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

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

For *E. coli*, the presence of carbapenemase producing strains, ESBL or AmpC enzyme producers was determined by assessing isolate MIC's against the microbiological breakpoints for meropenem, cefotaxime and ceftazidime.

Any isolates showing a meropenem MIC's greater than 0.125mg/l, cefotaxime MIC's greater than 0.25mg/l or ceftazidime MIC's greater than 0.5mg/l were tested against

a further panel of antimicrobials containing cefotaxime, ceftazidime, cefotaxime / clavulanate, ceftazidime / clavulanate, imipenem, ertapenem, temocillin, cefoxitin, cefepime and meropenem.

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

5.2.5 Determination of isolates as AmpC- or ESBL-phenotype

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/I against cefepime and also showed a reduction in MIC of \geq 8 fold against combined cefotaxime / clavulanate or ceftazidime / clavulanate when compared with the cephalosporin alone were considered to carry an ESBL.

AmpC-phenotype isolates were resistant to cefotaxime or ceftazidime but also to 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.

5.2.6 Identification of *bla*_{CTX-M}, *bla*_{OXA}, *bla*_{SHV} and *bla*_{TEM} and sequencing of *bla*_{CTX-M} genes

Isolates from CA-ESBL were tested for *bla*_{CTX-M}, *bla*_{OXA}, *bla*_{SHV} and *bla*_{TEM} genes using a multiplex PCR as previously described [1]. For *bla*_{CTX-M} positive isolates, the sequence type of the *bla*_{CTX-M} gene was determined by sequencing of *bla*_{CTX-M} amplicons as previously described [9, 10].

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

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

5.2.8 Statistical evaluation of positives (AMPC / EBSL *E. coli*) between years

The numbers of positive samples were too small to allow comparison between years using generic methods such as the chi-square contingency table test. Instead, the probability of the observed or more extreme outcomes was exactly calculated, assuming a null hypothesis that samples of each meat from different years were equivalent. Since the numbers of samples from each year were roughly equal, a given number of positive samples from any year was treated as an equivalent outcome to the same number of positive samples from either of the other two years. For example, the observed numbers of positive pork samples (6 in 2015, 1 in 2017 and 3 in 2019) were treated as 6 from any one year, 3 from another year and 1 from the remaining year.

6. Results

6.1 General considerations

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

6.2 Details of the meat samples tested.

The background details of the meat samples tested have been provided as part of the report produced by HallMark Veterinary Compliance Services [7]. The main details of each meat sample tested are listed in Tables 1 and 2 of this report for beef and pork respectively, with anonymised codes for all shops and brands if two or more samples were obtained for the brand.

As stated in the methods, the survey required representative random collection across the year of a range of different chilled, fresh beef and pork retail meats across the UK; totalling 300 of each meat type plus an additional 5% for contingency purposes [7].

Due to a technical issue with selective agar affecting some of the samples tested in December 2019, it was decided, following discussion with the FSA, to exclude all meat samples tested in that month from analysis. As such, of the 315 and 313 beef and pork samples tested for the entire year, 289 and 285 beef and pork samples (collected from January to November 2019) were considered eligible for reporting.

The different countries of origin of the meats samples is summarised in Table 3. Meat samples originated mainly in the UK, but were also from Argentina, Belgium, Denmark, the EU (country not stated), France, Germany, Ireland, the Netherlands and Spain. After the UK, the second highest number of beef samples came from Ireland, whilst the second highest number of pork samples came from Germany (Table 3). The origin of the meat samples in relation to the different supermarkets (in code) is shown in Table 4. There was a relatively even distribution of beef and pork obtained from the different supermarkets. Most meats samples (at least 30 samples of both beef and pork from one supermarket) originated from supermarkets B, C and H and the fewest of samples (less than 10) originated from supermarkets D and F.

As for supermarkets, there was also a relatively even spread of beef and pork across different brands (Table 5). Most samples (at least 20 for both beef and pork for a specific brand) were from brands C2, G1, H1 and Q2.

6.3 Samples positive on MacConkey agar + 1 mg/L cefotaxime – EU harmonised method

One beef sample (0.35%, 95% confidence interval 0.06% to 1.90%) and three pork samples (1.05%, 95% confidence interval 0.36% to 3.05%) tested were positive for AmpC- or ESBL-producing *E. coli* (third generation cephalosporin resistance) on MCA-CTX agar using a sensitive detection method (Table 6).

Based on MIC results, it was possible to determine if *E. coli* were AmpC- or ESBLphenotype. One *E. coli* from pork was AmpC-phenotype, whilst the remaining three *E. coli* were ESBL-phenotype (Table 6).

The percentages of 285 pork samples therefore that were positive for AmpC-phenotype *E. coli* were 0.35% (95% confidence interval 0.06% to 1.96%).

The percentages of 289 beef and 285 pork samples therefore that were positive for an ESBL-phenotype were 0.35% (95% confidence interval 0.06% to 1.90%) and 0.70% (95% confidence interval 0.19% to 2.52%), respectively.

6.4 Samples positive on CHROMagar™ ESBL - UK non-harmonised additional test

The beef sample and one of the pork samples positive on MCA-CTX agar were also positive on CA-ESBL agar.

The resulting isolates tested were found to be positive for CTX-M 14 (beef) and CTX-M 24 (pork) (Table 6). Two additional pork samples not positive on MCA-CTX was positive on CA-ESBL agar, and the resulting isolate tested was found to be positive for CTX-M 14 and CTX-1 (Table 6).

6.5 Samples positive for carbapenem resistance — EU harmonised method

None of the samples tested (n=574) were positive for carbapenem resistant *E. coli* on CARBA and OXA-48 agars by the EU harmonised method.

6.6 MIC results for isolates from MCA-CTX agar - EU harmonised method

A summary of resistances derived from MIC results for the different phenotypes of the four *E. coli* isolated from MCA-CTX agar shown in Table 7.

Individual MIC results for each of the four isolates tested are shown in Table 8.

As would be expected, since the isolates were obtained from agar containing 1 mg/L of the beta-lactam antibiotic cefotaxime, all isolates were resistant to the beta-lactam antibiotic ampicillin, and to the cephalosporin antibiotics cefepime, cefotaxime and ceftazidime (Tables 7 and 8).

None of the isolates were resistant to chloramphenicol, ciprofloxacin, gentamycin, nalidixic acid, temocillin or tigecycline (Tables 7 and 8).

Additionally, none of the isolates were resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin (Tables 7 and 8).

6.7 Bacterial counts - EU harmonised method

None of the beef and pork samples gave rise to viable counts of *E. coli* above the detection limit on MCA (total *E. coli*) and MCA-CTX (AmpC-/ESBL-phenotype *E. coli*) agars. This indicated that the numbers of total and AmpC-/ESBL-phenotype *E. coli* on these samples were either absent or low.

6.8 Samples positive for colistin resistant *E. coli* - UK non-harmonised additional test

A total of 45 samples gave rise to lactose fermenting colistin resistant colonies on colistin agar (MCA-COL).

Positives comprised 22/289 (7.60%, 95% confidence interval 5.08% to 11.26%) beef samples and 23/285 (8.07%, 95% confidence interval 5.44% to 11.82%) pork samples.

PCR tests from ~ 10 to 20 lactose fermenter colonies from this agar for plasmid mediated colistin resistance genes *mcr1*, *mcr2* or *mcr-3* were all negative.

6.8.1 Statistical evaluation of positives (AMPC / EBSL *E. coli*) between years

Overall, results were consistent with the 2015 and 2017 EU surveys, in that only one beef sample (0.35%, 95% confidence interval 0.017% to 1.69%) and three pork samples (1.05%, 95% confidence interval 0.27% to 2.84%) tested were positive for AmpC-/ESBL-producing *E. coli* (third generation cephalosporin resistance) on MCA-CTX.

There was no significant evidence that the frequency of positive samples differed between years: assuming the null hypothesis that all samples were equivalent regardless of year, exact calculation, using the binomial probability distribution and the observed overall frequency of positives, found that the probability that two years would have at least 2 positive samples, while one year would have no more than 1 positive sample, as observed in beef = 0.373. For the observations in pork, the probability that one year would have at least 6 positive samples, one year would

have no more than 1 positive sample, and the third year would have no more than 3 positive samples = 0.064, which, as the less likely of two test outcomes, cannot be considered significant.

7. Discussion

The discussion from the 2017 report on "EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in *E. coli* from Retail Meats (Year 3 – Beef and Pork)" is given in the appendix of this report [11]. Much of the discussion relating to previous studies of ESBL-producing *E. coli* in raw beef and pork in the UK and other countries is of relevance for this 2019 survey. It is recommended to read this previous discussion if a wider background overview is required. The discussion below focuses on results from the 2019 survey.

The EFSA have now reported the prevalence of AmpC and ESBL producing *E. coli* in beef and pork from 23/24 member states and 2/2 non-member states in 2015 [12] and from 28 member states and 3 non-member states in 2017 [6]. In total from all states, in 2015/2017, 5,329/6,621 retail beef samples and 5,350/6,803 retail pork samples were tested.

From beef, the EFSA data for all results shows that for 2015/2017, 5.0%/3.9%, 1.8%/1.1% and 0.3%/0.1% of all samples were positive for ESBL or AmpC or AmpC+ESBL phenotype *E. coli* respectively (Figure 1).

From pork, the EFSA data for all results shows that for 2015/2017, 7.0%/4.7%, 2.3%/1.6% and 0.4%/0.3% of all samples were positive for ESBL or AmpC or AmpC+ESBL phenotype *E. coli* respectively (Figure 1).

These results suggest a slight decline in AmpC-/ESBL-producing *E. coli* overall in beef and pork in the EU and member states that participated between 2015 and 2017.

In both 2015 and 2017 there was variation of levels or AMR *E. coli* isolated from beef and pork from different countries. For example, this ranged from no AmpC-/ESBL-producing *E. coli* detected from beef and or pork samples from some countries, to maximum levels of 11.5%/17.3% of beef samples from Bulgaria being positive for AmpC-/ESBL-producing *E. coli* in 2015 and in the same year 6.6%/21.3% of pork samples from the Czech Republic and Portugal respectively being positive for AmpC-/ESBL-producing *E. coli*.

In 2017 the maximum levels of beef and pork samples positive for AmpC-/ESBLproducing *E. coli* in different countries had fallen compared to the 2015 results.

As such in 2017 for beef, the maximum levels of samples positive for AmpC-/ESBLproducing *E. coli* per country was 5.0%/10.5% for the Czech Republic and Portugal respectively. For pork, these values in 2017 were 4.7%/10.1% for AmpC-/ESBLproducing *E. coli* from the Romania and the Czech Republic, respectively.

For beef this represent a fall in the maximum positivity of AmpC/ESBL *E. coli* of all countries tested of 11.5%/17.3% to 5.0%/10.5%, whilst for pork the fall was from 6.6%/21.3% to 4.7%/10.1%, between 2015 and 2017, as summarised in Figure 3.

Across the UK, the evidence shows that the levels of AmpC-/ESBL-phenotype *E. coli* in beef and pork have remained at a low stable level over the years 2015, 2017 and 2019 (no statistically significant differences in positives across these years). These levels have been 0.35% to 0.64% for beef and 0.32% to 1.92% for pork (Figure 3).

There was a drop in the numbers of samples that gave rise to lactose fermenters on colistin agar (MCA-COL) in 2019 compared to 2017. In 2017 21.4% of beef samples and 14.8% of pork samples were positive on MCA-COL agar. This dropped to 7.60% of beef samples and 8.07% of pork samples positive in 2019.

In 2017, one beef steak sample (expensive steak) of foreign providence was found to be positive for *mcr-1* plasmid mediated colistin resistant *E. coli*. However, for the 2019 samples, although some samples gave rise to lactose fermenters on MCA-COL agar, none were positive for *mcr-1,2,3* plasmid mediated colistin resistant *E. coli*.

Since the discovery of plasmid mediated colistin resistance in pigs in China in 2015[13], there has been a decrease of use of colistin in veterinary medicine in the UK.

In 2015, 0.12 mg/kg of colistin was used in food producing animals in the UK, but has reduced to 0.0007 mg/kg in 2018. In particular, colistin use in pigs dropped from 0.86 mg/kg in 2015 to 0.004 mg/kg in 2018, representing a reduction of 99.6% [14].

During this time period colistin was not reported to be used in beef cattle, although its use in dairy cattle dropped from 0.007 to 0 mg/kg between 2017 and 2018 [14].

It is possible that the overall reduction of use of colistin on farms in the last few years may have contributed to the slight reduction in colistin resistant lactose fermenting colonies isolated from beef and pork between 2017 and 2019.

As for the 2017 results, none of the meats were positive for carbapenem resistant *E. coli.*

With respect to bacterial counts, in 2017, for the 624 beef and pork retail samples tested, none gave rise to presumptive *E. coli* counts on MCA-CTX agar and / or CA-ESBL agars above the detection limit of 40 cfu/gram of meat. The count results were overall the same for 2019 samples as for 2017 samples, but there were differences in the method between these two years. For the 2019 meats, the bacterial counts method was harmonised to the EU testing method. As such, MCA-CTX agar was still used, but instead of CA-ESBL, MCA (MacConkey agar without supplements) was used.

MCA agar does not contain antibiotics, whilst both agars used for the 2017 study contained agents to select for either AmpC-/ESBL-producing *E. coli* or ESBL-producing *E. coli*. As such, for the 2019 counts, results show that the levels of both AmpC-/ESBL-producing *E. coli* and background *E. coli* were below detection levels.

Finally, for the 2017 report for beef and pork samples it was discussed that the predominant *E. coli* strain associated with human infections is the pandemic O25-ST131 CTX-M-15-producing clone [15, 16]. For 2019 samples, one beef sample was positive for CTX-M 14, and three pork samples were positive for CTX-M 1 or 14 or 24. As such none of the samples were positive for the human pandemic O25-ST131 CTX-M-15- producing *E. coli* clone. Previous UK studies have isolated CTX-M 14 from cattle [17], CTX-M 1 from pork [18] and CTX-M 1 and 14 from pigs [19]. A German study has isolated CTX-M 24 from pigs [20].

8. Conclusions

- Results of the UK 2019 EU harmonised surveillance of antimicrobial resistance (AMR) in retail beef and pork were similar (no statistically significant differences) to results from the 2015 and 2017 surveys, with ~ 1% of beef and pork samples tested positive for AmpC-/ESBL-phenotype *E. coli*.
- None of the beef and pork samples were positive for *E. coli* with resistance to last resort carbapenem or colistin antibiotics.
- None of the meat samples prior to enrichment had background or AmpC-/ESBLphenotype *E. coli* counts above the EU detection levels indicating very low numbers of these bacteria
- Results compare favourably to results from other countries that participated in these EU monitoring surveys in 2015 and 2017, as published by EFSA.

9. Tables

 Table 1. Beef samples (sorted by sample ID) tested at APHA (excludes December 2019 samples).

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
6194	08/11/2019	Beef steaks-expensive	United Kingdom	Q2	G
6276	10/10/2019	Beef steaks-less expensive	United Kingdom	11	1
6440	12/09/2019	Beef steaks-expensive	United Kingdom	X1	G
6491	08/10/2019	Beef steaks-less expensive	Ireland	G1	В
267422	12/07/2019	Beef steaks-less expensive	United Kingdom	H1	С
343397	12/08/2019	Beef steaks-less expensive	United Kingdom	E1	A
343432	20/08/2019	All other beef and veal	United Kingdom	C2	Н
381037	18/01/2019	Beef steaks-expensive	United Kingdom	Q2	G
381078	21/01/2019	Beef steaks-less expensive	United Kingdom	H1	С
540467	04/06/2019	Beef steaks-expensive	United Kingdom	11	I
540468	04/06/2019	Beef steaks-less expensive	United Kingdom	C2	Н
540490	15/04/2019	Beef steaks-expensive	United Kingdom	G1	В
540491	15/04/2019	Beef steaks-expensive	United Kingdom	H1	С
540492	15/04/2019	Beef steaks-less expensive	United Kingdom	A2	С
540663	10/06/2019	Beef steaks-expensive	United Kingdom	Z1	J
540668	12/04/2019	Beef steaks-expensive	United Kingdom	Q2	G
540669	12/04/2019	Beef steaks-less expensive	United Kingdom	A2	С
540701	08/05/2019	Beef steaks-less expensive	Ireland	G1	В
540766	08/05/2019	Beef steaks-expensive	United Kingdom	B1	Η

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
540771	07/05/2019	Beef steaks-less expensive	United Kingdom	C2	Н
540781	11/04/2019	Beef steaks-less expensive	United Kingdom	C2	Н
540799	11/04/2019	Beef steaks-less expensive	United Kingdom	B2	A
540800	11/04/2019	Beef steaks-expensive	United Kingdom	F1	G
540905	09/05/2019	Beef steaks-expensive	United Kingdom	H1	С
558274	07/05/2019	Beef steaks-less expensive	United Kingdom	C2	Н
558308	13/03/2019	Beef steaks-expensive	United Kingdom	B1	Н
558561	07/08/2019	Beef steaks-less expensive	United Kingdom	11	I
558576	06/03/2019	Beef steaks-less expensive	United Kingdom	G1	В
558578	06/03/2019	Beef steaks-less expensive	Ireland	G1	В
1562500	04/06/2019	Beef steaks-expensive	United Kingdom	P1	?
1562501	04/06/2019	Beef steaks-less expensive	United Kingdom	G1	В
1562505	04/06/2019	Beef steaks-expensive	United Kingdom	W1	E
1562508	04/06/2019	Beef steaks-less expensive	United Kingdom	X1	G
1562511	04/06/2019	Beef steaks-expensive	United Kingdom	Q2	J
1562512	04/06/2019	Beef steaks-less expensive	United Kingdom	11	I
1562513	04/06/2019	Beef steaks-less expensive	United Kingdom	H1	С
1562730	15/04/2019	Beef steaks-less expensive	United Kingdom	1	I
1563634	21/01/2019	Beef steaks-less expensive	United Kingdom	L1	F
1614500	15/04/2019	Beef steaks-expensive	United Kingdom	C2	Н
2447770	05/03/2019	Beef steaks-less expensive	United Kingdom	1	
2447771	05/03/2019	Beef steaks-expensive	United Kingdom	N1	F

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2447772	05/03/2019	Beef steaks-less expensive	United Kingdom	P1	?
2447782	05/03/2019	Beef steaks-expensive	United Kingdom	G1	В
2447783	05/03/2019	Beef steaks-less expensive	United Kingdom	?	?
2447784	05/03/2019	Beef steaks-less expensive	United Kingdom	W1	E
2447785	05/03/2019	Beef steaks-expensive	United Kingdom	12	С
2447786	12/03/2019	Beef steaks-expensive	United Kingdom	B2	A
2447789	12/03/2019	Beef steaks-expensive	United Kingdom	H1	С
2447794	05/03/2019	Beef steaks-less expensive	United Kingdom	M1	I
2447795	05/03/2019	Beef steaks-expensive	United Kingdom	Z1	J
2447797	05/03/2019	Beef steaks-expensive	United Kingdom	12	С
2447798	08/02/2019	Beef steaks-less expensive	United Kingdom	U1	В
2447799	08/02/2019	Beef steaks-less expensive	United Kingdom	C2	Η
2447800	08/02/2019	Beef steaks-less expensive	United Kingdom	W1	E
2447801	08/02/2019	Beef steaks-less expensive	United Kingdom	M1	I
2447802	08/02/2019	Beef steaks-less expensive	United Kingdom	11	I
2447807	12/03/2019	Beef steaks-expensive	United Kingdom	Z1	J
2447809	08/02/2019	Beef steaks-expensive	United Kingdom	Q2	G
2447811	08/02/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2447812	08/02/2019	Beef steaks-expensive	United Kingdom	L1	F
2447813	08/02/2019	Beef steaks-expensive	United Kingdom	F1	G
2447815	06/02/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2447817	06/02/2019	Beef steaks-less expensive	United Kingdom	W1	E

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2447818	06/02/2019	Beef steaks-expensive	United Kingdom	D1	?
2447820	07/02/2019	Beef steaks-expensive	United Kingdom	M2	D
2447821	07/02/2019	Beef steaks-expensive	United Kingdom	W1	E
2447823	07/02/2019	Beef steaks-less expensive	United Kingdom	?	?
2447830	12/03/2019	Beef steaks-expensive	United Kingdom	Q2	G
2447832	07/02/2019	Beef steaks-less expensive	United Kingdom	H1	С
2447833	07/02/2019	Beef steaks-expensive	United Kingdom	B2	A
2447834	12/03/2019	Beef steaks-expensive	United Kingdom	1	I
2447835	07/02/2019	Beef steaks-less expensive	United Kingdom	U1	В
2447836	07/02/2019	Beef steaks-expensive	United Kingdom	Q2	G
2447839	06/02/2019	Beef steaks-expensive	United Kingdom	Q2	J
2447840	06/02/2019	Beef steaks-expensive	United Kingdom	Q2	J
2447842	06/02/2019	Beef steaks-less expensive	United Kingdom	H1	С
2447843	06/02/2019	Beef steaks-less expensive	United Kingdom	M1	I
2447844	06/02/2019	Beef steaks-less expensive	United Kingdom	B2	A
2447845	07/02/2019	Beef steaks-less expensive	United Kingdom	W1	E
2447846	07/02/2019	Beef steaks-expensive	Ireland	H1	С
2447847	07/02/2019	Beef steaks-expensive	United Kingdom	Q2	J
2447850	06/03/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2447851	06/03/2019	Beef steaks-expensive	Ireland	H1	С
2447852	06/03/2019	Beef steaks-expensive	Ireland	G1	В
2447856	06/03/2019	Beef steaks-less expensive	Ireland	H1	С

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2447863	21/01/2019	Beef steaks-less expensive	United Kingdom	P1	?
2447864	21/01/2019	Beef steaks-expensive	United Kingdom	P1	?
2447867	18/01/2019	Beef steaks-less expensive	United Kingdom	G1	В
2447868	18/01/2019	Beef steaks-expensive	United Kingdom	W1	E
2447870	18/01/2019	Beef steaks-expensive	United Kingdom	G1	В
2447871	18/01/2019	Beef steaks-expensive	United Kingdom	H1	С
2447873	18/01/2019	Beef steaks-less expensive	United Kingdom	C1	?
2447878	18/01/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2447880	18/01/2019	Beef steaks-less expensive	United Kingdom	W1	E
2447881	18/01/2019	Beef steaks-expensive	United Kingdom	P1	?
2447883	18/01/2019	Beef steaks-less expensive	Ireland	G1	В
2447885	18/01/2019	Beef steaks-expensive	United Kingdom	C2	Н
2447886	18/01/2019	Beef steaks-expensive	United Kingdom	Q2	J
2447888	16/01/2019	Beef steaks-less expensive	United Kingdom	H1	С
2447889	16/01/2019	Beef steaks-expensive	United Kingdom	G2	E
2447890	16/01/2019	Beef steaks-expensive	United Kingdom	Z1	J
2447898	16/01/2019	Beef steaks-expensive	United Kingdom	12	С
2447899	16/01/2019	Beef steaks-expensive	United Kingdom	12	С
2447902	29/01/2019	Beef steaks-expensive	United Kingdom	E2	?
2447903	29/01/2019	Beef steaks-expensive	United Kingdom	E2	?
2447906	21/01/2019	Beef steaks-expensive	United Kingdom	H2	?
2447907	21/01/2019	Beef steaks-expensive	United Kingdom	H2	?
Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
---------------------	-------------------------------	----------------------------	-------------------	--------------------------	----------------------
2558197	12/03/2019	Beef steaks-expensive	United Kingdom	C2	Η
2558278	12/03/2019	Beef steaks-expensive	United Kingdom	B2	A
2558546	21/01/2019	Beef steaks-less expensive	Ireland	G1	В
2664450	05/11/2019	Beef steaks-expensive	United Kingdom	P1	?
2664451	05/11/2019	Beef steaks-less expensive	United Kingdom	Q2	G
2664461	05/11/2019	Beef steaks-less expensive	United Kingdom	R1	Н
2664464	05/11/2019	Beef steaks-less expensive	Ireland	M2	D
2664466	05/11/2019	Beef steaks-less expensive	United Kingdom	W1	E
2664468	05/11/2019	Beef steaks-expensive	Ireland	H1	С
2664469	05/11/2019	Beef steaks-less expensive	Ireland	H1	С
2664470	05/11/2019	Beef steaks-less expensive	Argentina	?	?
2664472	05/11/2019	Beef steaks-less expensive	United Kingdom	U1	В
2664476	05/11/2019	Beef steaks-expensive	United Kingdom	C2	Н
2664477	05/11/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2664482	08/11/2019	Beef steaks-expensive	United Kingdom	W1	E
2664485	08/11/2019	Beef steaks-expensive	United Kingdom	B2	A
2664486	08/11/2019	Beef steaks-expensive	United Kingdom	S1	?
2664487	08/11/2019	Beef steaks-expensive	United Kingdom	S1	?
2664492	11/11/2019	Beef steaks-expensive	United Kingdom	12	С
2664493	11/11/2019	Beef steaks-expensive	United Kingdom	12	С
2664498	11/11/2019	Beef steaks-expensive	United Kingdom	Z1	J
2664500	25/11/2019	Beef steaks-less expensive	United Kingdom	U1	В

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2664501	25/11/2019	Beef steaks-expensive	United Kingdom	B2	A
2664503	25/11/2019	Beef steaks-expensive	United Kingdom	P1	?
2664504	08/11/2019	All other beef and veal	United Kingdom	L1	F
2664508	08/11/2019	Beef steaks-expensive	United Kingdom	U1	В
2664510	08/11/2019	Beef steaks-expensive	United Kingdom	W1	E
2664511	08/11/2019	Beef steaks-expensive	Spain	?	?
2664516	10/10/2019	Beef steaks-expensive	United Kingdom	X1	G
2664517	10/10/2019	Beef steaks-expensive	United Kingdom	H1	С
2664518	10/10/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2664531	10/10/2019	Beef steaks-expensive	United Kingdom	C2	Н
2664532	10/10/2019	Beef steaks-expensive	United Kingdom	B2	A
2664534	10/10/2019	Beef steaks-less expensive	United Kingdom	X1	G
2664631	09/10/2019	Beef steaks-expensive	United Kingdom	?	?
2664633	09/10/2019	Beef steaks-less expensive	United Kingdom	A2	С
2664636	09/10/2019	Beef steaks-less expensive	United Kingdom	P1	?
2664637	09/10/2019	Beef steaks-less expensive	United Kingdom	P1	?
2664638	09/10/2019	Beef steaks-expensive	United Kingdom	Z1	J
2664639	09/10/2019	Beef steaks-expensive	United Kingdom	Z1	J
2664641	09/10/2019	Beef steaks-less expensive	Ireland	H1	С
2664646	08/10/2019	Beef steaks-less expensive	United Kingdom	?	?
2664647	08/10/2019	Beef steaks-expensive	United Kingdom	G2	E
2664649	08/10/2019	Beef steaks-expensive	United Kingdom	Z1	J

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2664652	09/10/2019	Beef steaks-expensive	United Kingdom	G2	E
2664653	09/10/2019	Beef steaks-expensive	United Kingdom	H1	С
2664654	12/09/2019	Beef steaks-less expensive	United Kingdom	P1	?
2664656	12/09/2019	Beef steaks-expensive	United Kingdom	U1	В
2664658	12/09/2019	Beef steaks-expensive	United Kingdom	Z1	J
2664663	12/09/2019	Beef steaks-expensive	United Kingdom	?	?
2664664	12/09/2019	Beef steaks-less expensive	United Kingdom	?	?
2664665	12/09/2019	All other beef and veal	United Kingdom	X1	G
2664682	12/09/2019	Beef steaks-expensive	United Kingdom	L2	?
2664683	12/09/2019	Beef steaks-expensive	United Kingdom	12	С
2664686	12/09/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2664687	12/09/2019	Beef steaks-less expensive	United Kingdom	W1	E
2664708	09/10/2019	Beef steaks-expensive	United Kingdom	11	I
2664709	09/10/2019	Beef steaks-expensive	United Kingdom	11	I
2672334	10/07/2019	Beef steaks-expensive	United Kingdom	12	С
2672335	10/07/2019	Beef steaks-less expensive	United Kingdom	M1	I
2672346	16/05/2019	Beef steaks-expensive	United Kingdom	C2	Н
2672350	14/05/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672351	16/05/2019	Beef steaks-expensive	United Kingdom	W1	E
2672353	16/05/2019	Beef steaks-expensive	United Kingdom	X1	G
2672357	16/09/2019	Beef steaks-expensive	United Kingdom	N1	F
2672358	16/09/2019	Beef steaks-expensive	United Kingdom	P2	?

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2672360	16/09/2019	Beef steaks-less expensive	United Kingdom	W1	E
2672361	16/09/2019	Beef steaks-expensive	Ireland	H1	С
2672364	14/05/2019	Beef steaks-expensive	United Kingdom	J1	I
2672365	14/05/2019	Beef steaks-less expensive	United Kingdom	H1	С
2672369	12/07/2019	Beef steaks-expensive	United Kingdom	?	?
2672370	12/07/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672371	12/07/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672373	10/06/2019	Beef steaks-expensive	United Kingdom	C2	Н
2672374	10/06/2019	Beef steaks-expensive	United Kingdom	C2	Н
2672376	10/06/2019	Beef steaks-expensive	United Kingdom	C2	Н
2672377	10/06/2019	Beef steaks-less expensive	United Kingdom	J2	D
2672381	15/05/2019	Beef steaks-less expensive	United Kingdom	P1	?
2672382	15/05/2019	Beef steaks-expensive	United Kingdom	P1	?
2672383	15/05/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672385	10/06/2019	Beef steaks-expensive	United Kingdom	G1	В
2672386	10/06/2019	Beef steaks-expensive	United Kingdom	Q2	J
2672398	11/07/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2672403	10/07/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2672405	10/07/2019	Beef steaks-less expensive	United Kingdom	W1	E
2672406	10/07/2019	Beef steaks-expensive	United Kingdom	11	
2672409	10/07/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672411	10/07/2019	Beef steaks-expensive	United Kingdom	Q2	J

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2672414	04/07/2019	Beef steaks-expensive	United Kingdom	1	I
2672415	04/07/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2672418	11/07/2019	Beef steaks-less expensive	United Kingdom	H1	С
2672420	11/07/2019	Beef steaks-less expensive	United Kingdom	U1	В
2672423	12/07/2019	Beef steaks-less expensive	United Kingdom	P1	?
2672426	12/07/2019	Beef steaks-expensive	United Kingdom	11	I
2672429	12/07/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672431	12/07/2019	Beef steaks-expensive	United Kingdom	G1	В
2672432	12/07/2019	Beef steaks-less expensive	United Kingdom	H1	С
2672469	11/07/2019	Beef steaks-expensive	United Kingdom	H1	С
2672470	11/07/2019	Beef steaks-expensive	United Kingdom	H1	С
2672472	11/07/2019	Beef steaks-expensive	United Kingdom	P1	?
2672473	11/07/2019	All other beef and veal	Ireland	P1	?
2672669	13/08/2019	Beef steaks-less expensive	United Kingdom	B2	A
2672672	13/08/2019	Beef steaks-less expensive	United Kingdom	M1	I
2672681	07/08/2019	Beef steaks-less expensive	United Kingdom	Q1	G
2672683	07/08/2019	Beef steaks-expensive	United Kingdom	P1	?
2672685	07/08/2019	Beef steaks-expensive	United Kingdom	12	С
2672687	07/08/2019	Beef steaks-expensive	United Kingdom	G1	В
2672689	20/08/2019	Beef steaks-less expensive	United Kingdom	H1	С
2672690	20/08/2019	Beef steaks-expensive	United Kingdom	B1	Н
2672692	20/08/2019	Beef steaks-expensive	United Kingdom	G1	В

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2672695	20/08/2019	Beef steaks-expensive	United Kingdom	B2	A
2672717	07/08/2019	Beef steaks-less expensive	Netherlands	?	?
2672722	07/08/2019	Beef steaks-expensive	United Kingdom	G2	E
2672723	07/08/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672735	12/08/2019	Beef steaks-expensive	United Kingdom	H1	С
2672737	12/08/2019	Beef steaks-less expensive	United Kingdom	J2	D
2672738	12/08/2019	Beef steaks-less expensive	United Kingdom	P1	?
2672740	12/08/2019	Beef steaks-expensive	United Kingdom	Z1	J
2672755	12/08/2019	Beef steaks-less expensive	United Kingdom	F2	E
2672756	12/08/2019	Beef steaks-expensive	United Kingdom	P1	?
2672760	07/08/2019	Beef steaks-expensive	United Kingdom	C2	Н
2672762	07/08/2019	Beef steaks-expensive	United Kingdom	C2	Н
2672765	07/08/2019	Beef steaks-less expensive	United Kingdom	?	?
2672766	07/08/2019	Beef steaks-less expensive	United Kingdom	Q2	G
2672800	13/09/2019	Beef steaks-expensive	United Kingdom	A1	?
2672807	16/09/2019	Beef steaks-expensive	United Kingdom	G1	В
2672808	16/09/2019	Beef steaks-less expensive	United Kingdom	X1	G
2672809	16/09/2019	Beef steaks-expensive	United Kingdom	Q2	G
2672810	16/09/2019	Beef steaks-expensive	United Kingdom	C2	Н
2672812	16/09/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2685587	09/05/2019	Beef steaks-expensive	United Kingdom	G1	В
2685589	09/05/2019	Beef steaks-expensive	United Kingdom	B1	Н

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2685590	07/06/2019	Beef steaks-expensive	United Kingdom	U1	В
2685592	07/05/2019	Beef steaks-expensive	United Kingdom	W1	E
2685594	07/06/2019	Beef steaks-expensive	United Kingdom	12	С
2685596	07/06/2019	Beef steaks-expensive	United Kingdom	H1	С
2685622	12/04/2019	Beef steaks-expensive	United Kingdom	L1	F
2685623	12/04/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2685624	12/04/2019	Beef steaks-less expensive	United Kingdom	U1	В
2685631	11/04/2019	Beef steaks-expensive	United Kingdom	D2	?
2685632	11/04/2019	Beef steaks-expensive	Ireland	O1	В
2685633	11/04/2019	Beef steaks-less expensive	United Kingdom	O2	?
2685639	08/05/2019	Beef steaks-less expensive	United Kingdom	G1	В
2685641	15/04/2019	Beef steaks-expensive	United Kingdom	G1	В
2685644	15/04/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2685645	15/04/2019	Beef steaks-expensive	United Kingdom	?	?
2685647	10/07/2019	Beef steaks-expensive	United Kingdom	C2	Н
2685656	12/04/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2685660	07/06/2019	Beef steaks-expensive	United Kingdom	W1	E
2685664	13/09/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2685665	13/09/2019	Beef steaks-expensive	United Kingdom	W1	E
2685667	13/09/2019	Beef steaks-expensive	United Kingdom	12	С
2685669	13/09/2019	Beef steaks-expensive	United Kingdom	C2	Н
2685671	13/09/2019	Beef steaks-less expensive	United Kingdom	X1	G

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2685673	12/04/2019	Beef steaks-expensive	United Kingdom	?	?
2685678	08/05/2019	Beef steaks-expensive	United Kingdom	Z1	J
2685680	08/05/2019	Beef steaks-less expensive	United Kingdom	12	С
2685683	09/05/2019	Beef steaks-expensive	United Kingdom	B1	Н
2685684	09/05/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2685685	09/05/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2685686	09/05/2019	Beef steaks-less expensive	United Kingdom	P1	?
2685687	09/05/2019	Beef steaks-less expensive	United Kingdom	P1	?
2685689	09/05/2019	Beef steaks-less expensive	Non European Union	P1	?
2685696	25/04/2019	Beef steaks-expensive	United Kingdom	1	I
2685699	25/04/2019	Beef steaks-expensive	United Kingdom	Z1	J
2685700	25/04/2019	Beef steaks-expensive	United Kingdom	Q2	J
2685705	04/06/2019	Beef steaks-less expensive	United Kingdom	Q2	J
2685706	04/06/2019	Beef steaks-less expensive	United Kingdom	H1	С
2685710	10/07/2019	Beef steaks-less expensive	United Kingdom	G1	В
2685713	15/05/2019	Beef steaks-less expensive	United Kingdom	W1	E
2685714	15/05/2019	All other beef and veal	United Kingdom	W1	E
2685725	10/06/2019	Beef steaks-expensive	United Kingdom	G1	В
2685737	10/06/2019	Beef steaks-expensive	United Kingdom	B2	A
2685739	10/06/2019	Beef steaks-expensive	United Kingdom	?	?
2685740	09/05/2019	Beef steaks-less expensive	United Kingdom	C2	Н
2685900	10/10/2019	Beef steaks-expensive	United Kingdom	N1	F

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super-market code
2685911	10/10/2019	Beef steaks-expensive	United Kingdom	T1	?
2685913	10/10/2019	Beef steaks-expensive	United Kingdom	B2	A
2686013	13/03/2019	Beef steaks-expensive	United Kingdom	G1	В
2686065	07/02/2019	Beef steaks-expensive	United Kingdom	U1	В
2686066	12/03/2019	Beef steaks-expensive	United Kingdom	11	I
2686069	12/03/2019	Beef steaks-less expensive	United Kingdom	C2	Н

? – Shop not on list

Table 2. Pork samples (sorted by sample ID) tested at APHA (excludes December2019 samples).

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
6196	08/11/2019	Pork chops	United Kingdom	W1	E
6207	11/11/2019	Pork fillets & steaks	Netherlands	H1	С
6208	08/11/2019	All other pork	United Kingdom	H1	С
6209	08/11/2019	Pork chops	United Kingdom	W1	E
6275	10/10/2019	All other pork	United Kingdom	Q2	J
6282	10/10/2019	Pork fillets & steaks	United Kingdom	X1	G
6315	10/10/2019	Pork fillets & steaks	United Kingdom	C2	Н
6441	12/09/2019	Pork fillets & steaks	United Kingdom	W1	E
6442	12/09/2019	Pork fillets & steaks	United Kingdom	X1	G
6443	12/09/2019	Pork fillets & steaks	Netherlands	H1	С
6444	12/09/2019	Pork chops	Netherlands	K2	С
6481	12/09/2019	Pork chops	United Kingdom	H1	С
6482	12/09/2019	Pork fillets & steaks	United Kingdom	H1	С
6486	09/10/2019	Pork chops	United Kingdom	Q2	J
6487	09/10/2019	Pork fillets & steaks	Germany	G1	В
6488	09/10/2019	Pork fillets & steaks	Spain	P1	?
6489	09/10/2019	All other pork	United Kingdom	C2	Н
6492	08/10/2019	Pork fillets & steaks	United Kingdom	W1	E
6493	12/09/2019	Pork chops	United Kingdom	?	?
6550	09/10/2019	Pork fillets & steaks	United Kingdom	W1	E
6551	09/10/2019	Pork fillets & steaks	United Kingdom	W1	E
343260	13/09/2019	Pork chops	United Kingdom	W1	E
343261	13/09/2019	Pork fillets & steaks	United Kingdom	W1	E
343298	16/09/2019	All other pork	United Kingdom	M1	I
343433	20/08/2019	All other pork	Netherlands	K2	С
343434	20/08/2019	Pork chops	Denmark	H1	С
343467	11/07/2019	All other pork	United Kingdom	E1	A
343468	11/07/2019	All other pork	Netherlands	K2	С
343592	12/07/2019	All other pork	Netherlands	K1	В
343593	12/07/2019	Pork chops	United Kingdom	H1	С
343619	07/08/2019	Pork fillets & steaks	United Kingdom	E1	A
343622	07/08/2019	Pork fillets & steaks	United Kingdom	G1	В
343648	10/07/2019	Pork fillets & steaks	United Kingdom	F1	G
364647	08/02/2019	All other pork	United Kingdom	K1	В
380952	05/03/2019	Pork fillets & steaks	United Kingdom	H1	С

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
380953	05/03/2019	Pork fillets & steaks	United Kingdom	C2	Н
380966	12/07/2019	All other pork	United Kingdom	W1	E
380968	12/07/2019	Pork chops	Netherlands	K1	В
380973	08/02/2019	Pork fillets & steaks	United Kingdom	J2	D
380974	08/02/2019	Pork chops	United Kingdom	W1	E
380975	08/02/2019	Pork chops	United Kingdom	Q2	J
380976	06/02/2019	Pork fillets & steaks	United Kingdom	G1	В
380977	06/02/2019	Pork chops	United Kingdom	H1	С
380978	06/02/2019	Pork fillets & steaks	Denmark	H1	С
380979	06/02/2019	Pork chops	United Kingdom	C2	Н
381029	06/03/2019	Pork fillets & steaks	United Kingdom	H1	С
381038	18/01/2019	Pork fillets & steaks	United Kingdom	B2	A
381041	16/01/2019	Pork chops	Germany	H1	С
381043	16/01/2019	Pork fillets & steaks	United Kingdom	K2	С
381044	16/01/2019	Pork fillets & steaks	United Kingdom	K2	С
381063	05/03/2019	Pork chops	United Kingdom	L1	F
381064	05/03/2019	Pork chops	United Kingdom	F1	G
381073	05/03/2019	Pork fillets & steaks	United Kingdom	H1	С
381113	29/01/2019	Pork chops	United Kingdom	W1	E
381114	29/01/2019	Pork chops	United Kingdom	W1	E
381235	08/02/2019	Pork chops	United Kingdom	W1	E
540425	14/05/2019	Pork chops	Germany	K1	В
540434	16/09/2019	All other pork	Netherlands	K1	В
540450	11/07/2019	All other pork	United Kingdom	H1	С
540451	11/07/2019	Pork chops	United Kingdom	H1	С
540458	16/05/2019	Pork fillets & steaks	Denmark	K2	С
540465	04/06/2019	Pork fillets & steaks	United Kingdom	P1	?
540466	04/06/2019	Pork fillets & steaks	United Kingdom	W1	E
540488	15/04/2019	Pork chops	United Kingdom	Q2	J
540489	15/04/2019	Pork chops	United Kingdom	F1	G
540493	15/04/2019	Pork fillets & steaks	United Kingdom	K2	С
540664	10/06/2019	All other pork	United Kingdom	C2	Н
540670	09/05/2019	Pork fillets & steaks	United Kingdom	C2	Η
540733	25/04/2019	All other pork	United Kingdom	E1	A
540764	15/04/2019	All other pork	United Kingdom	W1	E
540765	15/04/2019	Pork fillets & steaks	Germany	G1	В
540769	12/04/2019	All other pork	Denmark	H1	С
540770	12/04/2019	Pork chops	Germany	K1	В

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
540779	11/04/2019	Pork chops	United Kingdom	Q2	J
540780	11/04/2019	All other pork	Denmark	H1	С
540782	11/04/2019	Pork chops	United Kingdom	F1	G
540783	11/04/2019	Pork chops	Germany	G1	В
540797	11/04/2019	All other pork	Netherlands	K2	С
540798	11/04/2019	All other pork	Belgium	P1	?
540801	11/04/2019	Pork fillets & steaks	Germany	G1	В
540902	10/06/2019	Pork fillets & steaks	United Kingdom	F1	G
540906	09/05/2019	All other pork	Denmark	H1	С
540948	07/08/2019	All other pork	United Kingdom	C2	Н
540949	10/06/2019	Pork chops	United Kingdom	C2	Η
541031	08/05/2019	All other pork	United Kingdom	W1	E
541032	14/05/2019	All other pork	United Kingdom	F1	G
541033	14/05/2019	Pork chops	United Kingdom	C2	Н
558559	10/07/2019	Pork fillets & steaks	United Kingdom	G1	В
558560	07/08/2019	Pork fillets & steaks	United Kingdom	F1	G
558562	12/03/2019	Pork fillets & steaks	United Kingdom	K2	С
558563	12/03/2019	Pork fillets & steaks	United Kingdom	G1	В
558574	06/03/2019	Pork fillets & steaks	Germany	G1	В
561033	05/11/2019	Pork fillets & steaks	United Kingdom	G1	В
1562499	04/06/2019	All other pork	United Kingdom	?	G
1562502	04/06/2019	All other pork	United Kingdom	M1	l
1562503	04/06/2019	Pork fillets & steaks	United Kingdom	K2	С
1562504	04/06/2019	Pork fillets & steaks	United Kingdom	H1	С
1562506	04/06/2019	Pork fillets & steaks	United Kingdom	Q2	J
1562507	04/06/2019	All other pork	United Kingdom	J2	D
1562509	04/06/2019	All other pork	United Kingdom	Q2	J
1562510	04/06/2019	All other pork	United Kingdom	Q2	J
1562514	04/06/2019	Pork fillets & steaks	United Kingdom	P1	?
1563552	21/01/2019	Pork chops	United Kingdom	Q2	J
1563633	21/01/2019	Pork fillets & steaks	United Kingdom	P1	?
1614373	07/02/2019	Pork chops	United Kingdom	Q2	J
1614388	07/02/2019	Pork fillets & steaks	United Kingdom	W1	E
1614390	07/02/2019	Pork chops	Germany	G1	В
2447768	12/03/2019	Pork fillets & steaks	Denmark	H1	С
2447769	05/03/2019	Pork fillets & steaks	United Kingdom	F1	G
2447780	05/03/2019	All other pork	United Kingdom	F1	G
2447781	05/03/2019	Pork fillets & steaks	United Kingdom	E1	A

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
2447787	12/03/2019	Pork chops	United Kingdom	?	?
2447796	05/03/2019	Pork fillets & steaks	United Kingdom	B2	A
2447803	08/02/2019	Pork fillets & steaks	European Union	P1	?
2447804	08/02/2019	Pork fillets & steaks	United Kingdom	C2	Н
2447808	08/02/2019	All other pork	United Kingdom	C2	Н
2447810	08/02/2019	All other pork	United Kingdom	C2	Η
2447816	06/02/2019	Pork fillets & steaks	United Kingdom	L1	F
2447819	06/02/2019	Pork fillets & steaks	United Kingdom	D1	?
2447822	07/02/2019	Pork fillets & steaks	United Kingdom	G2	E
2447824	07/02/2019	All other pork	United Kingdom	K1	В
2447825	07/02/2019	Pork fillets & steaks	Germany	G1	В
2447827	06/02/2019	Pork chops	United Kingdom	G1	В
2447828	06/02/2019	Pork fillets & steaks	United Kingdom	K2	С
2447829	12/03/2019	Pork fillets & steaks	United Kingdom	?	?
2447831	12/03/2019	Pork fillets & steaks	France	J2	D
2447837	07/02/2019	All other pork	United Kingdom	H1	С
2447841	07/02/2019	Pork fillets & steaks	United Kingdom	F1	G
2447849	07/02/2019	Pork fillets & steaks	United Kingdom	Q2	J
2447853	06/03/2019	All other pork	United Kingdom	W1	E
2447854	06/03/2019	All other pork	United Kingdom	Q2	J
2447855	06/03/2019	Pork fillets & steaks	Germany	G1	В
2447861	21/01/2019	All other pork	United Kingdom	K2	С
2447862	21/01/2019	Pork fillets & steaks	United Kingdom	K2	С
2447869	18/01/2019	Pork fillets & steaks	United Kingdom	G1	В
2447872	18/01/2019	Pork fillets & steaks	United Kingdom	C1	?
2447874	18/01/2019	Pork fillets & steaks	Germany	G1	В
2447875	18/01/2019	Pork chops	United Kingdom	?	?
2447876	18/01/2019	Pork fillets & steaks	United Kingdom	W1	E
2447877	18/01/2019	Pork fillets & steaks	United Kingdom	H1	С
2447879	18/01/2019	Pork fillets & steaks	United Kingdom	Q2	J
2447882	18/01/2019	Pork fillets & steaks	United Kingdom	12	С
2447884	18/01/2019	All other pork	Ireland	G1	В
2447887	16/01/2019	All other pork	United Kingdom	W1	E
2447897	16/01/2019	Pork fillets & steaks	United Kingdom	Q2	J
2447908	21/01/2019	Pork fillets & steaks	United Kingdom	Q2	J
2448058	21/01/2019	Pork fillets & steaks	United Kingdom	H1	С
2558547	21/01/2019	All other pork	United Kingdom	M1	
2664460	05/11/2019	Pork fillets & steaks	United Kingdom	C2	Н

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
2664462	05/11/2019	Pork fillets & steaks	United Kingdom	E1	A
2664463	05/11/2019	All other pork	United Kingdom	C2	Н
2664465	05/11/2019	Pork fillets & steaks	United Kingdom	X1	G
2664467	05/11/2019	Pork fillets & steaks	United Kingdom	G2	E
2664471	05/11/2019	Pork fillets & steaks	United Kingdom	?	?
2664473	05/11/2019	Pork fillets & steaks	United Kingdom	?	?
2664478	05/11/2019	Pork fillets & steaks	United Kingdom	Q2	J
2664479	05/11/2019	Pork fillets & steaks	United Kingdom	Q2	J
2664480	05/11/2019	Pork fillets & steaks	United Kingdom	Q2	J
2664481	05/11/2019	Pork fillets & steaks	United Kingdom	X1	G
2664483	08/11/2019	Pork fillets & steaks	Germany	G1	В
2664484	08/11/2019	All other pork	United Kingdom	M1	I
2664491	11/11/2019	All other pork	United Kingdom	C2	Н
2664494	11/11/2019	Pork fillets & steaks	United Kingdom	X1	G
2664496	11/11/2019	All other pork	Netherlands	K2	С
2664497	11/11/2019	Pork fillets & steaks	United Kingdom	L1	F
2664499	11/11/2019	Pork fillets & steaks	United Kingdom	?	I
2664502	25/11/2019	All other pork	United Kingdom	11	I
2664505	08/11/2019	All other pork	Netherlands	K1	В
2664506	08/11/2019	All other pork	Netherlands	K2	С
2664507	08/11/2019	Pork fillets & steaks	United Kingdom	C2	Н
2664509	08/11/2019	All other pork	United Kingdom	C2	Н
2664514	10/10/2019	All other pork	United Kingdom	Y1	?
2664519	10/10/2019	Pork fillets & steaks	Germany	G1	В
2664533	10/10/2019	All other pork	United Kingdom	Y1	?
2664632	09/10/2019	All other pork	United Kingdom	H1	С
2664634	09/10/2019	Pork fillets & steaks	Spain	N2	?
2664635	09/10/2019	Pork chops	Germany	G1	В
2664640	09/10/2019	All other pork	United Kingdom	C2	Н
2664644	08/10/2019	All other pork	United Kingdom	H1	С
2664645	08/10/2019	Pork chops	France	J2	D
2664648	08/10/2019	All other pork	United Kingdom	W1	E
2664655	12/09/2019	Pork fillets & steaks	United Kingdom	P1	?
2664662	12/09/2019	Pork fillets & steaks	United Kingdom	11	
2664681	12/09/2019	Pork fillets & steaks	United Kingdom	L2	?
2664688	12/09/2019	All other pork	United Kingdom	H1	С
2664710	09/10/2019	All other pork	United Kingdom	W1	E
2664711	09/10/2019	Pork fillets & steaks	United Kingdom	P1	?

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
2672345	16/05/2019	Pork fillets & steaks	United Kingdom	C2	Η
2672348	14/05/2019	Pork fillets & steaks	United Kingdom	W1	E
2672349	14/05/2019	Pork fillets & steaks	Germany	G1	В
2672352	16/05/2019	All other pork	United Kingdom	W1	E
2672356	16/09/2019	Pork fillets & steaks	United Kingdom	X1	G
2672359	16/09/2019	Pork chops	United Kingdom	P2	?
2672362	16/09/2019	All other pork	United Kingdom	C2	Н
2672368	12/07/2019	Pork fillets & steaks	United Kingdom	M1	I
2672372	10/06/2019	Pork chops	United Kingdom	?	?
2672375	10/06/2019	All other pork	United Kingdom	C2	Н
2672379	10/06/2019	Pork fillets & steaks	Spain	?	?
2672380	15/05/2019	Pork chops	United Kingdom	?	?
2672384	15/05/2019	All other pork	United Kingdom	H1	С
2672387	10/06/2019	Pork fillets & steaks	United Kingdom	G2	E
2672388	10/06/2019	All other pork	United Kingdom	W1	E
2672390	10/06/2019	All other pork	United Kingdom	H1	С
2672404	10/07/2019	Pork fillets & steaks	United Kingdom	G2	E
2672407	10/07/2019	Pork fillets & steaks	Netherlands	G1	В
2672408	10/07/2019	Pork fillets & steaks	United Kingdom	C2	Н
2672410	10/07/2019	All other pork	United Kingdom	X1	G
2672413	04/07/2019	Pork fillets & steaks	United Kingdom	J1	I
2672419	11/07/2019	Pork chops	United Kingdom	H1	С
2672425	12/07/2019	All other pork	United Kingdom	M1	I
2672427	12/07/2019	Pork fillets & steaks	United Kingdom	?	?
2672428	12/07/2019	Pork fillets & steaks	United Kingdom	X1	G
2672430	12/07/2019	Pork chops	United Kingdom	B1	Н
2672471	11/07/2019	Pork fillets & steaks	United Kingdom	H1	С
2672667	13/08/2019	All other pork	Ireland	G1	В
2672668	13/08/2019	Pork fillets & steaks	Germany	J2	D
2672670	13/08/2019	Pork fillets & steaks	United Kingdom	11	I
2672671	13/08/2019	Pork fillets & steaks	United Kingdom	H1	С
2672682	07/08/2019	Pork fillets & steaks	United Kingdom	C2	Н
2672684	07/08/2019	Pork chops	United Kingdom	P1	?
2672686	07/08/2019	All other pork	Germany	K2	С
2672691	20/08/2019	Pork fillets & steaks	United Kingdom	C2	Н
2672693	20/08/2019	All other pork	Netherlands	K1	В
2672694	20/08/2019	Pork fillets & steaks	Denmark	G1	В
2672724	07/08/2019	All other pork	Belgium	?	?

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
2672733	12/08/2019	Pork fillets & steaks	United Kingdom	L1	F
2672734	12/08/2019	Pork chops	United Kingdom	M1	I
2672736	12/08/2019	Pork chops	Germany	G1	В
2672739	12/08/2019	Pork fillets & steaks	United Kingdom	W1	E
2672754	12/08/2019	Pork chops	Germany	G1	В
2672757	12/08/2019	Pork fillets & steaks	United Kingdom	Q2	J
2672758	12/08/2019	All other pork	United Kingdom	Q2	J
2672761	07/08/2019	Pork chops	United Kingdom	C2	Н
2672763	07/08/2019	Pork fillets & steaks	Germany	G1	В
2672764	07/08/2019	Pork fillets & steaks	Spain	N2	?
2672767	07/08/2019	Pork fillets & steaks	Germany	J2	D
2672799	13/09/2019	Pork fillets & steaks	United Kingdom	G1	В
2672801	13/09/2019	Pork fillets & steaks	United Kingdom	A1	?
2672802	13/09/2019	All other pork	United Kingdom	A1	?
2672804	16/09/2019	Pork fillets & steaks	United Kingdom	H1	С
2672805	16/09/2019	All other pork	United Kingdom	H1	С
2672806	16/09/2019	Pork fillets & steaks	United Kingdom	E1	A
2672811	16/09/2019	All other pork	United Kingdom	?	?
2685586	09/05/2019	Pork chops	Germany	G1	В
2685588	09/05/2019	All other pork	United Kingdom	W1	E
2685591	07/06/2019	Pork chops	Germany	G1	В
2685595	07/06/2019	All other pork	United Kingdom	Q2	J
2685597	07/06/2019	Pork chops	United Kingdom	P1	?
2685625	12/04/2019	Pork fillets & steaks	Belgium	?	?
2685626	12/04/2019	All other pork	Netherlands	K2	С
2685627	09/05/2019	Pork fillets & steaks	United Kingdom	C2	Н
2685628	11/04/2019	Pork fillets & steaks	Ireland	O1	В
2685629	11/04/2019	Pork fillets & steaks	United Kingdom	02	?
2685630	11/04/2019	All other pork	United Kingdom	D2	?
2685640	15/04/2019	Pork fillets & steaks	United Kingdom	G1	В
2685642	15/04/2019	Pork fillets & steaks	United Kingdom	H1	С
2685646	10/07/2019	Pork fillets & steaks	United Kingdom	L1	F
2685661	07/06/2019	All other pork	United Kingdom	F1	G
2685666	13/09/2019	All other pork	United Kingdom	?	?
2685668	13/09/2019	All other pork	United Kingdom	C2	H
2685670	13/09/2019	Pork fillets & steaks	United Kingdom	?	?
2685672	12/04/2019	All other pork	United Kingdom	H1	С
2685674	12/04/2019	All other pork	Germany	K1	В

Unique Sample ID	Date of testing at APHA	Food Category	Country of origin	Brands if ≥ 2 samples	Super- market code
2685675	12/04/2019	All other pork	United Kingdom	C2	Η
2685676	14/05/2019	All other pork	United Kingdom	P1	?
2685682	08/05/2019	All other pork	United Kingdom	Q2	J
2685688	09/05/2019	Pork fillets & steaks	United Kingdom	P1	?
2685690	14/05/2019	Pork chops	United Kingdom	Q2	J
2685698	25/04/2019	All other pork	United Kingdom	H1	С
2685701	25/04/2019	All other pork	Netherlands	H1	С
2685702	04/06/2019	All other pork	Netherlands	K2	С
2685704	04/06/2019	All other pork	United Kingdom	H1	С
2685707	11/07/2019	Pork chops	United Kingdom	C2	Н
2685709	10/07/2019	Pork chops	United Kingdom	C2	Н
2685711	15/05/2019	Pork fillets & steaks	United Kingdom	C2	Н
2685712	15/05/2019	All other pork	United Kingdom	W1	E
2685736	10/06/2019	Pork fillets & steaks	United Kingdom	G1	В
2685738	10/06/2019	All other pork	Denmark	H1	С
2685849	15/05/2019	All other pork	United Kingdom	C2	Η
2685912	10/10/2019	All other pork	United Kingdom	T1	?
2685914	10/10/2019	Pork fillets & steaks	United Kingdom	Q2	J
2685915	10/10/2019	All other pork	United Kingdom	Q1	G
2686012	13/03/2019	Pork chops	Germany	G1	В
2686022	13/03/2019	Pork fillets & steaks	United Kingdom	12	С
2686070	12/03/2019	Pork fillets & steaks	Denmark	H1	С

? - Shop not on list

Food Group (beef/ pork)	Country of Origin	No. of samples tested
Beef	Argentina	1
Beef	Ireland	16
Beef	Netherlands	1
Beef	Non-European Union	1
Beef	Spain	1
Beef	United Kingdom	269
Pork	Belgium	3
Pork	Denmark	10
Pork	European Union	1
Pork	France	2
Pork	Germany	26
Pork	Ireland	3
Pork	Netherlands	17
Pork	Spain	4
Pork	United Kingdom	219

 Table 3. Country of origin of samples (excludes December 2019 samples).

Supermarket code	Food Group (beef/ pork)	No. of samples tested
?	Beef	35
?	Pork	25
A	Beef	13
A	Pork	8
В	Beef	34
В	Pork	44
С	Beef	43
С	Pork	60
D	Beef	4
D	Pork	6
E	Beef	25
E	Pork	32
F	Beef	7
F	Pork	5
G	Beef	20
G	Pork	20
Н	Beef	37
Н	Pork	33
l	Beef	21
	Pork	11
J	Beef	36
J	Pork	24

Table 4. Number of beef and pork samples tested per supermarket (excludesDecember 2019 samples).

? - Shop not on list

Table 5. Number of beef and pork samples tested per brand if two or more samplesper brand (excludes December 2019 samples).

Brands if two or more samples per brand	Food Group (beef/ pork)	No. of samples tested
A1	Beef	1
A1	Pork	2
A2	Beef	3
B1	Beef	5
B1	Pork	1
B2	Beef	12
B2	Pork	2
C1	Beef	1
C1	Pork	1
C2	Beef	31
C2	Pork	32
D1	Beef	1
D1	Pork	1
D2	Beef	1
D2	Pork	1
E1	Beef	1
E1	Pork	6
E2	Beef	2
F1	Beef	2
F1	Pork	11
G1	Beef	23
G1	Pork	33
G2	Beef	4
G2	Pork	4
H1	Beef	28
H1	Pork	40
H2	Beef	2
11	Beef	15
11	Pork	3
12	Beef	12
12	Pork	2
J1	Beef	1
J1	Pork	1
J2	Beef	2
J2	Pork	6
K1	Pork	10
K2	Pork	18
L1	Beef	4

Brands if two or more samples per brand	Food Group (beef/ pork)	No. of samples tested
L1	Pork	5
L2	Beef	1
L2	Pork	1
M1	Beef	5
M1	Pork	7
M2	Beef	2
N1	Beef	3
N2	Pork	2
01	Beef	1
01	Pork	1
02	Beef	1
02	Pork	1
P1	Beef	21
P1	Pork	12
P2	Beef	1
P2	Pork	1
Q1	Beef	1
Q1	Pork	1
Q2	Beef	26
Q2	Pork	24
S1	Beef	2
T1	Beef	1
T1	Pork	1
U1	Beef	10
W1	Beef	20
W1	Pork	28
X1	Beef	8
X1	Pork	8
Y1	Pork	2
Z1	Beef	19

Table 6. Summary of samples positive for *E. coli* from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX) or CHROMagar™ ESBL (CA-ESBL)

Sample ID	Date tested APHA	Meat type	Meat cut	Brand code	Retail store code	Sampling Location	Country of Origin	Growth on MCA- CTX ^a	Resistance Phenotype ^a	Growth on CA- ESBL ^b	CA- ESBL PCR result	CA-ESBL CTX gene sequence	Batch/Lot Number
381029	06/03/2019	Pork	Pork fillets & steaks	H1	С	Kent Thames Gateway	UK	Yes	AmpC	No	NA	NA	2 001 058 18:47 1115
2685696	25/04/2019	Beef	Beef steaks- expensiv e	11	I	Buckinghamshire CC	UK	Yes	ESBL	Yes	С	CTX-M 14	A903 14:34 L7 108
2685676	14/05/2019	Pork	All other pork	P1	?	Tyneside	UK	Yes	ESBL	Yes	СТ	CTX-M 24	-
1562514	04/06/2019	Pork	Pork fillets & steaks	P1	?	Haringey and Islington	UK	No	NA	Yes	С	CTX-M 14	-
2664655	12/09/2019	Pork	Pork fillets & steaks	P1	?	Gloucestershire	UK	Yes	ESBL	No	NA	NA	-
2664465	05/11/2019	Pork	Pork fillets & steaks	X1	G	Birmingham	UK	No	NA	Yes	С	CTX-M 1	9295 19:15

a – EU harmonised test method

- *b* UK non-harmonised additional test
- ? Shop not on list
- NA Not applicable for the isolate, e.g. MICs are not performed for isolates from CA-ESBL
- C isolate positive for bla_{CTX-M} gene, T isolate positive for bla_{TEM} gene

	No. Resistant ^a / no. tested				
Antibiotic	Beef	Pork	Pork		
	ESBL	ESBL	AmpC		
Ampicillin	1/1	2/2	1/1		
Azithromycin	0/1	0/2	0/1		
Cefepime ^b	1/1	2/2	1/1		
Cefotaxime ^b	1/1	2/2	1/1		
Cefoxitin ^b	0/1	0/2	1/1		
Ceftazidime ^b	1/1	2/2	1/1		
Chloramphenicol	0/1	0/2	0/1		
Ciprofloxacin	0/1	0/2	0/1		
Colistin ^c	0/1	0/2	0/1		
Ertapenem ^c	0/1	0/2	0/1		
Gentamicin	0/1	0/2	0/1		
Imipenem ^c	0/1	0/2	0/1		
Meropenem ^c	0/1	0/2	0/1		
Nalidixic Acid	0/1	0/2	0/1		
Sulfamethoxazole	1/1	1/2	0/1		
Temocillin	0/1	0/2	0/1		
Tetracycline	0/1	1/2	1/1		
Tigecycline	0/1	0/2	0/1		
Trimethoprim	0/1	1/2	1/1		

Table 7. Summary of resistances of *E. coli* isolated on MCA CTX agar

a – Microbiologically resistant using EUCAST ECOFFS.

b – Crtitically important cephalosporin antibiotics

c – Last resort antibiotics - three carbapenem antibiotics ertapenem, imipenem and meropenem and colistin

Antibiotic	Indicator for MIC	MIC (ug/ml)	Interpretation of MIC ^a
Ampicillin	>	64	R
Azithromycin	=	8	S
Cefepime ^b	=	0.25	R
Cefotaxime ^b	>	16	R
Cefotaxime / Clavulanate ^c	=	8	No synergy
Cefoxitin ^b	>	16	R
Ceftazidime ^b	=	16	R
Ceftazidime / Clavulanate ^c	=	8	No synergy
Chloramphenicol	<=	8	S
Ciprofloxacin	<=	0.015	S
Colistin ^d	<=	1	S
Ertapenem ^d	=	0.03	S
Gentamicin	<=	0.5	S
Imipenem ^d	=	0.25	S
Meropenem ^d	<=	0.03	S
Nalidixic Acid	<=	4	S
Sulfamethoxazole	=	32	S
Temocillin	=	16	S
Tetracycline	>	64	R
Tigecycline	<=	0.25	S
Trimethoprim	<=	0.25	S

Table 8a. MIC results for AmpC *E. coli* isolate from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX) from UK pork sample 381029.

R- resistant, S – sensitive

a – Microbiologically resistant or sensitive using EUCAST ECOFFS.

b – Critically important cephalosporin antibiotics.

c – Last resort antibiotics - carbapenem antibiotics ertapenem, imipenem and meropenem and colistin.

Table 8b. MIC results for ESBL *E. coli* isolate from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX) from UK beef sample 2685696.

Antibiotic	Indicator	MIC	Interpretation of			
	if not =	(µg/ml)	MIC ^a			
Ampicillin	>	64	R			
Azithromycin	=	4	S			
Cefepime ^b	=	4	R			
Cefotaxime ^b	=	64	R			
Cefotaxime / Clavulanate ^c	<=	0.06	Synergy			
Cefoxitin ^b	=	4	S			
Ceftazidime ^b	=	1	R			
Ceftazidime / Clavulanate ^c	=	0.25	Synergy			
Chloramphenicol	<=	8	S			
Ciprofloxacin	<=	0.015	S			
Colistin ^d	<=	1	S			
Ertapenem ^d	<=	0.015	S			
Gentamicin	=	2	S			
Imipenem ^d	<=	0.12	S			
Meropenem ^d	<=	0.03	S			
Nalidixic Acid	<=	4	S			
Sulfamethoxazole	>	1024	R			
Temocillin	=	8	S			
Tetracycline	<=	2	S			
Tigecycline	<=	0.25	S			
Trimethoprim	<=	0.25	S			

R- resistant, S – sensitive

a – Microbiologically resistant or sensitive using EUCAST ECOFFS.

b – Critically important cephalosporin antibiotics.

c – Last resort antibiotics - carbapenem antibiotics ertapenem, imipenem and meropenem and colistin.

Table	e 8c.	MIC	results	for	ESBL	E.	coli	isolate	from	MacConkey	agar	+ ′	1 mg/L	cefotaxime
(MCA	-CT>	K) froi	m UK po	ork s	sample	26	6856	76.		-	-		-	

Antibiotic	Indicator	MIC	Interpretation of			
Antibiotic	if not =	(µg/ml)	MIC ^a			
Ampicillin	>	64	R			
Azithromycin	<=	2	S			
Cefepime ^b	=	4	R			
Cefotaxime ^b	=	32	R			
Cefotaxime / Clavulanate ^c	<=	0.06	Synergy			
Cefoxitin ^b	=	4	S			
Ceftazidime ^b	=	1	R			
Ceftazidime / Clavulanate ^c	<=	0.12	Synergy			
Chloramphenicol	<=	8	S			
Ciprofloxacin	<=	0.015	S			
Colistin ^d	<=	1	S			
Ertapenem ^d	<=	0.015	S			
Gentamicin	<=	0.5	S			
Imipenem ^d	<=	0.12	S			
Meropenem ^d	<=	0.03	S			
Nalidixic Acid	<=	4	S			
Sulfamethoxazole	>	1024	R			
Temocillin	=	4	S			
Tetracycline	=	64	R			
Tigecycline	<=	0.25	S			
Trimethoprim	>	32	R			

R- resistant, S - sensitive

a – Microbiologically resistant or sensitive using EUCAST ECOFFS.

b – Critically important cephalosporin antibiotics.

c – Last resort antibiotics - carbapenem antibiotics ertapenem, imipenem and meropenem and colistin.

Table 8d. MIC results for ESBL *E. coli* isolate from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX) from UK pork sample 2664655.

Antibiotic	Indicator	MIC	Interpretation of			
	If not =	(µg/mi)				
Ampiciilin	>	64	R			
Azithromycin	=	4	S			
Cefepime ^b	=	4	R			
Cefotaxime ^b	=	32	R			
Cefotaxime / Clavulanate ^c	<=	0.06	Synergy			
Cefoxitin ^b	=	8	S			
Ceftazidime ^b	=	1	R			
Ceftazidime / Clavulanate ^c	=	0.25	Synergy			
Chloramphenicol	<=	8	S			
Ciprofloxacin	<=	0.015	S			
Colistin ^d	<=	1	S			
Ertapenem ^d	<=	0.015	S			
Gentamicin	=	1	S			
Imipenem ^d	<=	0.12	S			
Meropenem ^d	<=	0.03	S			
Nalidixic Acid	<=	4	S			
Sulfamethoxazole	<=	8	S			
Temocillin	=	8	S			
Tetracycline	<=	2	S			
Tigecycline	<=	0.25	S			
Trimethoprim	=	0.5	S			

R- resistant, S – sensitive

a – Microbiologically resistant or sensitive using EUCAST ECOFFS.

b – Critically important cephalosporin antibiotics.

c – Last resort antibiotics - carbapenem antibiotics ertapenem, imipenem and meropenem and colistin.

Table 9. Samples positive on MacConkey agar + 2 mg/L colistin (excludes December 2019samples) sorted by date.

Date of testing at APHA	Unique Sample ID	Food Group (beef/ pork)	Country of Origin	Growth MCA - COL	Growth MCA- CTX	Growth CA- ESBL	<i>mcr</i> - 1 PCR	<i>mcr</i> - 2 PCR	<i>mcr</i> - 3 PCR	Super- market codes	Brands if two or more samples per brand
18/01/2019	2447871	Beef	UK	+	-	-	-	-	-	С	H1
18/01/2019	2447876	Pork	UK	+	-	-	-	-	-	E	W1
18/01/2019	2447884	Pork	Ireland	+	-	-	-	-	-	В	G1
18/01/2019	2447878	Beef	UK	+	-	-	-	-	-	J	Q2
21/01/2019	2447862	Pork	UK	+	-	-	-	-	-	С	K2
21/01/2019	1563634	Beef	UK	+	-	-	-	-	-	F	L1
21/01/2019	2558546	Beef	Ireland	+	-	-	-	-	-	В	G1
21/01/2019	381078	Beef	UK	+	-	-	-	-	-	С	H1
21/01/2019	2558547	Pork	UK	+	-	-	-	-	-	I	M1
06/02/2019	2447843	Beef	UK	+	-	-	-	-	-	I	M1
06/02/2019	2447816	Pork	UK	+	-	-	-	-	-	F	L1
06/02/2019	380976	Pork	UK	+	-	-	-	-	-	В	G1
07/02/2019	2447832	Beef	UK	+	-	-	-	-	-	С	H1
07/02/2019	2447846	Beef	Ireland	+	-	-	-	-	-	С	H1
07/02/2019	2447824	Pork	UK	+	-	-	-	-	-	В	K1
07/02/2019	2686065	Beef	UK	+	-	-	-	-	-	В	U1
07/02/2019	2447841	Pork	UK	+	-	-	-	-	-	G	F1
07/02/2019	1614373	Pork	UK	+	-	-	-	-	-	J	Q2
06/03/2019	2447855	Pork	Germany	+	-	-	-	-	-	В	G1
06/03/2019	2447856	Beef	Ireland	+	-	-	-	-	-	С	H1
06/03/2019	558578	Beef	Ireland	+	-	-	-	-	-	В	G1
12/03/2019	2558197	Beef	UK	+	-	-	-	-	-	Н	C2
12/03/2019	2447834	Beef	UK	+	-	-	-	-	-	I	11
12/03/2019	2447768	Pork	Denmark	+	-	-	-	-	-	С	H1
12/03/2019	2447831	Pork	France	+	-	-	-	-	-	D	J2
11/04/2019	540797	Pork	Netherlands	+	-	-	-	-	-	С	K2
11/04/2019	2685630	Pork	UK	+	-	-	-	-	-	?	D2
12/04/2019	2685622	Beef	UK	+	-	-	-	-	-	F	L1
12/04/2019	2685623	Beef	UK	+	-	-	-	-	-	Н	C2
15/04/2019	540490	Beef	UK	+	-	-	-	-	-	В	G1
15/04/2019	2685640	Pork	UK	+	-	-	-	-	-	В	G1
15/04/2019	2685644	Beef	UK	+	-	-	-	-	-	J	Q2
15/04/2019	2685641	Beef	UK	+	-	-	-	-	-	В	G1
25/04/2019	2685696	Beef	UK	+	+	+	-	-	-		11

Date of testing at APHA	Unique Sample ID	Food Group (beef/ pork)	Country of Origin	Growth MCA - COL	Growth MCA- CTX	Growth CA- ESBL	<i>mcr</i> - 1 PCR	<i>mcr</i> - 2 PCR	<i>mcr</i> - 3 PCR	Super- market codes	Brands if two or more samples per brand
25/04/2019	540733	Pork	UK	+	-	-	-	-	-	А	E1
08/05/2019	2685682	Pork	UK	+	-	-	-	-	-	J	Q2
14/05/2019	2672348	Pork	UK	+	-	-	-	-	-	E	W1
16/05/2019	2672345	Pork	UK	+	-	-	-	-	-	Η	C2
10/06/2019	2685738	Pork	Denmark	+	-	-	-	-	-	С	H1
10/06/2019	540664	Pork	UK	+	-	-	-	-	-	Н	C2
08/10/2019	2664647	Beef	UK	+	-	-	-	-	-	E	G2
08/10/2019	2664648	Pork	UK	+	-	-	-	-	-	E	W1
09/10/2019	2664653	Beef	UK	+	-	-	-	-	-	С	H1
09/10/2019	6489	Pork	UK	+	-	-	-	-	-	Н	C2
09/10/2019	2664639	Beef	UK	+	-	-	-	-	-	J	Z1

+ Growth on agar or positive for *mcr* gene.

- No growth on agar or negative for *mcr* gene.

10. Figures

Figure 1. Decrease in the percentage of EU beef and pork samples testing positive for AmpC, ESBL and AmpC + ESBL *E. coli* between 2015 and 2017– EU EFSA data.



Figure 2. Decrease in the maximum country levels (for all countries submitting results) of AmpC-ESBL-producing *E. coli* reported for beef and pork between 2015 and 2017 – EU EFSA data.





Figure 3. Percentages of UK beef and pork samples positive for AmpC-/ESBLphenotype *E. coli* on MCA-CTX agar from 2015 to 2019

11. References

[1] Fang H, Ataker F, Hedin G, Dornbusch K. Molecular epidemiology of extendedspectrum beta-lactamases among *Escherichia col*i isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. J Clin Microbiol. 2008;46:707-12.

[2] Duggett NA, Sayers E, AbuOun M, Ellis RJ, Nunez-Garcia J, Randall L, et al. Occurrence and characterization of mcr-1-harbouring *Escherichia coli* isolated from pigs in Great Britain from 2013 to 2015. J Antimicrob Chemother. 2017;72:691-5.

[3] DTU. Quantification of ESBL/AmpC-producing *Escherichia coli* in caecal content and fresh meat samples. 2017. https://www.eurl-

ar.eu/CustomerData/Files/Folders/21-protocols/399_esbl-ampc-quantificationprotocol-19-03-2018.pdf

[4] Anonymous. EFSA EUSR-AMR Workflow and Criteria for

ESBL/AmpC/Carbapenemase -Phenotypes. 2018a. https://www.eurlar.eu/CustomerData/Files/Folders/3-workshop-kgs-lyngby-april2016/25_efsa-eusramr-workflow-and-criteria-for-esbl-ampc-carbapenemase-phenotypes.pdf.

[5] EUCAST. MIC and zone diameter distributions and ECOFFs. 2018. http://www.eucast.org/mic_distributions_and_ecoffs/.

[6] EFSA. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. EFSA Journal. 2019;17:1-278.

[7] Anonymous. EU Harmonised Survey of Antimicrobial Resistance (AMR) on Retail Meats (Year 5: Beef and Pork) – retail sample collection, transportation and survey design services. Report of Hallmark Veterinary complinace services. 2020.

[8] Randall LP, Lemma F, Koylass M, Rogers J, Ayling RD, Worth D, et al. Evaluation of MALDI-ToF as a method for the identification of bacteria in the veterinary diagnostic laboratory. Res Vet Sci. 2015;101:42-9.

[9] Carattoli A, Garcia-Fernandez A, Varesi P, Fortini D, Gerardi S, Penni A, et al. Molecular epidemiology of *Escherichia coli* producing extended-spectrum betalactamases isolated in Rome, Italy. J Clin Microbiol. 2008;46:103-8. [10] Sabate M, Navarro F, Miro E, Campoy S, Mirelis B, Barbe J, et al. Novel complex sul1-type integron in *Escherichia coli* carrying bla(CTX-M-9). Antimicrob Agents Chemother. 2002;46:2656-61.

[11] FSA. RDFS102109 - EU Harmonised Surveillance of Antimicrobial Resistance(AMR) in E. coli from Retail Meats (Year 2 - Chicken). 2017.https://www.food.gov.uk/sites/default/files/media/document/fs102109y2.pdf.

[12] EFSA. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. EFSA Journal. 2017;15:1-212.

[13] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet infectious diseases. 2015;16:161–8.

[14] VMD. UK Veterinary Antibiotic Resistance and Sales Surveillance Report. 2019. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/58234 1/1051728-v53-UK-VARSS_2015.pdf.

[15] Pomba C, da Fonseca JD, Baptista BC, Correia JD, Martinez-Martinez L. Detection of the pandemic O25-ST131 human virulent *Escherichia coli* CTX-M-15-producing clone harboring the *qnrB2* and *aac(6')-lb-cr* genes in a dog. Antimicrob Agents Chemother. 2009;53:327-8.

[16] Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev. 2014;27:543-74.

[17] Horton RA, Duncan D, Randall LP, Chappell S, Brunton LA, Warner R, et al.
 Longitudinal study of CTX-M ESBL-producing *E. coli* strains on a UK dairy farm.
 Res Vet Sci. 2016;109:107-13.

[18] Randall LP, Lodge MP, Elviss NC, Lemma FL, Hopkins KL, Teale CJ, et al. Evaluation of meat, fruit and vegetables from retail stores in five United Kingdom regions as sources of extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant *Escherichia coli*. International Journal of Food Microbiology. 2017;241:283-90. [19] Randall LP, Lemma F, Rogers JP, Cheney TE, Powell LF, Teale CJ. Prevalence of extended-spectrum-beta-lactamase-producing *Escherichia coli* from pigs at slaughter in the UK in 2013. J Antimicrob Chemother. 2014;69:2947-50.

[20] Valentin L, Sharp H, Hille K, Seibt U, Fischer J, Pfeifer Y, et al. Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. Int J Med Microbiol. 2014;304:805-16.

[21] Petternel C, Galler H, Zarfel G, Luxner J, Haas D, Grisold AJ, et al. Isolation and characterization of multidrug-resistant bacteria from minced meat in Austria. Food microbiology. 2014;44:41-6.

[22] Geser N, Stephan R, Hachler H. Occurrence and characteristics of extendedspectrum beta-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC veterinary research. 2012;8:21.

[23] Carmo LP, Nielsen LR, da Costa PM, Alban L. Exposure assessment of extended-spectrum beta-lactamases/AmpC beta-lactamases-producing *Escherichia coli* in meat in Denmark. Infection ecology & epidemiology. 2014;4.

[24] FSA. FS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in Bacteria from Retail Meats (Year 1). 2016.

https://www.food.gov.uk/sites/default/files/media/document/fs102109finreport.pdf.

[25] El Garch F, de Jong A, Bertrand X, Hocquet D, Sauget M. mcr-1-like detection in commensal *Escherichia coli* and Salmonella spp. from food-producing animals at slaughter in Europe. Vet Microbiol. 2018;213:42-6.

[26] Randall LP, Horton RA, Lemma F, Martelli F, Duggett NAD, Smith RP, et al. Longitudinal study on the occurrence in pigs of colistin-resistant *Escherichia coli* carrying *mcr-1* following the cessation of use of colistin. J Appl Microbiol. 2018.

[27] Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clin Microbiol Rev. 2017;30:557-96.

[28] Cavaco LM, Abatih E, Aarestrup FM, Guardabassi L. Selection and persistence of CTX-M-producing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrob Agents Chemother. 2008;52:3612-6. [29] Agersø Y, Aarestrup FM. Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended-spectrum cephalosporinase-producing *Escherichia coli* in slaughter pigs. J Antimicrob Chemother. 2013;68:569-72.

[30] VMD. UK Veterinary Antibiotic Resistance and Sales Surveillance Report. 2015. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/58234 1/1051728-v53-UK-VARSS_2015.pdf.

[31] Anonymous. Guidelines for the prudent use of antimicrobials in veterinary medicine. Practical examples.

https://ec.europa.eu/health/sites/health/files/antimicrobial_resistance/docs/2015_pru dent_use_guidelines_annex_en.pdf. European commission notice. 2015. Brussels, 10.9.2015:1-54.

[32] FSA. Report on the presence of colistin resistant and *mcr-1* plasmid mediated colistin resistant *E. coli* and *Klebsiella* on five beef knuckle samples. 2017a.
12. Appendix – Discussion from the 2017 report

Previous published studies prior to commissioning of the current EU surveys showed that 20% of minced beef from Austria were positive for mainly CTX-M-1 ESBL-producing *E. coli* [21], whilst another study in Switzerland in 2012 found that none of 104 minced beef and pork samples were positive for ESBL-producing *Enterobacteriaceae*, although in this study 15.3% of the porcine, 13.7% of the bovine, 8.6% of the sheep and 63.4% of the chicken faecal samples yielded ESBL-producers after an enrichment step [22]. Another study in Denmark in 2014 found that 83.8% of broiler meat, 12.5% of pork and 3.7% of beef tested was contaminated with AmpC / ESBL *E. coli* [23]. However, these studies lack a uniform methodology across different countries that is employed in current EU harmonised studies such as reported here.

For 2017 UK beef and pork retail meat samples tested in this study using the EU harmonised method (MCA-CTX), 0.32% (for both meat types) were positive for AmpC phenotype *E. coli* whilst, 0.32% of beef samples only were positive for ESBL phenotype *E. coli*. These results exclude the one extra pork sample positive on CA-ESBL only, since this is an extra test outside the EU harmonised method.

In the EU survey of AMR in bacteria from UK retail meat in 2015 [24], the percentages of beef and pork samples that were positive for ESBL phenotype *E. coli* were 1.0% and 2.1% respectively, and the percentages of beef and pork samples therefore that were positive for AmpC phenotype *E. coli* were 1.0% and 0.4% respectively. As such, between 2015 and 2019, the percentage of retail samples of beef and pork in the UK contaminated with AmpC or ESBL phenotype *E. coli* has remained almost identical.

In a slightly earlier study in which retail beef (n = 159) and pork (n = 79) meat samples were collected and tested in 2013-2014 from 5 different regions in the UK, 1.9% and 2.5% of beef and pork samples respectively, were positive for ESBLproducing *E. coli*, whilst 0.8% of beef samples and 1.3% of pork samples were positive for *E. coli* carrying the AmpC *bla*_{CIT} genes, with *bla*_{CMY-2} the most frequent variant detected by sequencing [18]. This earlier study, whilst suggesting there has been a slight reduction in the numbers of beef and pork contaminated with AmpC or ESBL phenotype *E. coli* between 2013/14 and 2017, involved a different sampling strategy and different isolation agars, as discussed previously [11].

Results for the 2015 EU monitoring of beef and pork for presumptive ESBL-/AmpC-/carbapenemase-producing *E. coli* have now been published by EFSA [24]. In 2015, EU monitoring was performed on a mandatory basis by 22 member states (MSs) and two non-MSs on meat from pigs, and by 23 MSs and two non-MSs on meat from bovine animals [24]. Results for the UK compared favourably with results from other countries in that presumptive AmpC phenotype *E. coli* in beef in 2015 ranged from 0% in Switzerland to 11.5% in Bulgaria (1% UK), whilst ESBL phenotype *E. coli* in beef ranged from 0% in Switzerland to 17.3% in Bulgaria (1% UK) [24]. For pork, presumptive AmpC phenotype *E. coli* in 2015 ranged from 0% in Switzerland to 6.6% in in the Czech republic (0.4% UK), whilst ESBL phenotype *E. coli* in pork ranged from 0.3% in Sweden to 20.8% in Bulgaria (2.1% UK) [24].

The predominant *E. coli* strain associated with human infections is the pandemic O25-ST131 CTX-M-15-producing clone [15, 16]. Only three isolates from CA-ESBL agar were tested by multiplex PCR for *bla*_{CTX}, *bla*_{OXA}, *bla*_{TEM} and *bla*_{SHV} genes, of which two isolates (both beef isolates and both from samples also positive on MCA-CTX) were positive for *bla*_{CTX-M} of sequence type CTX-M 1. As such none of the samples were positive for the human pandemic O25-ST131 CTX-M-15- producing *E. coli* clone. Use of the additional CA-ESBL agar allowed for detection from one sample of an ESBL *E. coli* not isolated from MCA-CTX. As such this beef sample was positive for two different isolates of *E. coli*, one of which was an AmpC and the other an ESBL phenotype.

Whilst a total of three samples out of 624 tested in this study were positive for AmpC or ESBL-phenotype *E. coli* on MCA-CTX agar, none of these isolates were resistant to the last resort antibiotics such as colistin and the three carbapenem antibiotics tested. However, 39 of the beef samples (12.4%) and 46 of the pork samples (14.8%) gave rise to presumptive *E. coli* on MCA-COL agar (colistin resistance), and one of the beef samples was positive for *mcr-1* plasmid mediated colistin resistant *E. coli*, as previously reported. In a recent study, 10,206 isolates of *E. coli* from cattle, chickens and pigs from EU member states were tested for resistance to

colistin and for the presence of the plasmid mediated colistin resistance gene *mcr-1* [25]. Of the 10,206 *E. coli* isolates, only 1.4% were resistant to colistin, and 0.7% of isolates were positive for *mcr-1* [25]. Whilst the percentage of beef and pork samples in this study that yielded presumptive *E. coli* that were colistin resistant were much higher than reported from the 10,206 *E. coli* from cattle, chickens and pigs [25], the methodologies were not comparable. Isolates from cattle, chickens and pigs were not stated to be selected with media containing colistin, which will specifically select for colistin resistant *<u>E. coli</u>*. Additionally, isolates from beef and pork in this study were not biochemically confirmed as *E. coli* (beyond being lactose fermenters on MCA-COL agar), unless first confirmed to be *mcr-1* positive. If a single *E. coli* was isolated from each of the meat samples using non-selective media, the percentage of samples that were positive for colistin resistant *E. coli* would have been much lower. The mechanisms of colistin resistance in the *mcr-1* negative isolates selected on MCA-COL agar is likely to be due to chromosomal mutations [26, 27].

It has been suggested, as discussed in a previous EU report [11], that to reduce the occurrence of AmpC-/ESBL-producing *E. coli* in livestock and in retail meat, it might be prudent to avoid use of cephalosporin antibiotics and reduce the use of other antimicrobials to as little as possible, but as much as necessary in livestock; to improve biosecurity to reduce ESBL / AmpC-producing bacterial dissemination; to improve slaughter hygiene and to perform some type of decontamination after slaughter [23].

In pigs a previous study showed that use of ceftiofur and cefquinome exerted a selective pressure for ESBL *E. coli* [28], whilst another study showed reduction of ESBL *E. coli* in pigs following introduction of voluntary restrictions on cephalosporin use [29].

It is also interesting to note that there was a significant drop (odds ratio 0.45 p-value < 0.001) in the percentages of ESBL *E. coli* isolated from chicken meat in 2013/14 (65.4%) [18] compared to the UK EU survey of 2016 (29.7%) [11]. In 2012 the British Poultry council, which represents more than 90% of the UK poultry meat

production, banned the use of all cephalosporins in flocks used for poultry meat production [30, 31].

In conclusion, the results of the second year of EU monitoring of retail beef and pork for AmpC and ESBL-phenotype *E. coli* in the UK showed only a low level of < 1% of samples were positive for AmpC or ESBL *E. coli* following examination using sensitive detection methods, and these results are similar to results for these meats in 2015 [24]. With respect to resistance to last resort antibiotics, none of the samples were positive for carbapenem resistant *E. coli*. Some of the samples were positive for colistin resistant presumptive *E. coli* and one of these samples was positive for the *mcr-1* plasmid mediated colistin resistance gene as previously reported [32].