



# FS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in Bacteria from Retail Meats (Year 3 - Beef and Pork, 2017)

# **Final report**

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# **Table of contents**

1.	Liability statement	3
2.	Lay person's summary	4
3.	Project summary	7
4.	Glossary	13
5.	Materials and methods	14
6.	Discussion	23
7.	Conclusions	27
8.	Tables	28
9.	Figures	51
10.	References	52

# 1. Liability statement

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## 2. Lay person's summary

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

The requirements (with additional detailed guidance from the EU Reference Laboratory for Antimicrobial Resistance) state that 300 retail 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  $\beta$ -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 resistance to a group of antibiotics known as carbapenems. Carbapenem antibiotics are also really important in human medicine, as they are termed "last resort" antibiotics, used to treat infections when all or almost all other treatment options are non-viable, due to the target bacteria being resistant to most / all other relevant antibiotics.

Other agars used at the request of the FSA to test UK samples (UK non-harmonised additional test) included an agar to specifically isolate *E. coli* with ESBL type resistance and an agar to isolate colistin resistant *E. coli*. Colistin is another "last

resort" antibiotic, so it is important to monitor if resistance to this type of bacteria is occurring in food samples.

Other additional work, outside of the scope of the EU survey, 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 resistance genes referred to as *mcr-1* and *mcr-2* were discovered in the last few years, so colistin resistant *E. coli* were tested for these two genes. The *mcr* genes are considered particularly important as they encodes resistance to the "last resort" antibiotic colistin, and as they are mobile they have the potential to transfer resistance in the gut to other similar bacteria. Finally, extra work requested by the FSA included performing counts of antibiotic resistant (AmpC and ESBL type resistance) *E. coli* in each sample, as this provides useful information on whether AmpC and / or ESBL positive meat samples have a low or high number of antibiotic resistant bacteria on them.

The number of samples allocated in each area was proportional to the population and samples were collected from the 11 supermarket chains in the "Big Four" and "Other large supermarket" categories, as well as from "other" shops.

The product categories were well defined to ensure consistency between surveyors. The pork samples categories were chops, fillets & steaks or other diced/sliced pork. The beef samples categories were less expensive steaks, expensive steaks or other diced/sliced beef. Samples were collected on a monthly basis during 1 week per month to ensure an even distribution between January and December 2017.

Overall, results showed less than 1% of 2017 retail beef and pork samples in the UK that were tested were positive for AmpC or ESBL producing *E. coli* (third generation cephalosporin resistance) using a sensitive detection method and these results were similar to the previous UK survey in 2015. Additionally, none of the samples gave rise to viable counts of *E. coli* above the detection limit of 40 bacteria per gram of meat on the two selective agars used indicating numbers of resistant bacteria in these samples were low.

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.

A single beef sample was found to be contaminated with recently identified *mcr-1* plasmid mediated colistin resistant *E. coli*, but *E. coli* with this resistance was not detected in any of the pork samples.

In 2015 EU monitoring of beef and pork for presumptive ESBL-/AmpC-/ carbapenemase-producing *E. coli* 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. Results for the UK compared favourably with results from other countries.

## 3. Project summary

In accordance with European Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, Member States (MS) are obliged to ensure that procedures are in place to monitor and report on the occurrence of antimicrobial resistance (AMR) in zoonotic organisms. The European Commission Implementing Decision 2013/652/EU, which came into force 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 retail beef and pork in 2017 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.

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 and outside of the remit of Decision 2013/652/EU, samples were also plated to CHROMagar<sup>™</sup> 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* and *mcr2* [2]. Finally, viable counts as colony forming units per gram of meat (cfu/g) of AmpC and ESBL-phenotype *E. coli* for all samples were also determined on MCA-CTX and CA-ESBL agars.

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 samples to APHA, and have reported separately on the sample details.

As this was the second year of sampling for beef and pork, the sampling plan was the same in terms of the number of samples required and the meat cuts etc.

[3]. However, the most recent market share data for year 3 was used, rather than using the same for year 1, to provide the most representative data for beef and pork in 2017. Selection of Retailers (Table 1) was based on the Family Food Data 2014

[4] and related figures provided by the FSA.

The 2017 red meat sampling plan used "proportionate stratified sampling" to allocate samples to NUTS3 areas and the samples were distributed in proportion to population. Eighty NUTS-3 locations with representation of England, Scotland, Wales and Northern Ireland that covers at least 80% of the total population were selected [3]. The number of samples allocated in each NUTS-3 area were proportional to the population size. Only fresh meat cuts were collected and analysed. Processed meat, minced meat, joints or meat with added herbs/spices were all excluded from sampling.

The product categories were well defined to ensure consistency between surveyors. Each sample was randomly assigned to a cut category, according to consumption which maximise the power of detecting different AMR between these cut categories [3]. The pork samples categories were: chops, fillets & steaks or other diced/sliced pork. The beef samples categories were less expensive steaks, expensive steaks or other diced/sliced beef [3]. Steaks that cost under £2 plus/100g were considered less expensive [3]. Expensive steak is defined as steak equal or above £2/100g [3]. Samples were collected on a monthly basis during 1 week per month to ensure an

even distribution between January and December 2017. Samples were collected from different supermarkets and parts of the UK (Table 1).

**Table 1.** Completed beef and pork sampling per retailer vs. UK countries in 2017 – HallMark Veterinary Compliance Services report [3].

Shop				Northern	United
Description	England	Wales	Scotland	Ireland	Kingdom
Asda	12.74%	25.0%	3.6%	11.1%	12.5%
Morrisons	12.36%	12.5%	17.9%	0.0%	12.5%
Sainsburys	13.90%	6.3%	7.1%	11.1%	12.8%
Tesco	18.73%	18.8%	17.9%	22.2%	18.8%
Aldi	7.34%	12.5%	7.1%	0.0%	7.4%
Со-ор	2.70%	0.0%	3.6%	0.0%	2.6%
Iceland	1.35%	0.0%	0.0%	0.0%	1.1%
Lidl	4.63%	6.3%	7.1%	11.1%	5.1%
Marks and					
Spencer	2.90%	6.3%	7.1%	0.0%	3.4%
Spar	0.00%	0.0%	0.0%	11.1%	0.3%
Waitrose	6.18%	0.0%	0.0%	0.0%	5.1%
Shop not on list	17.18%	12.5%	28.6%	33.3%	18.4%
Total	100.00%	100.00%	100.00%	100.00%	100.00%

A bespoke in-house APHA Standard Operating Procedure (SOP) based on published EU test methods was used as per previous years. The method involved enrichment of 25 grams of meat in Buffered Peptone Water (BPW), before plating this enrichment broth to the different selective agars used. 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.

In total 314 beef and 310 pork samples were tested between January and December 2017. Only three (0.48%, 95% confidence interval 0.16% to 1.40%) of the 624 samples tested according to the EU harmonised methods yielded *E. coli* colonies on MCA-CTX agar (third generation cephalosporin resistance). These samples comprised two of the beef samples (both steaks, one expensive and one less expensive) and one of the pork samples (belly slices) positive on MCA-CTX agar, representing 0.64% (95% confidence interval 0.17% to 2.29%) of the beef samples and 0.32% (95% confidence interval 0.06% to 1.8%) of the pork samples.

The two beef samples that were positive on MCA-CTX agar were also positive on CA-ESBL agar (UK non-harmonised additional test), and the resulting isolates tested were found to be positive for CTX-M 1 type ESBL gene. One additional pork sample (pork chops) not positive on MCA-CTX was also positive on CA-ESBL agar, and the resulting isolate tested was found to be positive for *bla*<sub>TEM</sub> gene.

None of the samples (n=624) were found to have carbapenem resistant *E. coli* on CARBA and OXA-48 by the EU harmonized method.

None of the samples (n=624) gave rise to viable counts of *E. coli* on MCA-CTX agar or on CA-ESBL agar above the detection limit of 40 cfu/gram (UK non-harmonised additional test).

Additional UK non-harmonised tests found 39 of the beef samples (12.4%, 95% confidence interval 9.22% to 16.53%) and 46 of the pork samples (14.8%, 95% confidence interval 11.31% to 19.23%) yielded presumptive *E. coli* on MCA-COL agar (indicating colistin resistance). Presumptive *E. coli* from MCA-COL agar were tested by RT-PCR for *mcr1* or *mcr2* genes [2]. One beef steak sample (expensive steak, sample ID 01562581) was found to be positive for *mcr-1* plasmid mediated colistin resistant *E. coli*. This sample was submitted to APHA Weybridge for testing on 16th May 2017 and APHA performed whole genome sequencing on three *mcr-1* positive isolates from this original sample, and results have previously been reported to the FSA (Annex 1).

Due to one beef steak sample being found positive for *mcr-1* plasmid mediated colistin resistant *E. coli*, the FSA requested that five additional beef knuckle samples were taken. The selected samples were from two different slaughter dates, with different lot numbers to the original sample, and were subsequently sent to APHA and tested on the 14th July 2017 for *mcr-1* plasmid mediated colistin resistant *E. coli* and *Klebsiella*. These samples were processed to include enumeration for both colistin sensitive and resistant *E. coli* and *Klebsiella* following both swabbing and homogenisation of meat samples, and also detection of colistin sensitive and resistant *E. coli* and *Klebsiella* after enrichment of samples. Whilst the original meat sample was positive for *mcr-1 E. coli*, none of the subsequent five beef knuckle

samples (deemed related, but with different lot numbers) tested at a later date were positive. These results have previously been reported to the FSA in an interim report entitled "Report on the presence of colistin resistant and *mcr-1* plasmid mediated colistin resistant *E. coli* and *Klebsiella* on five beef knuckle samples" and submitted to the FSA on the 8<sup>th</sup> of December 2017 [5] (Annex 1).

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 [6]. An ESBL phenotype was inferred if the isolates were resistant to cefotaxime and / or ceftazidime, but susceptible to cefoxitin and the isolates showed clavulanate synergy with cefotaxime and / or ceftazidime [6]. An AmpC phenotype was inferred if cefotaxime/ clavulanate and ceftazidime/ clavulanate synergy was not shown and isolates were resistant to cefotaxime or ceftazidime and cefoxitin [6].

Two of the isolates from MCA-CTX agar (one from beef and one from pork) had an AmpC phenotype, whilst the remaining isolate from MCA-CTX agar from beef had an ESBL phenotype. The percentages of beef and pork samples therefore that were positive for AmpC phenotype *E. coli* were 0.32% (95% confidence interval 0.06% to 1.78%) and 0.32% (95% confidence interval 0.06% to 1.8%) respectively. The percentages of beef samples therefore that were positive for an ESBL phenotype was also 0.32% (95% confidence interval 0.06% to 1.78%).

Use of CA-ESBL allowed for detection from one sample of an ESBL *E. coli* not isolated from MCA-CTX, which only gave rise to an AmpC *E. coli* from this sample. As such, this one beef sample was positive for two different isolates of *E. coli*, one of which was an AmpC (from MCA-CTX) and the other an ESBL (from CA-ESBL) phenotype. It was beyond the designated EU remit to report on MICs from CA-ESBL, so this ESBL isolate has not been included in the numbers of samples positive from MCA-CTX agar, although the sample was positive for AmpC *E. coli* on MCA-CTX.

As would be expected, all isolates from MCA-CTX agar were microbiologically (using EUCAST ECOFFS) resistant, using EUCAST ECOFFs [7], 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 isolates to cefoxitin also.

None of the isolates from MCA-CTX agar were resistant to the last resort antibiotics colistin, ertapenem, imipenem or meropenem, as would also be expected, since none of the samples gave rise to *E. coli* on CARBA and OXA-48 agars.

Overall, results showed less than 1% of retail beef and pork samples in the UK that were tested were positive for AmpC or ESBL producing *E. coli* using a sensitive detection method and these results were similar to the previous UK survey in 2015. Additionally, none of the samples gave rise to viable counts of *E. coli* above the detection limit on the two selective agars used for detection of AmpC and ESBL phenotype *E. coli* and none of samples gave rise to isolates resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem. One beef sample did give rise to *mcr-1* plasmid mediated colistin resistant *E. coli* which has been investigated and reported elsewhere [5] (Annex1).

In 2015 EU monitoring of beef and pork for presumptive ESBL-/AmpC-/carbapenemase-producing *E. coli* 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 [8]. 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) [8]. 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) [8].

## 4. Glossary

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

AmpC enzyme – Enzyme conferring AmpC type resistance

AMR – Antimicrobial resistance

APHA – Animal and Plant Health Agency

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

CRL- Community Reference Laboratory

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

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

CTX - Cefotaxime

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

EN - Norme Européenne / Europäische Norm (European Standard)

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

EU - European Union

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

FSA – Food Standards Agency

HCCA - α-Cyano-4-hydroxycinnamic acid

ISO - International Organisation for Standardisation

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

MCA - MacConkey agar

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

MIC – Minimum Inhibitory Concentration

MS - Member State

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

QC - Quality control

SOP – Standard Operating Procedure

### 5. Materials and Methods

All the methodology with respect to the work performed is detailed in five internal APHA Standard operating procedures (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 EU and / or
   APHA protocols (CBU 0278).
- Microbank -70°C Bacterial Storage System (CBU 0155).
- Identification of Bacteria by MALDI ToF (BAC 0334).
- Minimum Inhibitory Concentration (MIC) The Sensititre Method (BA 0604).
- Oxidase test (BA 050) for confirmation of lactose fermenters as E. coli.
- Indole Spot Test a Rapid Method for Bacteria (BA 0130) for confirmation of lactose fermenters as E. coli.
- Real Time PCR for plasmid mediated colistin resistance genes mcr-1 and mcr-2 (BAC 0415).

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

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

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

- EU method Isolation of ESBL, AMPC and carbapenemase producing E. coli
   from fresh meat January 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 - January 2017

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

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

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

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

MCA-CTX and MCA-COL plates were incubated for 18-22 hours at  $44 \pm 0.5$  °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 (pink to red colonies) from MCA-CTX were assumed to be presumptive AmpC / EBSL *E. coli*, blue colonies from CA-ESBL were assumed to be presumptive ESBL-producing *E. coli* and pink to burgundy colour colonies from CARBA and OXA-48 agars were assumed to be presumptive carbapenem resistant *E. coli*. A single presumptive *E. coli* from each of these agars was plated again to the agar of origin to ensure purity prior to further tests and storage if required.

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

From MCA-COL plates, a sweep of ~ 10 to 20 lactose fermenters (according to SOP BAC0415) was used to prepare a crude DNA sample for detection of *mcr-1* and *mcr-2* plasmid mediated colistin resistance genes by real time PCR. A sweep was taken to increase the sensitivity of detection of the *mcr* genes. If the "sweep" was negative for *mcr-1* and *mcr-2* by PCR, then the isolates were discarded. If positive, a single colony was purified if possible, retested for *mcr-1* and *mcr-2*, and if positive the resulting isolate was stored pending further tests.

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

### Storage of purified isolates of interest

Isolates of interest will be stored for up to five years to comply with EU requirements. Isolates were stored on "bead" culture (frozen in cryogenic material) at -80°C.

For "beads," purified bacterial culture was aseptically transferred using a 10  $\mu$ l loop from the second agar plate to a commercial "beads" tube. The cryogenic liquid and bacterial growth was mixed in the tube, before removing most of the supernatant cryogenic liquid, and then storing the tube at - 80°C.

# 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 such isolates as *E. coli*. Presumptive *E. coli* from other agars, such as CA-ESBL, CARBA and OXA-48, were identified by plating isolates to MCA to test if such isolates were

lactose fermenters coupled with oxidase and indole tests and / or MALDI ToF as described by an in-house SOP and based on that previously described [9].

For the oxidase test and indole tests, a single well isolated colony was taken from MCA-CTX agar (or other relevant agars), 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.

Isolates prior to MALDI ToF were also grown on blood agar. A small amount of bacterial growth was applied to the metal target plate. Growth on the target plates was overlaid with 1 µl of 70% formic acid to perform a partial protein extraction, and allowed to dry. Each spot was then overlaid with 1 µl of HCCA matrix, and again this was allowed to dry before the target plate was loaded into the MALDI ToF machine. Using Biotyper software, resulting spectra from the MALDI ToF run were searched against the Bruker database of spectra, and if the resulting score was ≥ 2.000, this was taken as reliable identification to the species level.

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

MICs were performed as described in our in-house SOP (BA 0604), 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 [7].

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

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

Isolates resistant to one or both of cefotaxime and ceftazidime that 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 [6].

Isolates resistant to cefotaxime or ceftazidime that also had an MIC of greater than 8mg/l against cefoxitin and showed no reduction to MIC's or a reduction of less than three dilution steps for cefotaxime or ceftazidime in the presence of clavulanate were considered to be carrying an AmpC enzyme [6]. It is also possible for isolates to have a combined AmpC / ESBL phenotype.

PCR for *bla*<sub>CTX-M</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> and plasmid mediated *mcr-1* and *mcr-2* genes.

Isolates of presumptive ESBL *E. coli* from CA-ESBL were tested for *bla*<sub>CTX-M</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> genes using a multiplex PCR [1], and resulting *bla*<sub>CTX-M</sub> amplicons were sequenced to determine the CTX-M sequence type [10]. Lactose fermenting colonies on MCA-COL were tested for the presence of plasmid mediated colistin resistance genes *mcr-1* and *mcr-2* [2, 11] by real time (RT) PCR, according to an inhouse SOP. To make detection more sensitive for the *mcr-1* and *mcr-2* PCRs, a "sweep" of ~ 10 to 20 colonies was taken to prepare the crude DNA for RT-PCR for this PCR only.

### Results

### **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.

### 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 [3], and the main details of each meat sample are listed in Table 1 of this report with anonymised codes for shop and brand. In total 314 beef and 310 pork samples were tested between January and December 2017.

Samples positive for presumptive AmpC / ESBL *E. coli* on MacConkey agar + 1 mg/L cefotaxime and on CHROMagar™ ESBL.

Details of the samples positive for presumptive AmpC / ESBL *E. coli* on MCA-CTX agar or CA-ESBL are shown in Table 2 and Figure 1.

Only three (0.48%, 95% confidence interval 0.16% to 1.40%) of the 624 samples tested yielded *E. coli* colonies on MCA-CTX agar by the EU harmonised method. These samples comprised two of the beef samples (both steaks, one expensive and one less expensive) and one of the pork samples (belly slices) positive, representing 0.64% (95% confidence interval 0.17% to 2.29%) of the beef samples and 0.32% (95% confidence interval 0.06% to 1.8%) of the pork samples (Figure 1).

The two beef samples that were positive on MCA-CTX agar were also positive on the additional CA-ESBL agar (not required for EU survey), and the resulting isolates tested were found to be positive for CTX-M 1 type ESBL gene (Table 2). One additional pork sample (pork chops) was also positive on CA-ESBL agar, and the

resulting isolate tested was found to be positive for *bla*<sub>TEM</sub> (Table 2, UK non-harmonised additional test).

# Samples positive for presumptive *E. coli* resistant to last resort antibiotics (colistin and carbapenems)

None of the samples gave rise to carbapenem resistant *E. coli* on CARBA and OXA-48 agars by the EU harmonised method (Figure 1).

Additional testing of UK samples (UK non-harmonised additional test) found 39 of the beef samples (12.4%, 95% confidence interval 9.22% to 16.53%) and 46 of the pork samples (14.8%, 95% confidence interval 11.31% to 19.23%) yielded presumptive *E. coli* on MCA-COL agar. Presumptive *E. coli* from MCA-COL agar were tested by RT-PCR for *mcr1* or *mcr2* [2]. One beef steak sample (expensive steak, sample ID 01562581) was found to be positive for *mcr-1* plasmid mediated colistin resistant *E. coli*. This sample was submitted to APHA Weybridge for testing on 16th May 2017 and APHA performed whole genome sequencing on three *mcr-1* positive isolates from this original sample, and results have previously been reported to the FSA (Annex 1).

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

Of the 624 retail beef and pork samples tested from various supermarkets, none gave rise to presumptive *E. coli* counts on MCA-CTX agar and / or CA-ESBL agar above the detection limit of 40 cfu/gram of meat.

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

The summary interpretation of MIC results for *E. coli* isolates from MCA-CTX agar for the three positive samples is shown in Table 3, whilst the individual MIC results for each of the three isolates tested are shown in Table 4. The patterns of resistance were used to determine isolates as having an AmpC or ESBL phenotype, as described in the methods.

None of the isolates were resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin (Tables 3 and 4). 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 (Tables 3 and 4).

The isolate from MCA-CTX from beef designated as ESBLs was resistant to the cephalosporin antibiotics cefotaxime and ceftazidime, but sensitive to the cephalosporin antibiotic cefoxitin and the combinations cefotaxime and clavulanate or ceftazidime and clavulanate showed synergy against this isolate (Table 4 and Figure 1). Conversely, the isolates designated as having an AmpC phenotype was resistant to cefoxitin, but there was no synergy shown with clavulanate and the cephalosporin antibiotics ceftazidime and cefotaxime (Table 4 and Figure 1).

The isolate from beef designated as an ESBL, in addition to resistance to betalactam antibiotics tested except cefoxitin, was also resistant to the older antibiotics such as sulfamethoxazole and tetracycline and was resistant to the quinolone antibiotics nalidixic acid and ciprofloxacin (Table 3 and 4). The two isolates (one beef, one pork) designated as AmpC were sensitive to all antibiotics tested except ampicillin, cefoxitin, ceftazidime and cefotaxime (Table 3 and 4).

### MIC results for isolates from CA-ESBL agar - UK non-harmonised additional test.

It is beyond the remit of the work agreed to perform MICs against isolates from CA-ESBL, although isolates from this agar are tested by PCR for *bla*<sub>CTX-M</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> [1].

However, sample 1612846 (beef) designated as being positive for AmpC phenotype *E. coli* from MCA-CTX agar was also positive for a CTXM-1 isolate from CA-ESBL agar (Table 2), suggesting that this sample was positive for two different types of *E. coli*, one being an AmpC phenotype and the other being an ESBL phenotype. In view of this, MICs were performed against the isolate from CA-ESBL and it was confirmed to have an ESBL phenotype (Table 4). As such sample 1612846 was positive for both an AmpC *E. coli* and an ESBL *E. coli*.

### 6. Discussion

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* [12], 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 [13]. 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* [14]. However, these studies lack a uniform methodology across different countries that is employed in current EU harmonized 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 [8], 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 2017, 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 ESBL-producing *E. coli*, whilst 0.8% of beef samples and 1.3% of pork samples were positive for *E. coli* carrying the AmpC *bla*<sub>CIT</sub> genes, with *bla*<sub>CMY-2</sub> the most frequent variant detected by sequencing [15]. 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 [16].

Results for the 2015 EU monitoring of beef and pork for presumptive ESBL-/AmpC-/carbapenemase-producing *E. coli* have now been published by EFSA [8]. 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 [8]. 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) [8]. 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) [8].

The predominant *E. coli* strain associated with human infections is the pandemic O25-ST131 CTX-M-15-producing clone [17, 18]. 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 (Annex 1). 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 [19]. Of the 10,206 E. coli isolates, only 1.4% were resistant to colistin, and 0.7% of isolates were positive for mcr-1 [19]. 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 [19], 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 E. coli. 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 [20, 21].

It has been suggested, as discussed in a previous EU report [16], 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 [14].

In pigs a previous study showed that use of ceftiofur and cefquinome exerted a selective pressure for ESBL *E. coli* [22], whilst another study showed reduction of ESBL *E. coli* in pigs following introduction of voluntary restrictions on cephalosporin use [23].

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%) [15] compared to the UK EU survey of 2016 (29.7%) [16]. 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 [24, 25].

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 [8]. 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 [5] (Annex 1).

## 7. Conclusions

Results of the UK 2017 EU harmonised surveillance of antimicrobial resistance (AMR) in retail beef and pork showed that less than 1% of samples tested were positive for AmpC/ESBL phenotype *E. coli*. With respect to resistance to last resort antibiotics, carbapenem resistant *E. coli* were not isolated from any of the meat samples, although one beef sample was positive for *mcr-1* plasmid mediated colistin resistant *E. coli*. These results are similar to the UK results for retail beef and pork from the same EU survey, but for 2015 samples, and compared favourably with results from other countries in 2015, as published by EFSA [8].

#### 8. Tables

Table 1. Beef and pork samples (sorted by despatch date) tested at APHA

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562896	16/01/2017	Beef	Beef steaks-less expensive	GB	G	YY
1612817	16/01/2017	Beef	Beef steaks-less expensive	GB	J	Е
1612824	16/01/2017	Beef	Beef steaks-expensive	GB	J	Н
1612825	16/01/2017	Beef	Beef steaks-less expensive	GB	Е	НН
1612827	16/01/2017	Beef	Beef steaks-less expensive	GB	E	НН
1612818	16/01/2017	Pork	Pork fillets & steaks	GB	J	Н
1612819	16/01/2017	Pork	Pork fillets & steaks	GB	E	НН
1612820	16/01/2017	Pork	Other diced/sliced pork	GB	E	НН
1612821	16/01/2017	Pork	Pork chops	GB	E	НН
1562893	16/01/2017	Pork	Pork fillets & steaks	GB	G	L
1612823	16/01/2017	Beef	Beef steaks-less expensive	GB	J	Е
1562895	16/01/2017	Pork	Pork fillets & steaks	GB	В	DF
1612826	16/01/2017	Beef	Beef steaks-expensive	GB	E	НН
1562897	16/01/2017	Beef	Beef steaks-less expensive	GB	D	Q
1562898	16/01/2017	Beef	Beef steaks-expensive	GB	В	DF
1562909	16/01/2017	Pork	Other diced/sliced pork	GB	Α	V
1562908	16/01/2017	Pork	Pork fillet & steaks	GB	D	Q
1562914	16/01/2017	Pork	Pork chops	GB	?	?
1562916	16/01/2017	Pork	Pork fillets & steaks	GB	D	Q
1562915	16/01/2017	Beef	Beef steaks-less expensive	GB	?	?
1562917	16/01/2017	Beef	Beef steaks-expensive	GB	D	WW
1562910	16/01/2017	Beef	Beef steaks-less expensive	GB	Α	V
1562907	16/01/2017	Beef	Beef steaks-expensive	GB	D	WW
1562894	16/01/2017	Pork	Pork chops	GB	D	Р
1612822	16/01/2017	Pork	Pork fillets & steaks	GB	J	Е
1612846	17/01/2017	Beef	Beef steaks-less expensive	GB	С	AZ
1612840	17/01/2017	Beef	Beef steaks-expensive	GB	?	R
1612842	17/01/2017	Pork	Pork fillets & steaks	GB	В	DS
1612839	17/01/2017	Beef	Beef steaks-expensive	GB	D	VV
1612841	17/01/2017	Pork	Pork fillets & steaks	GB	?	R
1612847	17/01/2017	Pork	Pork fillets & steaks	GB	D	Q

<sup>&</sup>lt;sup>1</sup> Please note that this is the information provided at the time of sampling.

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1612845	17/01/2017	Pork	Pork chops	GB	С	KK
1612848	17/01/2017	Beef	Beef steaks-expensive	GB	В	DS
1612849	17/01/2017	Beef	Beef steaks-expensive	GB	?	?
1612843	17/01/2017	Pork	Pork fillets & steaks	GB	?	?
1612830	18/01/2017	Pork	Other diced/sliced pork	GB	J	E
1612835	18/01/2017	Beef	Beef steaks-expensive	GB	Н	ER
1612832	18/01/2017	Beef	Beef steaks-expensive	GB	E	NN
1612833	18/01/2017	Beef	Beef steaks-less expensive	GB	В	DS
1612834	18/01/2017	Beef	Beef steaks-expensive	GB	J	F
1612828	18/01/2017	Pork	Other diced/sliced pork	GB	Е	НН
1612829	18/01/2017	Pork	Pork chops	GB	В	DS
1612831	18/01/2017	Pork	Other diced/sliced pork	GB	Н	ER
1562892	23/01/2017	Beef	Beef steaks-expensive	GB	В	DF
1562886	23/01/2017	Beef	Beef steaks-expensive	GB	J	E
1562891	23/01/2017	Pork	Pork fillets & steaks	GB	J	AW
1562884	23/01/2017	Pork	Pork fillets & steaks	GB	Н	ER
1562885	23/01/2017	Pork	Pork chops	GB	J	E
1562888	23/01/2017	Pork	Other diced/sliced pork	GB	В	DS
1562889	23/01/2017	Pork	Pork chops	GB	В	DS
1562883	23/01/2017	Beef	Beef steaks-expensive	GB	Н	ER
1563499	23/01/2017	Pork	Pork fillets & steaks	GB	?	?
1563500	23/01/2017	Beef	Beef steaks-less expensive	GB	?	?
1562887	23/01/2017	Beef	Beef steaks-less expensive	GB	В	DS
1562882	23/01/2017	Beef	Beef steaks-less expensive	GB	J	Н
1562854	20/02/2017	Pork	Other diced/sliced pork	GB	E	НН
1612864	20/02/2017	Pork	Other diced/sliced pork	GB	E	НН
1612866	20/02/2017	Pork	Pork fillets & steaks	GB	Α	V
1612865	20/02/2017	Pork	Other diced/sliced pork	NL	В	GT
1364317	20/02/2017	Beef	Beef steaks-less expensive	GB	В	DS
1364319	20/02/2017	Beef	Beef steaks-expensive	GB	E	NN
1364316	20/02/2017	Beef	Beef steaks-expensive	GB	В	DF
1364340	20/02/2017	Beef	Beef steaks-expensive	GB	I	S
1364321	20/02/2017	Pork	Pork fillets & steaks	GB	В	DS
1612838	20/02/2017	Pork	Pork fillets & steaks	GB	D	Q
1562851	20/02/2017	Pork	Other diced/sliced pork	GB	F	II
1562853	20/02/2017	Beef	Beef steaks-expensive	GB	E	НН

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562852	20/02/2017	Beef	Beef steaks-expensive	GB	F	II
1563511	20/02/2017	Pork	Other diced/sliced pork	GB	D	Q
1612863	20/02/2017	Pork	Pork fillets & steaks	GB	J	Н
1612837	20/02/2017	Pork	Other diced/sliced pork	GB	E	НН
1563508	20/02/2017	Pork	Pork fillets & steaks	GB	Α	V
1612871	20/02/2017	Beef	Beef steaks-expensive	GB	E	NN
1563514	20/02/2017	Pork	Other diced/sliced pork	GB	В	GT
1563509	20/02/2017	Beef	Beef steaks-expensive	GB	D	WW
1563510	20/02/2017	Beef	Beef steaks-expensive	GB	D	WW
1563507	20/02/2017	Pork	Pork chops	GB	G	L
1563506	20/02/2017	Beef	Beef steaks-expensive	GB	G	L
1563505	20/02/2017	Beef	Beef steaks-expensive	GB	Α	FR
1612868	20/02/2017	Beef	Beef steaks-expensive	GB	Α	FR
1563512	20/02/2017	Pork	Other diced/sliced pork	GB	D	Q
1563504	20/02/2017	Beef	Beef steaks-less expensive	GB	I	S
1612872	20/02/2017	Beef	Beef steaks-less expensive	GB	J	W
1612869	20/02/2017	Beef	Beef steaks-expensive	GB	?	?
1563503	20/02/2017	Pork	Pork fillets & steaks	GB	I	S
1563513	20/02/2017	Beef	Beef steaks-expensive	GB	В	DF
1612870	20/02/2017	Beef	Beef steaks-expensive	GB	В	DF
1562675	06/03/2017	Pork	Pork fillets & steaks	GB	J	Н
1562673	06/03/2017	Pork	Pork fillets & steaks	GB	В	DS
1562668	06/03/2017	Pork	Pork chops	GB	D	Р
1562666	06/03/2017	Pork	Pork fillets & steaks	GB	F	II
1562676	06/03/2017	Pork	Other diced/sliced pork	GB	J	Н
1562670	06/03/2017	Pork	Other diced/sliced pork	GB	J	E
1562663	06/03/2017	Pork	Other diced/sliced pork	GB	?	?
1562661	06/03/2017	Pork	Pork fillets & steaks	GB	G	L
1562665	06/03/2017	Beef	Beef steaks-expensive	GB	D	Q
1562662	06/03/2017	Beef	Beef steaks-less expensive	GB	G	L
1562672	06/03/2017	Beef	Beef steaks-less expensive	GB	J	E
1562667	06/03/2017	Beef	Beef steaks-expensive	GB	F	II
1562671	06/03/2017	Beef	Beef steaks-less expensive	GB	J	F
1562674	06/03/2017	Beef	Beef steaks-less expensive	GB	В	DS
1562677	06/03/2017	Beef	Beef steaks-less expensive	GB	J	W
1512678	06/03/2017	Beef	Beef steaks-less expensive	GB		

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562669	06/03/2017	Pork	Pork fillets & steaks	GB	J	Е
1562664	06/03/2017	Beef	Beef steaks-expensive	GB	?	?
1563705	13/03/2017	Beef	Beef steaks-less expensive	GB	J	Е
1614554	13/03/2017	Beef	Beef steaks-expensive	GB	J	Е
1562858	13/03/2017	Pork	Pork fillets & steaks	GB	Е	НН
1563710	13/03/2017	Pork	Pork chops	GB	J	Е
1563701	13/03/2017	Pork	Pork chops	GB	Е	НН
1563709	13/03/2017	Pork	Other diced/sliced pork	GB	J	E
1563714	13/03/2017	Beef	Beef steaks-less expensive	GB	Н	ER
1562600	13/03/2017	Beef	Beef steaks-less expensive	GB	D	Q
1563702	13/03/2017	Beef	Beef steaks-less expensive	GB	Е	НН
1562602	13/03/2017	Pork	Pork chops	GB	?	?
1563712	13/03/2017	Beef	Beef steaks-less expensive	GB	В	М
1563715	13/03/2017	Beef	Beef steaks-less expensive	GB	Е	НН
1562959	13/03/2017	Beef	Beef steaks-expensive	GB	В	DF
1562961	13/03/2017	Beef	Beef steaks-expensive	GB	?	?
1562606	13/03/2017	Beef	Beef steaks-expensive	GB	E	НН
1562599	13/03/2017	Pork	Other diced/sliced pork	GB	D	Q
1562962	13/03/2017	Pork	Pork fillets & steaks	GB	?	?
1562963	13/03/2017	Pork	Pork fillets & steaks	GB	E	НН
1562857	13/03/2017	Pork	Pork chops	GB	?	D
1562913	13/03/2017	Pork	Other sliced/diced Pork	GB	?	FF
1562912	13/03/2017	Beef	Beef steaks-expensive	GB	?	D
1562911	13/03/2017	Beef	Beef steaks-less expensive	GB	?	FF
1563703	13/03/2017	Beef	Beef steaks-less expensive	GB	J	Е
1562601	13/03/2017	Beef	Beef steaks-less expensive	GB	?	?
1562960	13/03/2017	Pork	Pork chops	GB	В	DS
1612851	13/03/2017	Beef	Beef steaks-expensive	GB	D	WW
1614555	13/03/2017	Pork	Pork fillets & steaks	GB	J	Е
1612852	13/03/2017	Pork	Pork fillets & steaks	GB	В	DS
1612859	13/03/2017	Pork	Pork fillets & steaks	GB	В	DS
1612857	13/03/2017	Pork	Pork fillets & steaks	GB	В	DS
1612853	13/03/2017	Beef	Beef steaks-less expensive	GB	В	М
1612854	13/03/2017	Beef	Beef steaks-less expensive	GB	В	М
1612856	13/03/2017	Beef	Beef steaks-expensive	GB	В	DF
1612860	13/03/2017	Beef	Beef steaks-less expensive	GB	J	Е

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1612858	13/03/2017	Beef	Beef steaks-less expensive	GB	В	DS
1612850	13/03/2017	Pork	Pork chops	GB	D	Р
1612855	13/03/2017	Pork	Other diced/sliced pork	GB	В	DS
1612861	13/03/2017	Pork	Pork fillets & steaks	GB	J	E
1562905	14/03/2017	Pork	Other diced/sliced pork	GB	F	II
1563876	14/03/2017	Beef	Beef steaks-expensive	GB	В	DF
1562918	14/03/2017	Pork	Pork fillets & steaks	GB	В	DF
1563873	14/03/2017	Pork	Other diced/sliced pork	GB	J	Н
1562903	14/03/2017	Pork	Pork fillets & steaks	GB	J	Е
1562900	14/03/2017	Pork	Other diced/sliced pork	GB	J	Н
1562901	14/03/2017	Beef	Beef steaks-expensive	GB	J	AW
1562904	14/03/2017	Beef	Beef steaks-expensive	GB	?	CX
1563874	14/03/2017	Pork	Pork chops	GB	?	CX
1562902	14/03/2017	Beef	Beef steaks-less expensive	GB	J	Е
1562899	14/03/2017	Beef	Beef steaks-expensive	GB	F	AQ
1562906	14/03/2017	Beef	Beef steaks-expensive	GB	J	AW
1563711	13/04/2017	Pork	Pork fillets & steaks	GB	В	DS
1562628	24/04/2017	Pork	Other diced/sliced pork	GB	?	А
1562620	24/04/2017	Beef	Beef steaks-expensive	GB	F	II
1562622	24/04/2017	Beef	Beef steaks-less expensive	GB	G	U
1562653	24/04/2017	Pork	Pork fillets & steaks	GB	Е	НН
1562624	24/04/2017	Beef	Beef steaks-less expensive	GB	?	?
1562619	24/04/2017	Pork	Pork chops	GB	F	II
1562631	24/04/2017	Beef	Beef steaks-less expensive	GB	J	00
1562654	24/04/2017	Beef	Beef steaks-less expensive	GB	E	НН
1562627	24/04/2017	Beef	Beef steaks-less expensive	GB	E	НН
1562630	24/04/2017	Pork	Pork chops	GB	J	00
1562625	24/04/2017	Pork	Pork fillets & steaks	GB	?	?
1562621	24/04/2017	Pork	Pork fillets & steaks	GB	G	BG
1562658	24/04/2017	Beef	Beef steaks-expensive	GB	G	U
1562879	24/04/2017	Beef	Beef steaks-less expensive	GB	D	Q
1562880	24/04/2017	Beef	Beef steaks-expensive	IE	В	DS
1562656	24/04/2017	Pork	Pork chops	GB	D	Р
1562629	24/04/2017	Beef	Beef steaks-expensive	GB	?	А
1562655	24/04/2017	Pork	Pork fillets & steaks	GB	G	L
1562626	24/04/2017	Pork	Pork fillets & steaks	GB	E	НН

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562657	24/04/2017	Pork	Pork chops	DK	В	DS
1562975	25/04/2017	Pork	Pork fillets & steaks	GB	G	L
1562639	25/04/2017	Beef	Beef steaks-expensive	GB	В	DF
1562632	25/04/2017	Beef	Beef steaks-expensive	GB	J	Е
1562643	25/04/2017	Beef	Beef steaks-expensive	GB	В	DS
1562642	25/04/2017	Beef	Beef steaks-expensive	GB	D	WW
1562972	25/04/2017	Pork	Pork fillets & steaks	GB	J	E
1562974	25/04/2017	Pork	Pork fillets & steaks	GB	В	DS
1562931	25/04/2017	Pork	Pork chops	GB	J	Е
1562977	25/04/2017	Pork	Pork fillets & steaks	GB	D	Q
1562971	25/04/2017	Beef	Beef steaks-expensive	GB	J	F
1562659	25/04/2017	Pork	Pork fillets & steaks	GB	?	N
1562973	25/04/2017	Beef	Beef steaks-expensive	GB	В	DF
1562660	25/04/2017	Beef	Beef steaks-less expensive	GB	D	Q
1562976	25/04/2017	Beef	Beef steaks-expensive	GB	G	L
1614556	25/04/2017	Beef	Beef steaks-less expensive	GB	?	N
1562644	25/04/2017	Pork	Pork fillets & steaks	GB	В	DS
1614531	25/04/2017	Pork	Other diced/sliced pork	GB	Е	НН
1562633	25/04/2017	Beef	Beef steaks-expensive	GB	?	UU
1562635	25/04/2017	Beef	Beef steaks-less expensive	GB	?	BV
1614532	25/04/2017	Beef	Beef steaks-expensive	GB	Е	НН
1614533	25/04/2017	Beef	Pork chops	GB	В	DF
1562634	25/04/2017	Pork	Other diced/sliced pork	GB	?	UU
1562636	25/04/2017	Pork	Pork fillets & steaks	GB	?	BV
1562640	25/04/2017	Pork	Pork fillets & steaks	GB	В	DS
1562641	25/04/2017	Pork	Other diced/sliced pork	GB	D	Q
1562978	25/04/2017	Pork	Other diced/sliced pork	GB	В	DS
1562652	09/05/2017	Pork	Pork fillets & steaks	GB	?	?
1562645	09/05/2017	Pork	Pork fillets & steaks	GB	?	?
1363740	09/05/2017	Beef	Beef steaks-expensive	GB	?	?
1562646	09/05/2017	Beef	Beef steaks-expensive	GB	?	?
1562832	15/05/2017	Pork	Pork fillets & steaks	GB	D	Q
1562822	15/05/2017	Pork	Pork fillets & steaks	GB	?	?
1562820	15/05/2017	Pork	Pork fillets & steaks	DE	J	Е
1562837	15/05/2017	Beef	Beef steaks-less expensive	GB	?	PP
1562830	15/05/2017	Beef	Beef steaks-expensive	GB	В	DF

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562829	15/05/2017	Beef	Beef steaks-expensive	IE	В	DS
1562831	15/05/2017	Beef	Beef steaks-less expensive	GB	D	Q
1562835	15/05/2017	Beef	Beef steaks-expensive	GB	Е	NN
1562836	15/05/2017	Beef	Beef steaks-expensive	GB	Е	NN
1612862	15/05/2017	Beef	Beef steaks-expensive	GB	D	WW
1562838	15/05/2017	Pork	Pork fillets & steaks	GB	?	PP
1562821	15/05/2017	Beef	Beef steaks-less expensive	GB	?	?
1562828	15/05/2017	Pork	Beef steaks-less expensive	IE	В	М
1562616	15/05/2017	Beef	Beef steaks-expensive	GB	?	?
1562613	15/05/2017	Pork	Other diced/sliced pork	GB	?	?
1562612	15/05/2017	Pork	Pork fillets & steaks	GB	В	DS
1562827	15/05/2017	Pork	Pork fillets & steaks	GB	В	DS
1562819	15/05/2017	Beef	Beef steaks-less expensive	GB	J	Е
1562834	15/05/2017	Pork	Pork fillets & steaks	GB	Е	НН
1562615	15/05/2017	Beef	Beef steaks-expensive	GB	В	DS
1562598	15/05/2017	Beef	Beef steaks-expensive	GB	J	AW
1562614	15/05/2017	Beef	Beef steaks-expensive	GB	?	K
1562571	15/05/2017	Pork	Pork fillets & steaks	GB	J	AW
1614524	15/05/2017	Pork	Pork fillets & steaks	GB	D	Q
1562833	15/05/2017	Pork	Pork chops	GB	Е	НН
1562611	15/05/2017	Pork	Pork fillets & steaks	GB	?	?
1562574	16/05/2017	Pork	Pork fillets & steaks	GB	J	AW
1562609	16/05/2017	Beef	Beef steaks-less expensive	GB	G	U
1562576	16/05/2017	Pork	Pork fillets & steaks	GB	В	DS
1562608	16/05/2017	Pork	Pork fillets & steaks	NL	Н	ER
1562578	16/05/2017	Pork	Other diced/sliced pork	NL	J	G
1562573	16/05/2017	Beef	Beef steaks-expensive	GB	J	AW
1562577	16/05/2017	Beef	Beef steaks-less expensive	IE	В	DS
1562607	16/05/2017	Beef	Beef steaks-expensive	GB	Н	ER
1562575	16/05/2017	Beef	Beef steaks-less expensive	IE	J	E
1562610	16/05/2017	Pork	Pork chops	GB	G	L
1562591	17/05/2017	Beef	Beef steaks-expensive	GB	В	DF
1562585	17/05/2017	Beef	Beef steaks-expensive	GB	?	FT
1562592	17/05/2017	Beef	Beef steaks-expensive	GB	J	AW
1562589	17/05/2017	Beef	Beef steaks-expensive	GB	В	DF
1562581	17/05/2017	Beef	Beef steaks-expensive	GB	?	Т

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562594	17/05/2017	Beef	Beef steaks-less expensive	GB	J	Н
1562593	17/05/2017	Beef	Beef steaks-expensive	GB	J	E
1562587	17/05/2017	Pork	Pork fillets & steaks	GB	?	FT
1562590	17/05/2017	Pork	Pork fillets & steaks	GB	В	DS
1562596	17/05/2017	Pork	Pork fillets & steaks	GB	J	AW
1562586	17/05/2017	Pork	Pork fillets & steaks	GB	В	DS
1562583	17/05/2017	Pork	Pork chops	BE	?	Т
1562595	17/05/2017	Pork	Pork fillets & steaks	GB	J	Н
1562588	17/05/2017	Beef	Beef steaks-expensive	GB	G	U
1614525	17/05/2017	Pork	Other diced/sliced pork	DE	J	G
1562584	17/05/2017	Pork	Other diced/sliced pork	GB	G	L
1562755	19/06/2017	Pork	Pork fillets & steaks	GB	Α	V
1562569	19/06/2017	Beef	Beef steaks-expensive	GB	F	II
1562568	19/06/2017	Beef	Beef steaks-less expensive	GB	D	Q
1562758	19/06/2017	Pork	Other diced/sliced pork	GB	G	L
1562746	19/06/2017	Pork	Pork fillets & steaks	GB	?	RR
1562556	19/06/2017	Beef	Beef steaks-expensive	GB	D	WW
1614521	19/06/2017	Pork	Pork chops	NL	J	G
1562757	19/06/2017	Pork	Pork fillets & steaks	GB	В	DS
1562566	19/06/2017	Pork	Pork fillets & steaks	GB	D	Q
1562550	19/06/2017	Beef	Beef steaks-expensive	GB	F	AQ
1614520	19/06/2017	Beef	Beef steaks-expensive	GB	?	MM
1562548	19/06/2017	Beef	Beef steaks-less expensive	GB	G	BG
1562549	19/06/2017	Pork	Pork fillets & steaks	GB	F	II
1562750	19/06/2017	Pork	Pork fillets & steaks	GB	I	S
1562564	19/06/2017	Beef	Beef steaks-expensive	GB	?	GH
1562567	19/06/2017	Pork	Other diced/sliced pork	GB	F	II
1614514	19/06/2017	Pork	Other diced/sliced pork	GB	?	MM
1562555	19/06/2017	Pork	Other diced/sliced pork	GB	D	Q
1562560	19/06/2017	Pork	Other diced/sliced pork	GB	?	JJ
1562563	19/06/2017	Pork	Other diced/sliced pork	GB	?	GH
1562565	19/06/2017	Pork	Other diced/sliced pork	GB	I	S
1562826	19/06/2017	Pork	Pork fillets & steaks	GB	D	Q
1562561	19/06/2017	Beef	Beef steaks-less expensive	GB	?	JJ
1562559	19/06/2017	Beef	Beef steaks-expensive	GB	D	ww
1562547	19/06/2017	Pork	Pork chops	GB	G	BG

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562747	19/06/2017	Pork	Pork fillets & steaks	GB	E	НН
1562749	19/06/2017	Beef	Beef steaks-expensive	GB	I	S
1562748	19/06/2017	Beef	Beef steaks-expensive	GB	E	НН
1562751	19/06/2017	Beef	Beef steaks-expensive	GB	J	F
1562756	19/06/2017	Beef	Beef steaks-expensive	GB	Α	FG
1562815	19/06/2017	Beef	Beef steaks-less expensive	GB	?	RR
1562745	19/06/2017	Beef	Beef steaks-expensive	GB	В	DF
1562817	19/06/2017	Beef	Beef steaks-less expensive	GB	I	S
1562816	19/06/2017	Beef	Beef steaks-less expensive	GB	G	L
1562805	20/06/2017	Pork	Pork chops	GB	Е	HH
1562551	20/06/2017	Pork	Pork chops	GB	?	?
1562557	20/06/2017	Pork	Other diced/sliced pork	GB	I	S
1562739	20/06/2017	Pork	Pork fillets & steaks	GB	В	DS
1562553	20/06/2017	Pork	Other diced/sliced pork	GB	Е	НН
1562740	20/06/2017	Beef	Beef steaks-expensive	GB	В	DF
1562554	20/06/2017	Beef	Beef steaks-expensive	GB	Е	HH
1562552	20/06/2017	Beef	Beef steaks-expensive	GB	?	ВВ
1562558	20/06/2017	Beef	Beef steaks-less expensive	GB	I	S
1562810	20/06/2017	Beef	Beef steaks-expensive	GB	Е	NN
1562807	20/06/2017	Pork	Pork chops	GB	Е	НН
1562809	20/06/2017	Beef	Beef steaks-expensive	GB	Е	NN
1562812	20/06/2017	Pork	Pork fillets & steaks	GB	В	DS
1562811	20/06/2017	Pork	Other diced/sliced pork	NL	В	GT
1562802	20/06/2017	Pork	Pork chops	GB	Α	V
1562801	20/06/2017	Pork	Pork fillets & steaks	GB	Α	V
1562814	20/06/2017	Beef	Beef steaks-expensive	GB	В	DF
1562804	20/06/2017	Beef	Beef steaks-expensive	GB	Α	FG
1562806	20/06/2017	Beef	Beef steaks-less expensive	GB	E	НН
1562803	20/06/2017	Beef	Beef steaks-expensive	GB	Α	FR
1562808	20/06/2017	Pork	Pork fillets & steaks	GB	E	НН
1562813	20/06/2017	Beef	Beef steaks-less expensive	IE	В	М
1614508	17/07/2017	Pork	Pork fillets & steaks	GB	В	DS
1562582	17/07/2017	Pork	Pork fillets & steaks	GB	?	Z
1614507	17/07/2017	Pork	Other diced/sliced pork	GB	?	?
1562604	17/07/2017	Pork	Pork fillets & steaks	GB	?	CV
1562967	17/07/2017	Pork	Other diced/sliced pork	DK	J	Е

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562970	17/07/2017	Pork	Pork chops	GB	В	DS
1562965	17/07/2017	Pork	Other diced/sliced pork	GB	E	НН
1614510	17/07/2017	Beef	Beef steaks-expensive	GB	D	WW
1614506	17/07/2017	Beef	Beef steaks-less expensive	GB	?	?
1562764	17/07/2017	Beef	Beef steaks-less expensive	GB	Α	V
1563515	17/07/2017	Pork	Pork chops	GB	В	DS
1614509	17/07/2017	Pork	Pork chops	GB	D	Q
1562772	17/07/2017	Pork	Other diced/sliced pork	GB	F	II
1562763	17/07/2017	Pork	Other diced/sliced pork	GB	Α	V
1562537	17/07/2017	Pork	Other diced/sliced pork	GB	?	?
1562536	17/07/2017	Pork	Pork fillets & steaks	DK	J	Е
1614530	17/07/2017	Pork	Pork chops	GB	?	EE
1364272	17/07/2017	Pork	Pork chops	GB	J	Н
1562762	17/07/2017	Beef	Beef steaks-expensive	GB	В	DF
1562605	17/07/2017	Beef	Beef steaks-expensive	IE	?	?
1562538	17/07/2017	Beef	Beef steaks-expensive	GB	J	F
1562773	17/07/2017	Beef	Beef steaks-expensive	GB	F	II
1562775	17/07/2017	Beef	Beef steaks-expensive	GB	J	AW
1562761	17/07/2017	Beef	Beef steaks-expensive	GB	В	DF
1562777	17/07/2017	Pork	Other diced/sliced pork	GB	D	Q
1562776	17/07/2017	Beef	Beef steaks-expensive	GB	D	VV
1364270	17/07/2017	Beef	Beef steaks-expensive	GB	?	Z
1562603	17/07/2017	Beef	Beef steaks-expensive	GB	?	CV
1562890	17/07/2017	Beef	Beef steaks-expensive	GB	?	?
1562964	17/07/2017	Beef	Beef steaks-expensive	GB	J	С
1562968	17/07/2017	Beef	Beef steaks-expensive	GB	E	NN
1562778	17/07/2017	Beef	Beef steaks-less expensive	GB	?	QQ
1562969	17/07/2017	Beef	Beef steaks-expensive	GB	В	DF
1562774	17/07/2017	Pork	Other diced/sliced pork	GB	J	Н
1562966	17/07/2017	Beef	Beef steaks-expensive	GB	J	F
1614529	17/07/2017	Beef	Beef steaks-expensive	GB	F	II
1562539	17/07/2017	Pork	Pork fillets & steaks	PL	?	0
1562526	18/07/2017	Pork	Pork fillets & steaks	DE	J	Е
1562521	18/07/2017	Pork	Pork fillets & steaks	GB	I	S
1562524	18/07/2017	Beef	Beef steaks-expensive	GB	В	DF
1614527	18/07/2017	Pork	Pork fillets & steaks	EU	?	GG

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562519	18/07/2017	Pork	Pork fillets & steaks	Pork fillets & steaks GB		Q
1562528	18/07/2017	Beef	Beef steaks-expensive	GB	G	U
1562799	18/07/2017	Beef	Beef steaks-less expensive	GB	?	XX
1562527	18/07/2017	Beef	Beef steaks-expensive	GB	J	F
1562522	18/07/2017	Beef	Beef steaks-expensive	GB	1	S
1562520	18/07/2017	Beef	Beef steaks-expensive	GB	D	WW
1562529	18/07/2017	Pork	Other diced/sliced pork	GB	G	L
1562525	18/07/2017	Pork	Other diced/sliced pork	GB	В	GT
1562534	14/08/2017	Pork	Pork fillets & steaks	GB	В	DS
1562721	14/08/2017	Pork	Pork fillets & steaks	GB	?	?
1562780	14/08/2017	Beef	Beef steaks-less expensive	GB	Е	НН
1562737	14/08/2017	Beef	Beef steaks-expensive	GB	J	F
1562533	14/08/2017	Beef	Beef steaks-expensive	GB	Α	FG
1562720	14/08/2017	Pork	Pork chops GB		E	НН
1562736	14/08/2017	Pork	Pork chops	GB	E	НН
1562719	14/08/2017	Beef	Beef steaks-expensive	GB	E	НН
1562535	14/08/2017	Pork	Pork fillets & steaks	GB	Α	V
1562779	14/08/2017	Beef	Beef steaks-less expensive	IE	В	М
1562718	14/08/2017	Pork	Other diced/sliced pork	GB	?	?
1562723	14/08/2017	Pork	Other diced/sliced pork	GB	?	?
1562713	14/08/2017	Pork	Pork fillets & steaks	EU	?	SS
1562722	14/08/2017	Beef	Beef steaks-expensive	GB	?	?
1562725	14/08/2017	Beef	Beef steaks-less expensive	GB	?	?
1562724	14/08/2017	Beef	Beef steaks-expensive	GB	?	?
1614498	14/08/2017	Beef	Beef steaks-expensive	AR	?	?
1614499	14/08/2017	Beef	Beef steaks-expensive	GB	В	DF
1562738	14/08/2017	Pork	Pork chops	DE	J	E
1562712	14/08/2017	Pork	Pork chops	GB	В	DS
1562781	15/08/2017	Pork	Pork chops	GB	D	Q
1562792	15/08/2017	Beef	Beef steaks-expensive	GB	J	AW
1562787	15/08/2017	Pork	Other diced/sliced pork	GB	E	НН
1614504	15/08/2017	Pork	Pork fillets & steaks	GB	В	GT
1562789	15/08/2017	Pork	Pork chops	GB	?	CC
1562791	15/08/2017	Pork	Other diced/sliced pork	GB	J	С
1562785	15/08/2017	Pork	Pork chops	GB	?	AA
1562541	15/08/2017	Beef	Beef steaks-expensive	GB	G	U

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1562782	15/08/2017	Pork	Pork chops	GB	D	Р
1614505	15/08/2017	Pork	Pork fillets & steaks	GB	В	DS
1562790	15/08/2017	Beef	Beef steaks-expensive	GB	?	?
1562788	15/08/2017	Beef	Beef steaks-expensive	GB	E	NN
1562783	15/08/2017	Beef	Beef steaks-less expensive	GB	D	Q
1614513	15/08/2017	Pork	Other diced/sliced pork	GB	J	E
1562786	15/08/2017	Beef	Beef steaks-less expensive	GB	?	?
1614528	15/08/2017	Beef	Beef steaks-expensive	GB	В	DF
1562542	15/08/2017	Beef	Beef steaks-expensive	GB	Е	NN
1562530	15/08/2017	Beef	Beef steaks-expensive	GB	В	DF
1562546	15/08/2017	Pork	Other diced/sliced pork	GB	D	Q
1562544	15/08/2017	Beef	Beef steaks-expensive	GB	J	F
1562545	15/08/2017	Beef	Beef steaks-expensive	GB	D	WW
1562532	15/08/2017	Pork	Other diced/sliced pork	Other diced/sliced pork GB		L
1562784	15/08/2017	Beef	Beef steaks-expensive	GB	D	VV
1562543	15/08/2017	Pork	Pork fillets & steaks	GB	Е	HH
1562706	16/08/2017	Beef	Beef steaks-expensive	GB	А	V
1562705	16/08/2017	Beef	Beef steaks-expensive	GB	D	Q
1562703	16/08/2017	Beef	Beef steaks-expensive	GB	?	?
1562702	16/08/2017	Pork	Pork chops	GB	А	V
1562701	16/08/2017	Pork	Pork chops	GB	D	Q
1562700	16/08/2017	Pork	Pork chops	GB	?	?
1562699	16/08/2017	Pork	Other diced/sliced pork	GB	D	Q
1562704	16/08/2017	Beef	Beef steaks-expensive	GB	D	WW
2558507	11/09/2017	Beef	Beef steaks-expensive	GB	В	DF
1562714	11/09/2017	Beef	Beef steaks-expensive	GB	?	DD
2558489	11/09/2017	Pork	Pork chops	GB	В	GT
2558496	11/09/2017	Pork	Other diced/sliced pork	GB	I	S
2558530	11/09/2017	Pork	Pork fillets & steaks	GB	?	Υ
2558492	11/09/2017	Pork	Other diced/sliced pork	GB	J	Н
2558498	11/09/2017	Pork	Pork chops	GB	?	В
1562715	11/09/2017	Pork	Other diced/sliced pork	GB	?	J
2558551	11/09/2017	Beef	Beef steaks-expensive	IE	?	Υ
2558497	11/09/2017	Beef	Beef steaks-expensive	GB	?	?
2558499	11/09/2017	Pork	Other diced/sliced pork	GB	Į	S
2558500	11/09/2017	Pork	Pork fillets & steaks	DE	J	E

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
2558501	11/09/2017	Pork	Other diced/sliced pork	NL	В	GT
2558502	11/09/2017	Beef	Beef steaks-less expensive	GB	I	S
2558506	11/09/2017	Beef	Beef steaks-less expensive	GB	J	E
2558531	11/09/2017	Beef	Beef steaks-less expensive	GB	J	Н
2558488	11/09/2017	Beef	Beef steaks-expensive	GB	D	Q
2558563	12/09/2017	Beef	Beef steaks-expensive	GB	D	WW
2558534	12/09/2017	Beef	Beef steaks-less expensive	GB	В	DS
2558537	12/09/2017	Beef	Beef steaks-expensive	GB	?	?
2558533	12/09/2017	Beef	Beef steaks-less expensive	GB	J	Н
2558539	12/09/2017	Beef	Beef steaks-expensive	GB	J	Е
2558535	12/09/2017	Pork	Pork fillets & steaks	GB	В	DF
2558538	12/09/2017	Pork	Other diced/sliced pork	GB	?	?
1614887	12/09/2017	Pork	Pork fillets & steaks	GB	J	Е
2558552	12/09/2017	Beef	Beef steaks-expensive	GB	Е	NN
2558553	12/09/2017	Beef	Beef steaks-expensive	GB	Е	NN
1562796	12/09/2017	Beef	Beef steaks-less expensive	IE	В	DS
2558554	12/09/2017	Pork	Pork fillets & steaks	GB	E	НН
1562798	12/09/2017	Pork	Pork fillets & steaks	GB	?	?
2558540	12/09/2017	Pork	Pork fillets & steaks	GB	J	AW
2558555	12/09/2017	Pork	Pork chops	GB	Е	НН
1562797	12/09/2017	Beef	Beef steaks-expensive	GB	?	?
2558556	12/09/2017	Pork	Other diced/sliced pork	NL	J	E
2558560	12/09/2017	Pork	Pork fillets & steaks	GB	G	L
2558557	12/09/2017	Pork	Other diced/sliced pork	DE	J	G
2558562	12/09/2017	Pork	Pork chops	GB	D	Q
2558558	12/09/2017	Beef	Beef steaks-expensive	GB	J	F
2558561	12/09/2017	Beef	Beef steaks-less expensive	GB	G	YY
2558559	12/09/2017	Beef	Beef steaks-less expensive	IE	J	G
1562795	12/09/2017	Pork	Pork fillets & steaks	GB	В	DS
2558505	13/09/2017	Pork	Pork chops	GB	?	Х
2558504	13/09/2017	Beef	Beef steaks-expensive	GB	?	Х
2558494	13/09/2017	Beef	Beef steaks-expensive	GB	J	E
1613143	13/09/2017	Pork	Pork chops	GB	G	L
1613142	13/09/2017	Pork	Other diced/sliced pork	GB	F	II
2558550	13/09/2017	Beef	Beef steaks-expensive	GB	G	U
2558503	13/09/2017	Beef	Beef steaks-less expensive	GB	F	II

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
1614479	13/09/2017	Pork	Pork chops	GB	D	Q
2558545	13/09/2017	Pork	Other diced/sliced pork	GB	В	DS
2558548	13/09/2017	Beef	Beef steaks-expensive	GB	В	DF
2558495	13/09/2017	Pork	Pork chops	DE	J	E
2558542	13/09/2017	Pork	Other diced/sliced pork	GB	?	?
2558544	13/09/2017	Pork	Pork fillets & steaks	GB	Е	НН
2558493	13/09/2017	Pork	Pork chops	DE	J	E
2558541	13/09/2017	Beef	Beef steaks-expensive	GB	?	?
2558543	13/09/2017	Beef	Beef steaks-expensive	GB	Е	НН
2558549	13/09/2017	Beef	Beef steaks-expensive	GB	D	ww
2558235	09/10/2017	Pork	Pork fillets & steaks	GB	Е	HH
2558227	09/10/2017	Pork	Pork fillets & steaks	GB	D	Q
2558229	09/10/2017	Pork	Pork chops	GB	?	?
2558226	09/10/2017	Beef	Beef steaks-expensive GB		D	ww
2558228	09/10/2017	Beef	Beef steaks-expensive	GB	?	?
2558233	09/10/2017	Pork	Pork fillets & steaks	GB	В	GT
2558237	09/10/2017	Pork	Other diced/sliced pork	GB	?	AS
2558232	09/10/2017	Beef	Other sliced/diced beef	GB	J	00
2558234	09/10/2017	Beef	Beef steaks-less expensive	GB	В	DS
2558236	09/10/2017	Beef	Beef steaks-expensive	GB	Е	НН
2558238	09/10/2017	Beef	Beef steaks-expensive	GB	?	BN
2558231	09/10/2017	Pork	Pork fillets & steaks	GB	J	00
2558242	10/10/2017	Beef	Beef steaks-expensive	GB	В	DS
2558223	10/10/2017	Beef	Beef steaks-expensive	GB	D	ww
2558217	10/10/2017	Beef	Beef steaks-less expensive	GB	В	М
2558243	10/10/2017	Pork	Pork fillets & steaks	GB	Е	HH
2558239	10/10/2017	Pork	Other diced/sliced pork	GB	?	LL
1614470	10/10/2017	Pork	Pork chops	DK	В	GT
2558240	10/10/2017	Beef	Beef steaks-expensive	GB	?	?
2558221	10/10/2017	Beef	Beef steaks-expensive	GB	I	S
2558222	10/10/2017	Pork	Other diced/sliced pork	GB	I	S
2558244	10/10/2017	Beef	Beef steaks-expensive	GB	Е	НН
2558215	10/10/2017	Beef	Beef steaks-expensive	GB	?	ZZ
2558209	10/10/2017	Pork	Pork fillets & steaks	GB	Α	V
2588214	10/10/2017	Pork	Other diced/sliced pork	GB	?	I
2558212	10/10/2017	Pork	Pork chops	GB	Е	НН

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
2558220	10/10/2017	Pork	Other diced/sliced pork	GB	В	GT
2558216	10/10/2017	Pork	Other diced/sliced pork	GB	?	ZZ
2558224	10/10/2017	Pork	Other diced/sliced pork	GB	D	Q
2558218	10/10/2017	Pork	Other diced/sliced pork	GB	В	DS
2558210	10/10/2017	Beef	Beef steaks-expensive	GB	Α	FR
2558213	10/10/2017	Beef	Beef steaks-less expensive	GB	?	I
2558211	10/10/2017	Beef	Beef steaks-expensive	GB	Е	NN
2558219	10/10/2017	Beef	Beef steaks-less expensive	IE	В	М
2558208	10/10/2017	Beef	Beef steaks-less expensive	GB	Α	V
2558207	10/10/2017	Pork	Pork chops	GB	Α	V
2558205	11/10/2017	Beef	Beef steaks-expensive	GB	?	TT
2558206	11/10/2017	Pork	Pork fillets & steaks	GB	?	TT
2558202	11/10/2017	Pork	Other diced/sliced pork	GB	J	Н
2558203	11/10/2017	Pork	Other diced/sliced pork GB		В	GT
2558198	11/10/2017	Beef	Beef steaks-expensive	GB	J	F
2558199	11/10/2017	Pork	Pork fillets & steaks	GB	J	E
2558204	11/10/2017	Beef	Beef steaks-less expensive	GB	В	DS
2558201	11/10/2017	Beef	Beef steaks-expensive	GB	J	AW
2448107	06/11/2017	Beef	Beef steaks-expensive	GB	J	F
2448157	06/11/2017	Pork	Pork fillets & steaks	GB	?	?
2448118	06/11/2017	Beef	Beef steaks-expensive	GB	В	DS
1614417	06/11/2017	Pork	Pork chops	GB	Е	NN
2448154	06/11/2017	Beef	Beef steaks-expensive	GB	E	НН
2448156	06/11/2017	Pork	Pork fillets & steaks	GB	В	DS
2448162	06/11/2017	Beef	Beef steaks-expensive	GB	D	Q
2448155	06/11/2017	Beef	Beef steaks-less expensive	GB	?	?
2448103	06/11/2017	Beef	Beef steaks-expensive	GB	J	Н
2448111	06/11/2017	Pork	Pork fillets & steaks	GB	Е	НН
2448106	06/11/2017	Pork	Other diced/sliced pork	GB	J	Е
2448102	06/11/2017	Pork	Pork fillets & steaks	GB	D	Q
2448104	06/11/2017	Pork	Other diced/sliced pork	GB	J	Н
2448109	06/11/2017	Beef	Beef steaks-expensive	GB	В	DF
2448105	06/11/2017	Beef	Beef steaks-expensive	GB	В	DS
2448101	06/11/2017	Beef	Beef steaks-expensive	GB	D	Q
2448108	06/11/2017	Pork	Other diced/sliced pork	GB	В	DS
2448110	06/11/2017	Beef	Beef steaks-expensive	GB	E	НН

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
2448177	07/11/2017	Pork	Pork fillets & steaks	GB	В	DS
2448217	07/11/2017	Pork	Pork fillets & steaks	GB	J	Н
1614447	07/11/2017	Pork	Pork fillets & steaks	GB	J	E
2448074	07/11/2017	Pork	Pork fillets & steaks	GB	Α	V
2448070	07/11/2017	Pork	Pork fillets & steaks	GB	J	E
2558225	07/11/2017	Beef	Beef steaks-expensive	GB	J	AW
2448176	07/11/2017	Beef	Beef steaks-expensive	IE	В	DS
2448072	07/11/2017	Beef	Beef steaks-expensive	GB	J	F
2448073	07/11/2017	Beef	Beef steaks-expensive	GB	J	F
2448075	07/11/2017	Beef	Beef steaks-expensive	GB	Α	V
2448166	08/11/2017	Beef	Beef steaks-expensive	GB	D	WW
2448082	08/11/2017	Pork	Other diced/sliced pork	GB	Α	FR
2448088	08/11/2017	Beef	Beef steaks-expensive	GB	E	NN
2448100	08/11/2017	Pork	Other diced/sliced pork	GB	В	DS
1614416	08/11/2017	Pork	Pork fillets & steaks	GB	D	Q
2448112	08/11/2017	Pork	Pork fillets & steaks	GB	G	L
2448113	08/11/2017	Pork	Pork fillets & steaks	GB	D	Q
2448114	08/11/2017	Beef	Beef steaks-less expensive	GB	D	Q
2448115	08/11/2017	Beef	Beef steaks-less expensive	GB	G	L
2448116	08/11/2017	Beef	Beef steaks-expensive	GB	D	WW
2448165	08/11/2017	Pork	Pork fillets & steaks	GB	D	Q
2448164	08/11/2017	Pork	Other diced/sliced pork	GB	D	Q
1614418	08/11/2017	Beef	Beef steaks-expensive	GB	D	Q
1614415	08/11/2017	Pork	Pork fillets & steaks	GB	J	Е
1562531	08/11/2017	Beef	Beef steaks-less expensive	GB	В	DS
2448086	08/11/2017	Beef	Beef steaks-less expensive	IE	J	G
2448090	08/11/2017	Beef	Beef steaks-expensive	GB	Е	NN
2448085	08/11/2017	Beef	Beef steaks-expensive	GB	J	AW
2448091	08/11/2017	Beef	Beef steaks-expensive	GB	Е	НН
1363864	08/11/2017	Pork	Other diced/sliced pork	GB	D	WW
2448083	08/11/2017	Beef	Beef steaks-expensive	GB	J	F
2448087	08/11/2017	Pork	Pork chops	GB	E	НН
1614419	08/11/2017	Pork	Pork fillets & steaks	GB	J	E
2448089	08/11/2017	Pork	Other diced/sliced pork	GB	E	НН
2448084	08/11/2017	Pork	Other diced/sliced pork	GB	J	Н
1614501	08/11/2017	Pork	Pork chops	GB	E	НН

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
2448081	08/11/2017	Beef	Beef steaks-expensive	GB	Α	V
2448031	04/12/2017	Beef	Beef steaks-expensive	GB	D	WW
1614412	04/12/2017	Pork	Other diced/sliced pork	GB	J	G
2448027	04/12/2017	Pork	Other diced/sliced pork	GB	В	DS
2448029	04/12/2017	Beef	Beef steaks-expensive	GB	J	F
2448028	04/12/2017	Pork	Other diced/sliced pork	GB	D	Q
2448030	04/12/2017	Beef	Beef steaks-expensive	IE	В	DS
2448044	05/12/2017	Pork	Other diced/sliced pork	GB	D	Q
2448066	05/12/2017	Beef	Beef steaks-less expensive	GB	?	?
2448040	05/12/2017	Pork	Pork fillets & steaks	GB	F	II
2448080	05/12/2017	Pork	Pork chops	NL	J	E
2448076	05/12/2017	Pork	Pork chops	GB	Е	НН
2448078	05/12/2017	Pork	Other diced/sliced pork	GB	D	Q
2448039	05/12/2017	Beef	Beef steaks-expensive	GB	F	II
2448077	05/12/2017	Beef	Beef steaks-expensive	GB	Е	NN
2448079	05/12/2017	Beef	Beef steaks-expensive	GB	D	Q
2448046	05/12/2017	Pork	Other diced/sliced pork	GB	Α	V
2448018	05/12/2017	Pork	Other diced/sliced pork	GB	J	Н
2448042	05/12/2017	Pork	Other diced/sliced pork	GB	E	НН
2448048	05/12/2017	Beef	Beef steaks-expensive	GB	Α	FR
2448049	05/12/2017	Beef	Beef steaks-expensive	GB	Α	FR
2448045	05/12/2017	Beef	Beef steaks-expensive	GB	D	Q
2448043	05/12/2017	Beef	Beef steaks-expensive	GB	Е	NN
2448041	05/12/2017	Beef	Beef steaks-expensive	GB	J	F
2448065	05/12/2017	Pork	Other diced/sliced pork	GB	?	?
2448063	05/12/2017	Beef	Beef steaks-less expensive	GB	J	E
2448047	05/12/2017	Pork	Pork chops	GB	Α	FG
2448016	05/12/2017	Pork	Other diced/sliced pork	GB	В	DS
2448024	05/12/2017	Pork	Pork fillets & steaks	NL	J	E
1614411	05/12/2017	Pork	Other diced/sliced pork	GB	J	G
2448061	05/12/2017	Beef	Beef steaks-expensive	GB	G	ВН
2448025	05/12/2017	Pork	Other diced/sliced pork	NL	В	DS
2448019	05/12/2017	Beef	Beef steaks-expensive	GB	В	DF
2448022	05/12/2017	Beef	Beef steaks-expensive	GB	J	F
2448017	05/12/2017	Beef	Beef steaks-expensive	GB	J	AW
2448023	05/12/2017	Beef	Beef steaks-expensive	GB	J	F

Unique Sample number	Dispatch Date	Food Group	Food Category	Country of Origin <sup>1</sup>	Anon shop	Anon brand
2448026	05/12/2017	Beef	Beef steaks-expensive	GB	В	DF
2448060	05/12/2017	Pork	Other diced/sliced pork	GB	В	DS
2448062	05/12/2017	Pork	Other diced/sliced pork	GB	G	ВН
2448064	05/12/2017	Pork	Pork fillets & steaks	GB	J	E
2448059	05/12/2017	Beef	Beef steaks-expensive	GB	В	М
2448015	05/12/2017	Beef	Beef steaks-expensive	GB	В	DF
2448020	05/12/2017	Pork	Other diced/sliced pork	DK	В	DS
2448055	06/12/2017	Beef	Beef steaks-expensive	GB	E	NN
1614896	06/12/2017	Pork	Other diced/sliced pork	GB	J	G
2448034	06/12/2017	Pork	Pork fillets & steaks	GB	F	II
2448033	06/12/2017	Pork	Other diced/sliced pork	GB	Α	V
2448036	06/12/2017	Beef	Beef steaks-less expensive	GB	J	E
2448035	06/12/2017	Beef	Beef steaks-expensive	GB	F	II
2448037	06/12/2017	Beef	Beef steaks-expensive	GB	Α	EW
2448056	06/12/2017	Beef	Beef steaks-expensive	GB	D	WW
1363938	06/12/2017	Pork	Pork fillets & steaks	GB	E	НН
2448117	06/12/2017	Pork	Pork fillets & steaks	GB	D	Q

**Table 2**. Summary of samples positive for *E. coli* from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX) or CHROMagar™ ESBL (CA-ESBL)

Sample ID	Date sent	Meat type	Meat cut	Brand	Retail store	Sampling Location	Country of Origin	Growth on MCA-CTX <sup>a</sup>	Resistance Phenotype <sup>a</sup>	Growth on CA-ESBL	CA-ESBL PCR result <sup>b</sup>	CA-ESBL CTX gene sequence <sup>b</sup>	Batch/Lot Number
1612828	18/01/201 7	Pork	Belly Slices	НН	Е	Greater Manchester North East	United Kingdom	Yes	AmpC	No	ND		32 13 10:43
1612840	17/01/201 7	Beef	Fillet Steak	R	Unknown	West and South of Northern Ireland	United Kingdom	Yes	ESBL	Yes	CTX	CTXM-1	4583
1612845	17/01/201 7	Pork	Chops	KK	С	West and South of Northern Ireland	United Kingdom	No	ND	Yes	TEM		506001000410
1612846	17/01/201 7	Beef	Fillet Steak	AZ	С	West and South of Northern Ireland	United Kingdom	Yes	AmpC	Yes	CTX	CTXM-1	50117

ND – Not determined

*a* – EU harmonised test method

b - UK non-harmonised additional test

**Table 3.** Summary of resistance phenotypes of *E. coli* isolated by growth on MCA CTX agar coli from retail meats, and resistances to antibiotics tested

	No. Re	sistant <sup>a</sup> / no.	tested
Antibiotic		Beef	Pork
	Beef ESBL	AmpC	AmpC
Ampicillin	1/1	1/1	1/1
Azithromycin	0/1	0/1	0/1
Cefepime	1/1	1/1	0/1
Cefotaxime	1/1	1/1	1/1
Cefoxitin	0/1	1/1	1/1
Ceftazidime	1/1	1/1	1/1
Chloramphenicol	0/1	0/1	0/1
Ciprofloxacin	1/1	0/1	0/1
Colistin	0/1	0/1	0/1
Ertapenem	0/1	0/1	0/1
Gentamicin	0/1	0/1	0/1
Imipenem	0/1	0/1	0/1
Meropenem	0/1	0/1	0/1
Nalidixic Acid	1/1	0/1	0/1
Sulfamethoxazole	1/1	0/1	0/1
Temocillin	0/1	0/1	0/1
Tetracycline	1/1	0/1	0/1
Tigecycline	0/1	0/1	0/1
Trimethoprim	0/1	0/1	0/1

Orange highlight denotes the four different cephalosporin antibiotics. These are cefepime, cefotaxime, cefoxitin, ceftazidime which were tested.

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

a – Microbiologically resistant using EUCAST ECOFFS.

**Table 4.** MIC results for *E. coli* isolates with an AmpC or ESBL phenotype from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX)

Results included for one isolate from CA-ESBL – additional isolate from sample 01612846

Isolate details	Antibiotic	Indicator	MIC (μg/ml)	Interpretation of MIC <sup>a</sup>
	Ampicillin	>	64	R
	Azithromycin		4	S
	Cefepime		16	R
Sample ID	Cefotaxime	>	64	R
01612840	Cefotaxime / Clavulanate	<=	0.06	Synergy
Meat type	Cefoxitin		8	S
Beef	Ceftazidime		8	R
Meat cut	Ceftazidime / Clavulanate		0.25	Synergy
Expensive steak	Chloramphenicol	<=	8	S
<b>Brand</b> R	Ciprofloxacin		0.5	R
	Colistin	<=	1	S
Retail store Not known	Ertapenem	<=	0.015	S
	Gentamicin	<=	0.5	S
Purchase area Northern Ireland	Imipenem		0.25	S
Country of	Meropenem	<=	0.03	S
Country of origin UK	Nalidixic Acid	>	128	R
ESBL	Sulfamethoxazole	>	1024	R
phenotype	Temocillin		8	S
	Tetracycline	>	64	R
	Tigecycline	<=	0.25	S
	Trimethoprim	<=	0.25	S
Isolate details	Antibiotic	Indicator	MIC (μg/ml)	Interpretation of MIC <sup>a</sup>
Sample ID	Ampicillin	>	64	R
01612846 MCA-CTX <sup>b</sup>	Azithromycin		8	S
<b>Meat type</b> Beef	Cefepime		0.25	R
Beei	Cefotaxime		8	R
Meat cut Less expensive	Cefotaxime / Clavulanate		4	No synergy
steak	Cefoxitin		64	R
Brand	Ceftazidime		8	R
AZ	Ceftazidime / Clavulanate		8	No synergy
Retail store	Chloramphenicol	<=	8	S
С	Ciprofloxacin	<=	0.015	S
Purchase area	Colistin	<=	1	S

Isolate details	Antibiotic	Indicator	MIC (μg/ml)	Interpretation of MIC <sup>a</sup>
Northern Ireland	Ertapenem		0.03	S
Country of origin	Gentamicin	<=	0.5	S
	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
AmpC phenotype	Nalidixic Acid	<=	4	S
	Sulfamethoxazole	<=	8	S
	Temocillin		8	S
	Tetracycline	<=	2	S
	Tigecycline	<=	0.25	S
	Trimethoprim	<=	0.25	S
Isolate details	Antibiotic	Indicator	MIC (μg/ml)	Interpretation of MIC <sup>a</sup>
Sample ID	Ampicillin	>	64	R
01612828	Azithromycin		4	S
Meat type Pork	Cefepime		0.25	S
	Cefotaxime		16	R
Meat cut Diced / sliced  Brand  HH	Cefotaxime / Clavulanate		8	No synergy
	Cefoxitin	>	64	R
	Ceftazidime		32	R
	Ceftazidime / Clavulanate		16	No synergy
Retail store E	Chloramphenicol	<=	8	S
Purchase area Greater Manchester	Ciprofloxacin	<=	0.015	S
	Colistin	<=	1	S
	Ertapenem		0.03	S
Country of origin UK AmpC phenotype	Gentamicin		2	S
	Imipenem		0.25	S
	Meropenem	<=	0.03	S
	Nalidixic Acid	<=	4	S
	Sulfamethoxazole		16	S
	Temocillin		8	S
	Tetracycline		64	R
	Tigecycline	<=	0.25	S
	Trimethoprim		0.5	S
Isolate details	Antibiotic	Indicator	MIC (μg/ml)	Interpretation of MIC <sup>a</sup>
Sample ID	Ampicillin	>	64	R
01612846 CA-ESBL <sup>b</sup>	Azithromycin		4	S
Meat type	Cefepime		16	R

Isolate details	Antibiotic	Indicator	MIC (μg/ml)	Interpretation of MIC <sup>a</sup>
Beef	Cefotaxime	>	64	R
<b>Meat cut</b> Less expensive steak	Cefotaxime / Clavulanate	<=	0.06	Synergy
	Cefoxitin		8	S
	Ceftazidime		16	R
<b>Brand</b> AZ	Ceftazidime / Clavulanate		0.25	Synergy
Retail store	Chloramphenicol	<=	8	S
	Ciprofloxacin	<=	0.015	S
Purchase area Northern Ireland	Colistin	<=	1	S
	Ertapenem		0.03	S
Country of origin	Gentamicin	<=	0.5	S
	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
ESBL phenotype	Nalidixic Acid	<=	4	S
	Sulfamethoxazole	>	1024	R
	Temocillin		4	S
	Tetracycline	>	64	R
	Tigecycline	<=	0.25	S
	Trimethoprim	<= <del></del>	0.25	S

Orange highlight denotes four different cephalosporin antibiotics. These are cefepime, cefotaxime, cefoxitin, ceftazidime.

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

Green highlight denotes cephalosporins with the beta-lactamase inhibitor clavulanic acid. These are cefotaxime/ clavulanate, ceftazidime/ clavulanate.

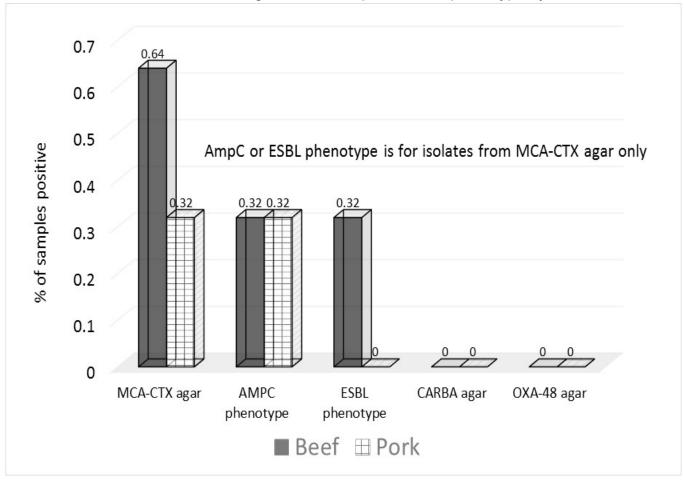
## R- resistant, S – sensitive.

- a Microbiologically resistant or sensitive using EUCAST ECOFFS.
- b Sample 01612846 was positive for two different *E. coli* on different agars, one an AmpC from the EU MCA CTX agar method and the other an ESBL phenotype from the additional CA-ESBL agar.

## 9. Figures

**Figure 1.** Percentages of beef and pork samples positive for AMR *E. coli* on different agars, and % of isolates from MCA-CTX agar with an AmpC or ESBL phenotype by MICs.

Percentages of beef and pork samples positive for AMR *E. coli* on different agars, and % of isolates from MCA-CTX agar with an AmpC or ESBL phenotype by MICs.



## 10. References

- [1] Fang H, Ataker F, Hedin G, Dornbusch K. Molecular epidemiology of extended-spectrum beta-lactamases among *Escherichia coli* 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] Anonymous. Final Report for FS102109 EU Harmonised Survey of Antimicrobial Resistance (AMR) on Retail Meats (Year 3: Beef and Pork). Report of Hallmark Veterinary complinace services. 2018b.
- [4] Anonymous. Family Food 2014. Annual report on household purchases of food and drink. 2018c. https://www.gov.uk/government/statistics/family-food-2014.
- [5] 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.
- [6] Anonymous. EFSA EUSR-AMR Workflow and Criteria for ESBL/AmpC/Carbapenemase -Phenotypes. 2018a. <a href="https://www.eurl-ar.eu/CustomerData/Files/Folders/3-workshop-kgs-lyngby-april2016/25">https://www.eurl-ar.eu/CustomerData/Files/Folders/3-workshop-kgs-lyngby-april2016/25</a> efsa-eusr-amr-workflow-and-criteria-for-esbl-ampc-carbapenemase-phenotypes.pdf.
- [7] EUCAST. MIC and zone diameter distributions and ECOFFs. 2018. http://www.eucast.org/mic distributions and ecoffs/.
- [8] 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.
- [9] 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.
- [10] Randall LP, Clouting C, Horton RA, Coldham NG, Wu G, Clifton-Hadley FA, et al. Prevalence of *Escherichia coli* carrying extended-spectrum beta-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. J Antimicrob Chemother. 2011;66:86-95.
- [11] Hu Y, Liu F, Lin IY, Gao GF, Zhu B. Dissemination of the *mcr-1* colistin resistance gene. The Lancet infectious diseases. 2015;16:146-7.
- [12] 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.

- [13] 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.
- [14] 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.
- [15] 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.
- [16] FSA. RDFS102109 EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in *E. coli* from Retail Meats (Year 2 Chicken). 2017b. https://www.food.gov.uk/sites/default/files/fs102109y2.pdf.
- [17] 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.
- [18] Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev. 2014;27:543-74.
- [19] 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.
- [20] 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.
- [21] 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.
- [22] 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.
- [23] 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.
- [24] VMD. UK Veterinary Antibiotic Resistance and Sales Surveillance Report. 2015; <a href="https://assets.publishing.service.gov.uk/government/uploads/system/uploads/a">https://assets.publishing.service.gov.uk/government/uploads/system/uploads/a</a> ttachment data/file/582341/1051728-v53-UK-VARSS 2015.pdf.

[25] 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.