Surveillance of antimicrobial resistance (AMR) in E. coli on beef and pork meat on retail sale in the UK (October to December 2021)

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Executive Summary

1.1 Background

Antibiotic resistance (AMR) in microorganisms is a growing problem. While it is a natural process, the extensive use of antimicrobials in humans and animals has been a significant driving force in its development. Antimicrobials are used in the livestock industry to prevent and control bacterial disease. The use of subtherapeutic levels of antibiotics in animal feed (as growth promotors) since the 1950's has caused an expansion of the pool of AMR bacteria. In 2006 the use of these was banned in the EU, and also in the UK.

Escherichia coli (E. coli) is a normal inhabitant of the mammalian gut (termed a commensal) and most isolates do not cause observable clinical disease in healthy animals or humans. However

commensal bacteria can be reservoirs of AMR genes. Horizontal gene transfer among bacteria allows them to exchange their genetic material including antibiotic resistance genes. *E. coli* isolates are therefore useful ‘indicators’ of AMR. They are ubiquitous in animals, and they allow us to monitor the presence of AMR typically circulating in food producing animals.

If the bacteria possess a resistance to three or more different classes of antibiotics, they are called multidrug resistant (MDR). MDR bacteria pose a health risk because fewer therapeutic agents are active against them. This is a particular concern if the MDR includes resistance to certain classes of antibiotics (such as the carbapenems) which are used to treat severe bacterial infections when other treatment options are ineffective.

There are several mechanisms by which bacteria can develop resistance to antimicrobials; the production of enzymes which break-down the drug; inactivation of the drug by modification; mutation of the drug target site or by transport of the drug out of the bacterial cell. Resistance to 3rd and 4th generation cephalosporins occurs by the production of ?-lactamase enzymes. Additionally, *E. coli* can possess resistance to carbapenems the ‘last resort’ antibiotics. Surveillance of AMR bacteria in humans, environments and food producing animals is crucial to monitor and understand the threat posed to public and animal health.

1.2 Introduction

Surveys to monitor the presence of AMR bacteria in foods of animal origin is a requirement of the European Directive 2003/99/EC and the commission implementing decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance (AMR) in zoonotic and commensal bacteria. Each year a survey has been conducted to monitor AMR *E. coli* in retail meat, alternating between testing 300 beef and pork samples in one year and then turkey and chicken meat the next year. In 2021, beef and pork on retail sale in the UK was sampled between October and December and investigated for the presence of *E. coli*.

The methodology used in this survey was based on current EU protocols for the testing of retail beef, chicken and pork. These involve culture of *E. coli* on selective agar media containing cephalosporin or carbapenem antimicrobial drugs. Growth of *E. coli* on selective media generally indicates resistance to the selective agent.

The *E. coli* isolates are screened against a panel of antimicrobials to determine their susceptibility. The pattern of resistance is characteristic of the ?-lactamase enzymes produced by the *E. coli* isolate, of which there are 3 main AMR phenotypes, AmpC, ESBL or carbapenemase-producers. ESBL and AmpC enzymes confer resistance to cephalosporins, whilst carbapenemase enzymes confer resistance to the ‘last resort’ carbapenem antibiotics.

At the request of the FSA (non-harmonised testing outside the remit of Decision 2013/652/EU) further screening was performed for *E. coli* resistant to colistin (an important drug in the treatment of highly resistant bacterial infections in humans). Colistin-resistant strains may harbour *mcr* resistance genes, which are located on plasmids that can transfer among bacteria.

1.3 Sampling

Samples were collected from retail premises across England, Scotland, Wales and Northern Ireland. As for previous years, the sampling plan used ‘proportionate stratified sampling’ from 80 locations (defined by the EU as the NUTS-3 areas) from all parts of the UK, and the proportion taken was according to population size of the area. Samples were taken from all but the smallest locations, and this covered at least 80% of the total population. The plan included the usual 11 largest supermarkets plus a ‘Shops not on the list’ group. A total of 105 samples each of eligible fresh beef and pork meat were collected from October 2021 to December 2021. In general, in previous surveys, 300 samples were tested (following EU recommendations) and these were collected and tested throughout one full year. The reduced numbers in this study were a

consequence of the delayed start following the UK exit from the EU, and because of the lab capacity of this period.

1.4 Culture and analysis methods

Methods were in line with EU protocols and / or APHA internal Standard Operating Procedures. Briefly this involved homogenising 27 grams of meat in 270 mL of a liquid bacterial recovery medium and plating an aliquot onto agar that is selective for *E. coli*. This selective media was used with and without cefotaxime antibiotic added. Cefotaxime is a 3rd generation cephalosporin and is used as a marker of AMR in *E. coli*. Plating on these 2 agars allows the estimation of the numbers of total *E. coli*, and also estimation of cefotaxime resistant *E. coli* present per gram of meat sample.

The remaining 250 mL of the homogenate was incubated for 18 to 22 hours aerobically, to allow recovery and multiplication of bacteria (enrichment), before being inoculated onto an antibiotic-containing selective agar plate. The media employed were - MacConkey (for background *E. coli*), MacConkey plus cefotaxime or colistin (for strains resistant to third-generation cephalosporin antimicrobials or to colistin), chromID® CARBA and chromID® OXA-48 (for carbapenem-resistant strains), and CHROMagar™ ESBL (for presumptive ESBL-phenotype isolates).

Up to three bacterial colonies from each of the different media types, were sub-cultured onto a fresh agar plate and tested biochemically to confirm that they were *E. coli*. The pattern of resistance to antimicrobials (the AMR phenotype) of the *E. coli* isolates were determined using a microbroth dilution method (with twenty different antibiotics) following standard methods according to Commission Implementing Decision 2013/652/EU. The Minimum inhibitory concentration (MIC) antibiotic breakpoints used to determine the AMR phenotype were stipulated by EU decision 2020/1729/EU. In previous years the breakpoints were stipulated by EU decision 2013/652/EU. This change resulted in the breakpoints for nalidixic acid, temocillin and tigecycline lowering (by half) compared to those used in previous years.

A PCR test for the detection of *mcr*1-5 genes which confers resistance to colistin was performed on *E. coli* isolates that grew on agar containing colistin. This was to confirm the presence of the plasmid-mediated colistin resistance genes. *E. coli* isolated on CHROMagar™ ESBL agar were tested for the presence and sequence type of blaCTX-M, blaOXA, blaSHV and blaTEM ESBL genes by whole-genome sequencing (WGS).

1.5 Results

A total of 105 beef and 105 pork samples were tested, and all yielded no growth of *E. coli* (total and resistant *E. coli*) on initial culture prior to bacterial enrichment. It should be noted that the enumeration method has a detection limit of 3,000 colony forming units per gram (cfu/g) using the EU protocol. The samples post enrichment however did yield *E. coli*, with one beef sample (0.95%, 95% confidence interval 0.02% to 5.19%) and four pork samples (3.81%, 95% confidence interval 1.05% to 9.47%) yielding AMR *E. coli*. These isolates were recovered using cefotaxime containing agar plates.

On CHROMagar™ ESBL plates none of the beef samples were positive, but two pork samples were positive (1.90%, 95% confidence interval 0.23% to 6.71%) after pre-enrichment.

No carbapenem-resistant *E. coli* were isolated on carbapenem-selective agar plates from beef or pork samples after enrichment.

Although 72 pre-enriched beef (68.57%, 95% confidence interval 58.78% to 77.28%) and 73 pre-enriched pork (69.52%,95% confidence interval 59.78% to 78.13%) samples yielded presumptive *E. coli* colonies on colistin-supplemented agar plates, none were shown to contain any of the *mcr* 1-5 genes.

1.6 AMR phenotypes

Microbroth dilution testing against a panel of 20 antimicrobials allowed the AMR phenotypes to be determined. Two of the pork samples (1.90%, 95% confidence interval 0.23% to 6.71%) were positive for AmpC-producing *E. coli* and two pork samples were positive for ESBL-producing *E. coli.* The beef isolate had an *E. coli* with an AmpC + ESBL-expressing phenotype (0.95%, 95% confidence interval 0.02% to 5.19%).

None of the five *E. coli* were resistant to any of the carbapenem antimicrobials tested (ertapenem, imipenem and meropenem), or to the antimicrobial colistin. Resistances however were seen to some of the cephalosporin antibiotics. The beef isolate was resistant to all four of the cephalosporin antibiotics it was tested against (cefepime, cefotaxime, cefoxitin, ceftazidime), whilst the pork isolates were resistant to at least two of these antibiotics. All five *E. coli* isolates were resistant to ampicillin, but showed no resistance to amikacin, temocillin or tigecycline.

1.7 Whole genome sequencing (WGS)

WGS of the two pork samples positive on CHROMagar ESBL, revealed that both isolates carried the blaCTX-M1 and blaCTX-M14 genes which encodes resistance to cephalosporin antibiotics (such as cefotaxime) and as such gives the isolates an ESBL-phenotype. The strains of *E. coli* involved were determined by multilocus sequence typing. The pork isolates were ST410 (CTX-M-1) and ST6745 (CTX-M-14).

1.8 Summary

This 2021 survey has shown that the prevalence of AMR *E. coli* in retail beef and pork samples is low, with less than 1% and less than 4% of beef and pork samples respectively possessing an ESBL- or AmpC-expressing *E.?coli*. None of the meat samples prior to enrichment had ‘background’ or AmpC-/ESBL-phenotype *E. coli* counts above the EU detection levels, indicating low numbers of these bacteria on meat samples.

This survey has used the same methodology as the surveys previously performed in 2015, 2017 and 2019. As such there is now data available for AMR in *E. coli* recovered from retail beef and pork over a seven-year period. The prevalence of AMR *E.?coli* during this time has remained low. Carbapenem resistant *E. coli* have not been detected in any of the years, and only one beef sample (of non-EU origin in 2017) has been positive for a colistin-resistant *E. coli*. As in previous years, our survey results compare favourably with results from other EU countries. The prevalence in the UK is lower than the average prevalence for all 28 member states combined.

In conclusion, these studies indicate that resistance to 3rd generation cephalosporins is very low in *E. coli* and has shown little variation in prevalence since 2015 (the start of the monitoring period). Resistance to the ‘last resort’ antibiotics is even lower, with no carbapenem resistant *E.? coli* isolates discovered in the seven years of monitoring, and only one isolate in this period has shown resistance to the antimicrobial colistin.

Glossary

Term	Definition
AmpC phenotype	Antimicrobial resistance profile type with resistance typically to cephalosporin antimicrobials including cefoxitin and also to ?-lactamase inhibitor-?-lactam combinations
AmpC enzyme	Enzyme conferring AmpC type resistance
AMR	Antimicrobial Resistance
APHA	Animal and Plant Health Agency

Term	Definition
BPW	Buffered Peptone broth, a liquid media widely used to grow bacteria
CRL	Community Reference Library
CTX-M	Group of ESBL enzymes that give bacteria resistance to cephalosporin antimicrobials.
Enterobacteriaceae	Family of bacteria including many common gut bacteria such as Escherichia coli or E. coli
CA-ESBL	CHROMagar™ ESBL, for isolation of ESBL-producing E. coli
CARBA	ChromID® CARBA agar, for isolation of carbapenemase resistant E. coli
COL	Colistin
CTX	Cefotaxime
ECOFF	Epidemiological Cut Off value (with respect to antimicrobial resistance)
EN	Norme Européenne /Europäische Norm (European Standard)
ESBL	Extended Spectrum ?-lactamase. Enzymes that are capable of breaking down many penicillin type antimicrobials, including cephalosporin antimicrobials.
ESBL-phenotype	Antimicrobial resistance profile type with resistance typically to cephalosporin antimicrobials but excluding resistance to cefoxitin and ?-lactamase inhibitor-?-lactam combinations
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EURL	European Union reference laboratories
FSA	Food Standards Agency
HCCA	?-Cyano-4-hydroxycinnamic acid
ISO	International Organisation for Standardisation
MALDI-ToF	Matrix-Assisted Laser Desorption / Ionization Time-of-Flight
MCA	MacConkey agar
MCA-COL	MacConkey agar + 2 mg/L colistin
MCA-CTX	MacConkey agar + 1 mg/L cefotaxime
MIC	Minimum Inhibitory Concentration
MS	Member States
NUTS	Nomenclature of Units for Territorial Statistics
OXA-48	ChromID® OXA-48 agar, for isolation of carbapenemase resistant E. coli
PBS	Phosphate Buffered saline
PCR	Polymerase Chain Reaction
PHENOTYPE	In this context, antimicrobial resistance type
QC	Quality control
SOP	Standard Operating Procedure
WGS	Whole Genome Sequencing

Materials and methods

1.9 Sampling criteria

The survey was based on the requirements of the European Directive **2003/99/EC** and the Commission Implementing Decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria – **2013/652/EU**. Rather than EU recommended 300 of each meat type collected across the entire year as occurred for beef and pork in 2015, 2017 and 2019 [1], this study collected 100 of each meat type in October, November and December of 2021. During the above time period a range of different chilled, fresh beef and pork retail meats were collected across the UK and tested as below.

1.9.1 From the ‘HallMark Veterinary and Compliance Services’ report with permission [2]

As in previous years, the sampling plan used ‘proportionate stratified sampling’ to allocate samples to NUTS-3 areas and the samples were distributed in proportion to population size.

Eighty NUTS-3 locations with representation of England, Scotland, Wales and Northern Ireland that covers at least 80% of the total population were selected. Samples were taken from all but the smallest NUTS-3 regions in the UK.

The plan included the usual 11 top supermarkets plus a 'Shops not on the list' group for comparability in proportion to the market share data provided by the FSA. In previous years, 'Shops not on the list' represented a single retailor category from the Family Food data. Based on the alternatives, 'Shops not on the list' are defined as being all retailors of red meat as described in the HallMark report [2].

The study sampled the same fresh meat cut categories as previous AMR studies. It included only fresh/chilled beef and pork, not frozen or cooked, and excluding processed, pre-prepared, ready-basted, marinated, seasoned, herbed, stuffed, cook in the bag, breaded, battered, etc. The product categories were well defined to ensure consistency between surveyors.

The pork samples categories were pork chops, pork fillets and steaks, and all other pork. Each sample was randomly assigned to a cut category, according to consumption data which maximise the power of detecting different AMR between these cut categories.

The beef samples categories were beef steak-more expensive, beef steak-less expensive and all other beef and veal. Steaks that cost under £2+/100g were considered less expensive. 'Expensive steak' is defined as steak equal or above £2/100g. Each sample was randomly assigned to a cut category according to the consumption data. The sampling of the beef and pork cuts were based on the proportions provided by FSA.

1.9.2 Work performed at APHA Weybridge

The methodology for the laboratory work is detailed in the 7 internal APHA standard operating procedures (SOP) below. These SOPs are based on EU methods (which are also listed below). PDF files of the most recent versions of the above EU methods can be found on-line.

APHA SOPs are: -

- Isolation of background (indicator commensal) and antimicrobial resistant Enterobacteriales from meats and caecal contents according to EU and / or APHA protocols (CBU 0278, version 9 – 20-05-2020).
- Microbank -70ºC Bacterial Storage System (CBU 0155).
- Identification of Bacteria by Oxidase (BA 050) and Indole Spot Test – a rapid method for bacteria (BA0130) and by MALDI-ToF (BAC 0334).
- Minimum Inhibitory Concentration (MIC) – The Sensititre Method (BA0604).
- Oxidase (BA 050).
- Indole Spot Test – a Rapid Method for Bacteria (BA 0130).
- Identification of bacteria by MALDI-ToF (BAC0334).

EU Methods (on which APHA SOPs are based) are:-

- **EU method** - Isolation of ESBL, AmpC and carbapenemase-producing *E. coli* from fresh meat – Version 7, December 2019.

- **EU method** - Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AmpC-producing *E. coli* in meat and animals – Version 3, November 2017?.

- **EU method** – Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli* – Version 2, January 2015.

- **EU method** - Quantification of ESBL/AmpC-producing *E. coli* in caecal content and fresh meat samples – Version 1, December 2017.

In addition to the APHA protocols, the PCR method for the detection of *mcr*1-5 genes directly follows the EU method 'PCR for plasmid-mediated colistin resistance genes *mcr-1, mcr-2, mcr-3, mcr-4, mcr-5* and variants (multiplex).'

In brief, 27 ± 0.5 grams of the retail meat sample that had been collected, transported and stored under conditions as stipulated by the EU protocols was homogenised. Homogenisation was in ~ 100 ml (from 243 mL) of sterile chilled BPW, before adding this homogenate to the remaining BPW and gently mixing, providing 270 mL of BPW homogenate.

From this 270 ml BPW homogenate, 20 mL was taken for the viable bacterial counts. Viable counts were performed according to the EU protocol with slight variation. This variation was homogenisation of one meat portion per sample in chilled BPW only, not one portion for counts in chilled saline and another portion for enrichment in chilled BPW. The full rationale and validation of this variation, which was approved by the FSA and the Danish Technical University (DTU), has been provided in a previous report.

For counts, the method involved plating 100 ?L BPW homogenate (prior to incubation) on to MacConkey agar with and without 1 mg/L cefotaxime. These two agars are used to enumerate the number of presumptive *E. coli* and the number of presumptive AmpC/ESBL-producing *E. coli* on the meat samples. The EU method states that at least 30 colonies must be counted to give an accurate estimate of the viable counts, and this limits the detection level to 3,000 cfu/g of meat. Because of the low numbers of *E. coli* in the meat samples, in general it is not necessary to further dilute the initial BPW homogenate for counts beyond the initial tenfold dilution.

The remaining 250 mL of BPW homogenate (e.g. 25 grams of meat and 225 mL of BPW as per EU protocols) was incubated at 37 ± 1ºC for 18-22 hours.

The incubated BPW / meat homogenate was used to inoculate 3 different media types (with 10µL). These are MacConkey agar containing 1 mg/L cefotaxime (MCA-CTX), chromID® CARBA (CARBA) and chromID® OXA-48 (OXA-48).

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

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

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

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

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

1.9.2.1 PCR for detection of plasmid-mediated mcr-1-5 genes

Samples that gave rise to pink to red colonies (likely lactose fermenters) on MCA-COL plates were tested for the presence of plasmid-mediated colistin resistance genes *mcr*-1-5 using the EU multiplex gel-based PCR. To make detection more sensitive, a 'sweep' of ~ 10 to 20 colonies was taken to prepare the crude DNA for the PCR. If the initial 'sweep' was PCR positive for any of *mcr* -1-5 genes, then multiple individual suspect *E. coli* colonies (up to 10 as available) were further examined by the *mcr*-1-5 PCR.

It should be noted that only pink to red colonies (likely lactose fermenters) were investigated. As such it is possible that *mcr*-1-5 genes detected in the original 'sweep' but not in the individual isolated colonies, could be due to the presence of other bacterial genera. This might include non-target lactose fermenters such as *Klebsiella spp.* and *Citrobacter spp*. [7] as well as non-lactose fermenters present in the 'sweep'.

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

All *E. coli* isolates from MCA-CTX, CA-ESBL and from CARBA and OXA-48 agars were stored in duplicate on 'beads' (frozen in cryogenic material at -70ºC). They will be stored for up to five years.

To prepare the 'beads', purified bacterial culture was aseptically transferred using a 10 µl loop from the pure culture on agar to a commercial 'beads' tube. The cryogenic liquid and bacterial growth were mixed in the tube, before removing most of the supernatant cryogenic liquid, and then storing the tube at - 70ºC.

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

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

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

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

MALDI-ToF was used for identification of problem isolates giving equivocal results by other tests only if required, and was used as described by an in-house SOP [3]. For MALDI-ToF identifications if required, isolates were also grown on blood agar. A small amount of bacterial growth was applied to the metal target plate. Growth on the target plates was overlaid with 1 µl of 70% formic acid to perform a partial protein extraction and allowed to dry. Each spot was then overlaid with 1 µl of HCCA matrix, and again this was allowed to dry before the target plate was loaded into the MALDI-ToF machine. Using Biotyper software, resulting spectra from the MALDI-ToF run were searched against the Bruker database of spectra, and if the resulting score was ?

2.000, this was taken as reliable identification to the species level, dependant also on consistency score and caveats that might apply.

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

As isolates for MIC testing were obtained from MCA-CTX which contains 1 mg/L cefotaxime, they were assumed to be resistant to cefotaxime and MICs were performed using both the initial screening plate and the plate for isolates showing meropenem MIC's greater than 0.125mg/L, cefotaxime MICs greater than 0.25mg/L or ceftazidime MIC's greater than 0.5mg/L. The second plate includes a further panel of antimicrobials containing cefotaxime, ceftazidime, cefotaxime / clavulanate, ceftazidime / clavulanate, imipenem, ertapenem, temocillin, cefoxitin, cefepime and meropenem.

In total, MICs of amikacin, ampicillin, azithromycin, cefepime, cefotaxime ± clavulanic acid, cefoxitin, ceftazidime ± clavulanic acid, chloramphenicol, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, meropenem, nalidixic acid, sulfamethoxazole, temocillin, tetracycline, tigecycline and trimethoprim were performed as described in our 'in-house' SOP (BA0604), based on EN ISO 20776-1:2006. The MIC plates used for testing included the antibiotic amikacin not used in previous plates in previous years.

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 2020/1729/EU [4]. After incubation at 37oC for 18 hours, the plates were examined, and growth end points established for each antimicrobial to provide MIC's.

It should be noted that a new EU Decision 2020/1729 repealing the EU decision 2013/652/EU was issued on the 17th November 2020 [4]. This decision affects the ECOFFs for some antibiotics, such as nalidixic acid (was >16 now >8).

For interpretation on whether an *E. coli* isolate was sensitive or resistant to an antibiotic, ECOFF values were used. ECOFFs are published by EUCAST and defined in Decision 2020/1729/EU. If an ECOFF was not available under this decision, then the ECOFF established by EFSA at the time of the EU monitoring survey was used.

For *E. coli*, the presence of carbapenemase-producing strains, Extended Spectrum Beta-Lactamase producers (ESBL) and AmpC ?-lactamase producers was determined initially by comparing isolate MICs against the microbiological breakpoints for meropenem, cefotaxime and ceftazidime.

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

1.9.2.5 Interpretation of phenotype from MICs

Isolates were determined to have an AmpC-, ESBL- or AmpC+ESBL-phenotype based on MIC results as follows:-

4.1.2.5.1 *E. coli* with an ESBL-phenotype:-
Isolates resistant to one or both of cefotaxime and ceftazidime that also showed a reduction in MIC of ? 8-fold against combined cefotaxime / clavulanate or ceftazidime / clavulanate when compared with the cephalosporin alone were considered to possess and ESBL-phenotype.

4.1.2.5.2 *E. coli* with an AmpC phenotype:-
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 possess an AmpC

phenotype.

4.1.2.5.3 *E. coli* with an AmpC+ESBL-phenotype:-
Isolates resistant to cefotaxime or ceftazidime that also had an MIC of greater than 8mg/L against cefoxitin that also showed a reduction in MIC of ? 8-fold against combined cefotaxime / clavulanate or ceftazidime / clavulanate when compared with the cephalosporin alone were considered to possess an AmpC +ESBL-phenotype.

1.9.2.6 Detection and sequencing of blaCTX-M, blaOXA, blaSHV and blaTEM

Presence of blaCTX-M, blaOXA, blaSHV and blaTEM from CA-ESBL was performed by Illumina WGS. Resulting FASTQ files were assembled using 'SPAdes - St Petersburg aligner' [5] and analysed using the DTU pipelines 'ResFinder 4.1'[6]

1.9.2.7 Statistical evaluation of samples positives on MCA-CTX between years and between beef and pork samples

Chi-square tests and Fisher's exact tests were used to evaluate if the variations in samples positive on MCA-CTX were significantly different over the years 2015, 2017, 2019 and 2021, and over all these years between beef and pork samples.

Results

General considerations

An excellent working partnership continued with HallMark Veterinary and Compliance Services, which was the company contracted by FSA to collect and deliver the meat samples. Communication between the two organisations and all other aspects of the partnership were highly satisfactory.

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 [2]. The main details of each meat sample tested are listed in Appendix 1 (beef samples) and Appendix 2 (pork samples), with anonymised codes for all shops.

The details of the different countries of origin for meat samples is shown in Table 1. Most of the beef samples were from the UK (n=85). Other beef samples came from Ireland (n=15), Brazil (n=2), Poland (n=1), Scotland (n=1) and Spain (n=1) (Table 1). Most of the pork samples were from the UK (n=89). Other pork samples were from Germany (n=10), Denmark (n=2), and one sample from each of Belgium, Holland, Ireland and the Netherlands (Table 2). The details of the different countries of origin for meat samples from different retailers is shown in Table 2 for beef and Table 3 for pork.

For beef samples, there was a greater diversity of country of origin for samples from retailer code I which represents 'Shops not on the list' such as independent butchers (Table 2). For pork samples there was a greater diversity of country of origin for samples from retailer code E, but this was probably because most of the pork samples collected came from retailer E (Table 3).

Table 1. Country of origin of beef and pork samples.

A table showing the country of origin for beef and pork samples. This shows that 81.91 percent of beef and 84.76 percent of pork samples were from the UK. After this Ireland was the most

common origin for beef, and Germany for pork samples. Very low percentages of samples were from the other EU countries and Brazil.

Country of origin	Number of samples beef (%)	Number of samples pork (%)	Total per country
Belgium	0 (0)	1 (0.95)	1
Brazil	2 (1.90)	0 (0)	2
Denmark	0 (0)	2 (1.90)	2
Germany	0 (0)	10 (9.52)	10
Holland	0 (0)	1 (0.95)	1
Ireland	15 (14.29)	1 (0.95)	16
Netherlands	0 (0)	1 (0.95)	1
Poland	1 (0.95)	0 (0)	1
Spain	1 (0.99)	0 (0)	1
UK	86 (81.91)	89 (84.76)	175
Total per meat	105 (100)	105 (100)	-

Table 2. Origin of beef samples based on retailer and country of origin.

A table showing the percentage of beef tested according to retailer, and the country of origin. All beef that was sampled from the supermarkets originated from UK and the Republic of Ireland. Meat from ‘not on the list’ shops came from the UK and 4 other countries. The last column shows the percentage of samples that were positive for AMR *E. coli* according to retailer.

Retailer code	Number	% of all samples	% of retailer UK	% of retailer Ireland	% of retailer Brazil	% of retailer Poland	% of retailer Spain	% of retailer + MAC-CTX
A	2	1.90	100	0	0	0	0	0
B	16	15.24	75	25	0	0	0	0
C	15	14.29	100	0	0	0	0	0
D	2	1.90	100	0	0	0	0	0
E	22	20.95	59.2	40.9	0	0	0	0
F	15	14.29	100	0	0	0	0	6.7
G	6	5.71	100	0	0	0	0	0
H	4	3.81	100	0	0	0	0	0
I	13	12.38	61.54	7.69	15.38	7.69	7.69	0
J	9	8.57	100	0	0	0	0	0
K	1	0.95	0	100	0	0	0	0

Table 3. Origin of pork samples based on retailer and country of origin.

A table showing the percentage of pork tested according to retailer, and the country of origin. The pork mainly originated from the UK, however for retailer E the pork came from 5 other countries as well as from the UK. Meat from ‘not on the list’ shops came from the UK and Belgium. The last column shows the percentage of samples that were positive for AMR *E. coli* according to retailer.

Retailer code	Number	% of all samples	% of retailer UK	% of retailer Germany	% of retailer Belgium	% of retailer Denmark	% of retailer Holland	% of retailer Ireland	% of retailer Netherlands	% of retailer + MAC-CTX
A	1	0.95	100	0	0	0	0	0	0	0
B	15	14.29	100	0	0	0	0	0	0	6.7
C	16	15.24	37.5	56.3	0	6.3	0	0	0	0
D	3	2.86	100	0	0	0	0	0	0	0
E	23	21.90	78.3	4.3	0	4.3	4.3	4.3	4.3	8.7

Retailer code	Number	% of all samples	% of retailer UK	% of retailer Germany	% of retailer Belgium	% of retailer Denmark	% of retailer Holland	% of retailer Ireland	% of retailer Netherlands	% of retailer + MAC-CTX
F	19	18.10	100	0	0	0	0	0	0	5.3
G	4	3.81	100	0	0	0	0	0	0	0
H	2	1.90	100	0	0	0	0	0	0	0
I	13	12.38	92.31	0	8.3	0	0	0	0	0
J	9	8.57	100	0	0	0	0	0	0	0

Retail codes I denotes shop not on list.

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

One beef sample (0.95%, 95% confidence interval 0.02% to 5.19%) and four pork samples (3.81%, 95% confidence interval 1.05% to 9.47%) tested were positive for AmpC- and / or ESBL-producing *E. coli* (third generation cephalosporin resistance) on MCA-CTX agar using a sensitive detection method (Table 4).

Based on interpretation of MIC results (**section 4**), two of the pork samples (1.90%, 95% confidence interval 0.23% to 6.71%) had an AmpC-phenotype and two an ESBL-phenotype, whilst the isolates from beef had an AmpC/ESBL-phenotype (0.95%, 95% confidence interval 0.02% to 5.19%).

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

Two of the pork samples which were positive on MCA-CTX agar were also positive on CA-ESBL agar (Table 4). These were analysed by WGS to determine the presence and sequence type of any genes likely to confirm the ESBL-phenotype of resistance.

The resulting isolates were both found to be positive by WGS for the blaTEM-1b gene which encodes beta-lactam resistance to antimicrobials such as ampicillin and for blaCTX-M genes (CTX-M-1 and CTX-M-14), which encode resistance to cephalosporin antimicrobials such as cefotaxime (Table 4) and as such confers the ESBL-phenotype. Additionally, the MLSTs of the isolates were ST410 and ST6745.

Samples positive for carbapenem resistance – EU harmonised method

None of the beef (n=105) or pork (n=105) samples tested yielded carbapenem-resistant *E. coli* on CARBA and OXA-48 agars by the EU harmonised method.

Table 4. Summary of samples positive for E. coli from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX) or CHROMagar™ ESBL (CA-ESBL).

Sample ID	Date tested APHA	Meat type	Meat cut	Retail store code	Sampling location	Country of origin	Growth on MCA-CTX	Resistance phenotype	Growth on CA-ESBL	CA-ESBL gene sequence	MLST
B02797587	22/10/2021	Beef	Braising Steak	F	Warwickshire CC	UK	Yes	ESBL +AmpC	No	Not applicable	Not applicable
P00462677	07/10/2021	Pork	Pork Belly	B	North Nottinghamshire	UK	Yes	ESBL	Yes	1	410
P02797689	06/10/2021	Pork	Pork Belly	E	Hertfordshire CC	Ireland	Yes	AmpC	No	Not applicable	Not applicable
P02898776	03/12/2021	Pork	Pork Belly	E	North Hampshire	UK	Yes	ESBL	Yes	14	6745

Sample ID	Datetested APHA	Meat type	Meat cut	Retailstore code	Sampling location	Country of origin	Growth on MCA-CTX	Resistance phenotype	Growth on CA-ESBL	CA-ESBL gene sequence	MLST
P02898865	22/10/2021	Pork	Pork Loin Medalliions	F	North Yorkshire CC	UK	Yes	AmpC	No	Not applicable	Not applicable

a – EU harmonised test method. Resistance phenotype by MICs is based on isolates from MCA-CTX agar.

b – UK non-harmonised additional test. CTX gene is based on isolates from CA-ESBL for which phenotype from MICs has not been derived.

NA – Not applicable for these isolates, for example, MICs are not performed for isolates from CA-ESBL.

Breakpoints for MICs

Table 5 shows the MIC breakpoints under the old EU Decision 2013/652/EU applied to previous reports, the breakpoints applied in this report under the new EU Decision 2020/1729/EU [4] and current EUCAST ECOFFs [8].

Both EU Decisions used the EUCAST ECOFFs available at the time. It needs to be considered that since the EU decision 2020/1729/EU, EUCAST has made some further amendments to the ECOFFs (Table 5), so neither decision is now completely in accordance with current EUCAST ECOFFs (Table 5).

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

The resistant / sensitive interpretations of the MIC results for twenty antimicrobials based on EU decision 2020/1729/EU breakpoints is shown in Table 5.

As would be expected, since the isolates were obtained from agar containing 1 mg/L of the beta-lactam antimicrobial cefotaxime, all isolates were resistant to the beta-lactam antimicrobial ampicillin, and to the cephalosporin antimicrobial cefotaxime (Table 6).

None of the isolates were resistant to amikacin, tigecycline or temocillin or to the ‘last resort’ antimicrobials colistin, ertapenem, imipenem or meropenem.

For these results, as there are only five isolates and as all were sensitive to amikacin, tigecycline, temocillin and to the ‘last resort’ antimicrobials colistin, ertapenem, imipenem or meropenem, the differences between the EU Decision 2013/652/EU and 2020/1729/EU breakpoints does not affect interpretation of MIC results.

Although the breakpoint for nalidixic acid changed from > 16 mg/L to > 8 mg/L between these two Decisions, as the nalidixic acid MICs against the five beef and pork *E. coli* isolates were 4 mg/L and 64 mg/L for sensitive and resistant isolates respectively, the change in breakpoints does not affect interpretation of MIC results. Additionally, the MICs of meropenem against all isolates of *E. coli* from beef and pork *E. coli* were <= 0.03 mg/L. As such the five *E. coli* isolates would be considered sensitive based on the EU decision 2020/1729/EU breakpoint of > 0.125 mg/L and the current EUCAST ECOFF of > 0.06 mg/L (Table 5).

Bacterial counts - EU harmonised method

Using the EU method ‘Quantification of ESBL/AmpC-producing Escherichia coli in caecal content and fresh meat samples’ none of the beef or pork meat samples pre-enrichment gave rise to background *E. coli* on MCA or to presumptive ESBL/AmpC-producing *E. coli* on MCA-CTX above

the limit of detection of the method (3,000 cfu/gram).

Table 5. MIC breakpoints (mg/L) according to EU decisions 2013/652/EU and 2020/1729/EU.

This table shows the MIC breakpoints according to EU decision 2013/652/EU which was applied to the previous reports from the monitoring surveys, and also the breakpoints applied in this report under the new EU decision 2020/1729/EU [4].

Antibiotic	Breakpoints under EU decision 2013/652/EU	Breakpoints under EU decision 2020/1729/EU	Difference between two decision breakpoints	Current EUCAST ECOFFs
Amikacin	Not used	> 8	None	> 8
Ampicillin	> 8	> 8	None	> 8
Azithromycin	> 16	> 16	None	> 16*
Cefepime	> 0.125	> 0.125	None	> 0.25†
Cefotaxime	> 25	> 25	None	> 25
Cefoxitin	> 8	> 8	None	> 8
Ceftazidime	> 0.5	> 0.5	None	> 0.5
Chloramphenicol	> 16	> 16	None	> 16
Ciprofloxacin	> 0.064	> 0.06	None	> 0.064
Colistin	> 2	> 2	None	> 2
Ertapenem	> 0.06	> 0.06	None	> 0.03*†
Gentamicin	> 2	> 2	None	> 2
Imipenem	> 0.5	> 0.5	None	> 0.5
Meropenem	> 0.125	> 0.125	None	> 0.06†
Nalidixic acid	> 16	> 8	New halved	> 8†
Sulfamethoxazole	> 64	> 64	None	None
Temocillin	> 32	> 16	New halved	> 16
Tetracycline	> 8	> 8	None	> 8
Tigecycline	> 1	> 0.5	New halved	> 0.5
Trimethoprim	> 2	> 2	None	> 2

* Current ECOFF tentative.

† – Current ECOFF different from EU decision 2020/1729/EU breakpoint.

The old and new EU Decisions used EUCAST ECOFFs available from EUCAST at that time. Since the new Decision EUCAST has made some further amendments to the ECOFFs, so neither Decision is now completely in accordance with current EUCAST ECOFFs. For example, the meropenem ECOFF in the latest Decision is >0.125 mg/L. However, EUCAST have now set the meropenem ECOFF at >0.06 mg/L.

Table 6. Phenotypes and resistance profiles of beef and pork *E. coli* isolates from MacConkey agar + 1 mg/L cefotaxime (MCA-CTX).

Phenotype and MIC results as R or S	B02797587	P02898776	P02898865	P00462677	P02797689	Count of R for beef isolate/total tested	Count of R for pork isolates/total tested
Phenotype	ESBL + AmpC	ESBL	AmpC	ESBL	AmpC	-	-
Amikacin	S	S	S	S	S	0/1	0/4
Ampicillin	R	R	R	R	R	1/1	4/4
Azithromycin	R	R	S	R	S	1/1	2/4
Cefepime †	R	R	S	R	S	1/1	2/4

Phenotype and MIC results as R or S	B02797587	P02898776	P02898865	P00462677	P02797689	Count of R for beef isolate/total tested	Count of R for pork isolates/total tested
Cefotaxime †	R	R	R	R	R	1/1	4/4
Cefoxitin †	R	S	R	S	R	1/1	2/4
Ceftazidime †	R	S	R	R	R	1/1	3/4
Chloramphenicol	S	R	S	S	S	0/1	1/4
Ciprofloxacin	R	S	S	R	S	1/1	1/4
Colistin ‡	S	S	S	S	S	0/1	0/4
Ertapenem ‡	S	S	S	S	S	0/1	0/4
Gentamicin	S	R	S	S	S	0/1	1/4
Imipenem ‡	S	S	S	S	S	0/1	0/4
Meropenem ‡	S	S	S	S	S	0/1	0/4
Nalidixic acid	R	S	S	R	S	1/1	1/4
Sulfamethoxazole	R	R	S	R	S	1/1	2/4
Temocillin	S	S	S	S	S	0/1	0/4
Tetracycline	R	R	S	R	S	1/1	2/4
Tigecycline	S	S	S	S	S	0/1	0/4
Trimethoprim	R	R	S	R	S	1/1	3/4

Key to Table 6

R – Resistant; S – Sensitive. Any isolates with an ESBL-phenotype would have shown synergy with cefotaxime and or ceftazidime + clavulanic acid – not shown in above.
Amikacin (R > 8 mg/L); Ampicillin (R > 8 mg/L); Azithromycin (R > 16 mg/L); Cefepime (R > 0.125 mg/L); Cefotaxime (R > 0.25 mg/L); Cefoxitin (R > 8); Ceftazidime (R > 0.5 mg/L); Chloramphenicol (R > 16 mg/L); Ciprofloxacin (R > 0.06 mg/L); Colistin (R > 2 mg/L); Ertapenem (R > 0.06 mg/L); Gentamicin (R > 2 mg/L); Imipenem (R > 0.5 mg/L); Meropenem (R > 0.125 mg/L); Nalidixic acid (R > 8 mg/L); Sulfamethoxazole (R > 64 mg/L); Temocillin (R > 16 mg/L); Tetracycline (R > 8); Tigecycline (R > 0.5); Trimethoprim (R > 2 mg/L).
Interpretative criteria according to tables 1and 4 in Commission Implementing Decision 2020/1729/EU [4].

Cephalosporin antimicrobials †

'Last resort' antimicrobials †

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

Although 72 pre-enriched beef (68.57%, 95% confidence interval 58.78% to 77.28%) and 73 pre-enriched pork (69.52% [95% confidence interval 59.78% to 78.13%) samples yielded pink to red colonies (potential *E. coli*) on colistin-supplemented MacConkey agar, none of these were positive for *mcr*1-5 by PCR.

Statistical evaluation of samples positive on MCA-CTX (AmpC-/ESBL-phenotype *E. coli*) between years and between beef and pork samples

The observations for beef and pork samples positive for AmpC-/ESBL-phenotype *E. coli* on MCA-CTX agar is presented in Table 7. Overall, 14/1012 pork samples but only 6/1020 beef samples were positive on this agar. This contrast was evaluated by comparing with the null hypothesis that the probability of a sample being positive was the same for pork and beef, which generated expected numbers of about 10 positive samples from each type of meat.

A chi-square test ('tabi' command in Stata 15) found that the probability of observing such a large contrast if AmpC-/ESBL-phenotype *E. coli* on MCA-CTX agar occurs in pork and beef samples at

equal frequency = 0.069. As such the difference was not significant at a threshold of $p < 0.05$. As the p value was < 0.1 and in view of the small sample size the observations were considered consistent with AmpC-/ESBL-phenotype *E. coli* being more frequent in pork samples, but the evidence was uncertain.

Of the 14 pork samples positive for AmpC-/ESBL-phenotype *E. coli* on MCA-CTX agar (Table 7), 10 were observed in 2015 or 2021, even though only 105 samples were gathered in 2021. The distribution of positive pork samples across years was compared with a null hypothesis that the frequency of positive samples was independent of year, which generated expected numbers of positive samples of about 4.2 in 2015, 2017 and 2019, and 1.4 in 2021. Because the expected numbers were low, Fisher's exact test was used to evaluate the deviation of the observed distribution of positive samples from the expected distribution ('tabi' command with 'exact' option in Stata 15).

The Fisher's exact test found that the observed distribution of positive samples between years or more contrasting distributions would be observed with probability 0.041 if the actual frequency of positives was independent of year and the number of positives in a year followed the binomial distribution. The observations suggest that the frequency of positive pork samples differed between years, since there was evidence marginally below the $p < 0.05$ threshold against the null hypothesis that the frequency of positive samples was the same every year from 2015 to 2021. The lowest frequency was in 2017 and the highest in 2021. However, the contrast could have been due to a random fluctuation, which would be more likely if, for example, the frequency of positives could be dependent on the timing of sampling within a year.

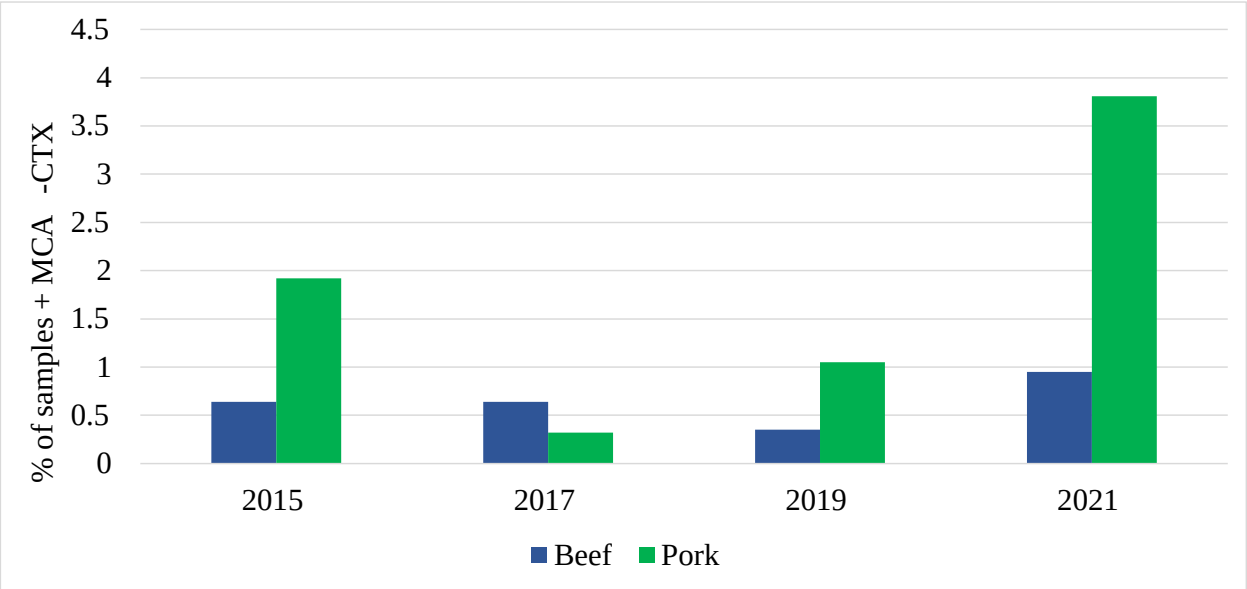
Only 6 beef samples were positive across all years, but at least one positive sample was observed every year, providing no evidence of difference between years.

Table 7. Numbers of samples of beef and pork positive on MCA-CTX agar by year.

Year	Beef + MCA-CTX	Beef - MCA-CTX	Pork + MCA-CTX	Pork - MCA-CTX
2015	2	310	6	306
2017	2	312	1	309
2019	1	288	3	282
2021	1	104	4	101
Total	6	1014	14	998

Figure 1. Percentage of retail beef and pork samples obtained in the UK positive for AmpC and or ESBL *E. coli* from 2015 to 2021.

For years 2015, 2017, 2019 (n = ~ 300 samples per year per meat type tested) and 2021 (n= ~ 100 samples tested per meat type).



Discussion

Overall findings

In 2015, the APHA tested for the first year retail beef and pork using EU protocols for the 'isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from fresh meat' [9]. Prior to this commissioning of the current EU surveys for the isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli*, there were relatively few studies that tested for of ESBL-, AmpC- and carbapenemase-producing *E. coli* from fresh beef and pork and there was no uniformity of methods used across the different studies.

An earlier UK study found that 1.9% of beef (n=159) and 2.5% of pork (n=79) samples collected in 2013-2014 from 5 different regions in the UK 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 blaCIT genes, with blaCMY-2 the most frequent variant detected by sequencing [10].

Other studies prior to commissioning of the current EU surveys showed, for example, that 20% of Austrian minced beef was positive for ESBL-producing *E. coli* (mainly CTX-M-1) [11]. A 2012 study in Switzerland found that none of 104 minced beef and pork samples were positive for ESBL-producing Enterobacteriaceae, although the same study showed that 15.3% of the porcine, 13.7% of the bovine, 8.6% of the ovine meat samples [12]. A 2014 study in Denmark found that 83.8% of broiler meat, 12.5% of pork and 3.7% of beef tested was contaminated with AmpC / ESBL *E. coli* [13].

EU surveys have provided a wealth of data for the presence of 'ESBL, AmpC- and carbapenemase-producing *E. coli*' from retail meats and animals. These findings are published in annual EFSA reports [14-17].

A comparison of UK beef and pork samples positive on MCA-CTX agar over the years 2015, 2017, 2019 and 2021 can be seen in Figure 1. For all these years the proportions of beef and pork samples positive on this agar for AmpC and / or ESBL *E. coli* remained at less than one and four percent respectively. It should be noted however that in 2015 to 2019 ~ 300 samples were tested each year (2015, 2017 and 2019), across the entire year, whilst for only 105 each of beef and pork samples were tested in October, November and December in 2021.

The 2021 EFSA report showed that EU monitoring was conducted by 28 Member States Member States (MSs) for beef and pork samples in 2019 [13]. Of a total of 6,308 and 6,793 beef and pork samples tested in 2019, a total of 5.2% (beef) and 6.8% (pork) samples were positive for

‘presumptive ESBL and / or AmpC producers’ [17]. As such, results for UK beef and pork samples for 2019 and 2021 compare favourably with these wider EU 2019 results.

The majority of the isolates from the 2021 EFSA report were determined to have a presumptive ESBL-phenotype [17], which correlates with the results from the UK in this and previous years.

The EFSA report also showed that in 2019 the frequency of samples positive for ‘presumptive ESBL and / or AmpC producers’ in beef samples ranged from 0.3% in the UK and Switzerland, to 24.0% in Bulgaria. The frequency of ‘presumptive ESBL and / or AmpC producers’ in pork samples ranged from 0% for Finland and the Netherlands to 24.4% for Bulgaria [17].

WGS analysis of the two *E. coli* isolates from CHROMagar™ ESBL agar found them to have CTX-M sequence types 1 and 14. Both of these CTX-M types have previously been reported in UK pigs [18] and from UK retail pork as part of previous EU surveys [1]. Additionally, neither of these were CTX-M-15 which is associated with the human pandemic O25:H4-ST131 CTX-M-15-producing clone [19, 20].

With respect to the two different MLSTs in these isolates, *E. coli* ST410 has been reported in 2018 as a worldwide extraintestinal pathogen associated with resistance to fluoroquinolones, third generation cephalosporins, and carbapenems [21]. In 2020 it was reported in a carbapenemase resistant (NDM) *E. coli* in hospitalised children in China [22]. E.?coli ST410 has also been reported in 2016 in pigs from South America [23]. A previous 2011 to 2013 German study investigating CTX-M-15 ESBL-producing *E. coli* from animal derived foods found that four isolates from pork (n=1), chicken (n=1) and turkey (n=2) meat were ST410 [11]. Another German study that focused on clinical *E. coli* from patients in 2011-12 found that ST410 comprised 3.8% of 160 clinical ESBL-producing *E. coli* [12].

There appears to be less published data about *E. coli* ST6745 although it has been isolated from *mcr*-1 *E. coli* from a crab from Asian aquaculture products [13].

None of the meat samples tested were positive on the two agars that screened for carbapenem-resistant *E. coli*. This finding is in general agreement with findings of the 2021 EFSA report which stated that only one pork sample from Germany in 2019 was positive for blaVIM-1 carbapenem-resistant *E. coli* [17]. Additionally, none of the five AmpC / ESBL-phenotype isolates obtained from MCA-CTX agar were resistant to the ‘last resort’ antimicrobials ertapenem, imipenem, meropenem or colistin.

In the current study seventy-two of the beef samples (67.9%) and 73 of the pork samples (70.2%) yielded pink to red colonies (potential *E. coli*) on colistin-supplemented MacConkey agar, but none of these were positive for *mcr*1-5 by PCR.

In a study of retail meat samples in Czechia (or Czech Republic) that originated from Czechia (n= 9), Poland (n=19), Hungary (n=8), Germany (n=6), Slovakia (n=4), France (n=4), Austria (n=2), Spain (n=1), Netherlands (n=1), Belgium (n=1), Great Britain (n=1), Brazil (n=8), and China (n=2), bacteria of the Enterobacteriaceae family carrying the *mcr*-1 gene were detected in 21% (18/86) of the examined samples, especially in turkey meat and turkey liver (16/24 positive for *mcr*-1 or 66.7%) [7]. None of the beef or pork samples in that study were however positive for *mcr*-1 [24].

The high incidence of pink to red colonies that were colistin-resistant from 2021 UK beef and pork samples suggests that many such colonies were probably not *E. coli*. It is possible that some of these pink to red colonies may be non-target lactose fermenters such as *Klebsiella spp*. and *Citrobacter spp.* [7], or possibly *Proteus, Morganella morganii, Pseudomonas mallei* or *Serratia marcescens*. *Serratia marcescens* is considered a slow lactose fermenter [25]. The presence of any of these other bacteria in the sweep could be responsible for a positive PCR *mcr*1-5 result. For any sweeps that were PCR positive, a PCR was conducted on the single colonies. None however were *mcr* PCR positive on further investigation.

Comparisons of the prevalence of ESBL- and AmpC-producing *E. coli* in beef and pork compared to poultry (for caecal contents and retail meat)

Since the EU monitoring of AMR started in retail meat in 2015, the prevalence of presumptive ESBL- and AmpC-producing *E. coli* is lower in beef and pork than in retail chicken meat [1].

In the 2018 EU survey, the prevalence (average of all 28 member states) of presumptive ESBL- and AmpC-producing *E. coli* in the caecal contents of broilers was found to be 48.3% (of 9,049 samples tested) and in pigs (in 2019) the prevalence was 42.7% (of 6,79) [17]. A similar figure of 46.4% can be seen in 2019 for AmpC-/ESBL-producing *E. coli* in 2,688 bovine animals of less than one year old.

If we compare the prevalence of AmpC-/ESBL-producing *E. coli* in animals compared to the prevalence in retail meat, it is lower in the meat compared to that seen in the caecal contents of the animals. In the 2018 EU survey, broiler caecal contents had a prevalence of 48.3 % of AmpC-/ESBL-producing *E. coli* compared to 39.8 % in broiler meat [17]. In pigs, caecal contents had a prevalence of 42.7 % compared to 6.8 % in pork meat. For bovines the prevalence of AmpC-/ESBL-producing *E. coli* was 46.2 % compared to 5.2 % prevalence in the retail meat [17].

The difference in prevalence of AmpC-/ESBL-producing *E. coli* between caecal contents and retail meat is much smaller in chicken than in pig and bovine samples. This may be a consequence of the processing methods used for the respective meats, with chicken meat more easily contaminated with intestinal contents in the abattoir than in the processing of beef and pork.

As discussed in a previous report [9], there are several ways in which to reduce the occurrence of AmpC-/ESBL-producing *E. coli* in livestock and in retail meat, such as avoiding/reducing the use of cephalosporin antimicrobials (as well as reduce the use of other antimicrobials). A previous study in pigs, showed that use of ceftiofur and cefquinome exerted a selective pressure for ESBL-producing *E. coli* [26], whilst another study showed reduction of ESBL- producing *E. coli* in pigs following introduction of voluntary restrictions on cephalosporin use [27].

Other improvements to reduce the occurrence of AmpC-/ESBL-producing *E. coli* in livestock and in retail meat, is in biosecurity to reduce the dissemination of ESBL / AmpC-producing *E.?coli* at the farm; improvement of slaughter hygiene and performing decontamination after slaughter [9].

Conclusions

- Results of the UK 2021 surveillance of antimicrobial resistance (AMR) mirroring the EU harmonised survey in beef and pork were similar to results from the 2015, 2017 and 2019 surveys, in that < 1% of beef samples and < 4% of pork samples tested positive for AmpC-/ESBL-producing *E. coli*.

- None of the beef and pork samples were positive for *E. coli* with resistance to ‘last resort’ carbapenem or colistin antimicrobials.

- Using WGS, two ESBL-producing *E. coli* isolates from pork were tested for CTX-M gene sequence and were CTX-M-1 and CTX-M-14. As such neither of these were CTX-M-15 which is associated with the human pandemic O25-ST131 CTX-M-15-producing clone [19, 20].

- None of the meat samples prior to enrichment had ‘background’ or AmpC-/ESBL-phenotype *E. coli* counts above the EU detection levels, indicating low numbers of these bacteria on meat samples.

- Results compare favourably to results from other countries that participated in EU monitoring surveys in 2019, as published by EFSA.

- The change in breakpoints for three antimicrobials between 2019 and 2021 should not affect interpretation of MICs (as resistant or sensitive) between these two sampling times.

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Appendix 1 - Details of all beef samples tested, sorted by date

ODT
Gweld Appendix 1 - Details of all beef samples tested, sorted by date as ODT(Open in a new window) (43.91 KB)

Appendix 2 - Details of all pork samples tested, sorted by date

ODT
Gweld Appendix 2 - Details of all pork samples tested, sorted by date as ODT(Open in a new window) (44.94 KB)