FINAL REPORT

FS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in Bacteria from Retail Meats (Year 1)

29th March 2016

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

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2. 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 beta-lactamase (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 in the UK 2015 for AmpC and ESBL *E. coli*, following 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 should be tested by culture for *E. coli* on MacConkey agar containing 1 mg/L of the cephalosporin antibiotic cefotaxime. *E. coli* isolates cultured from such media are expected to show third generation cephalosporin resistance which may include ESBL or AmpC type resistance and should be further tested by performing Minimum Inhibitory Concentrations (MICs) to determine their susceptibility to a panel of antibiotics.

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.

Only fresh meat cuts were collected and analysed. Processed meat (such as burgers, sausages), minced meat, joints or meat with added herbs/spices were all excluded from sampling. Each sample was randomly assigned to a cut category with 105 to 106 samples in each category. Cut catagories were beef steaks-expensive (£2 and over), beef steaks – less expensive (£1.99 and under), other sliced/diced beef, pork chops, pork fillet & steaks and other sliced/diced pork. Samples were collected on a quarterly basis (averaging 79 pork and 79 beef) during 1 week per month to ensure an even distribution, between January and December 2015.[1] Samples were mainly obtained in England, but were also obtained in

Northern Ireland, Scotland and Wales. [1] Samples were collected from the five major supermarket chains, the eight largest convenience store groups and other convenience stores (smaller retailers). [1] This was based on the market share data of meat sale spending (according to 2012 UK spending on meat and fish by type of retailers in the Mintel 2013 UK report). Both expensive and inexpensive cuts were selected.[1]

A bespoke in-house SOP based on published EU methods was written by the APHA for the purpose of this study and agreed with the FSA before commencement of work. The method involved enrichment of 25 grams of meat in Buffered Peptone Water (BPW), before plating this enrichment broth to the EU recommended selective agar. The method has the theoretical potential to detect one AmpC or ESBL *E. coli* in 25 grams of meat.

In total 312 beef and 312 pork samples were tested between January and December 2015. Only eight (1.28%) of the 624 samples tested yielded *E. coli* colonies on MacConkey agar + 1 mg/L cefotaxime. These samples comprised two of the beef samples and six of the pork samples which were positive on the selective agar, representing 0.64% (95% confidence interval 0.08% to 2.30%) of the beef samples and 1.92% (95% confidence interval 0.71% to 4.14%) of the pork samples.

Determination of the susceptibility of isolates to a panel of relevant antibiotics allowed phenotypic characterisation of third generation cephalosporin resistance. An ESBL phenotype was inferred if the isolates were resistant to cefotaxime and / or ceftazidime but susceptible to cefoxitin and the isolates showed clavulanate synergy with cefotaxime and / or ceftazidime. An AmpC phenotype was inferred if cefotaxime/ clavulanate and ceftazidime/ clavulanate synergy was not shown and isolates were resistant to cefotaxime, ceftazidime and cefoxitin.

Two of the isolates (one from beef and one from pork) had an AmpC phenotype, whilst all the others had an ESBL phenotype. The percentages of beef and pork samples therefore that were positive for ESBL phenotype *E. coli* were 0.32% (95% confidence interval 0.01% to 1.77%) and 1.6% (95% confidence interval 0.52% to 3.7%) respectively. The percentages of beef and pork samples therefore that were positive for AmpC phenotype *E. coli* were 0.32% (95% confidence interval 0.01% to 1.77%) and 0.32% (95% confidence interval 0.01% to 1.77%) respectively.

None of the isolates were resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin. As would be expected, all isolates were resistant 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 designated as ESBLs were microbiologically (using EUCAST ECOFFS) resistant to the cephalosporin antibiotics cefepime, cefotaxime and ceftazidime, but were sensitive to the cephalosporin antibiotic cefoxitin. Conversely, the isolates designated at AmpC phenotype were resistant to cefoxitin.
Several of the isolates were resistant to the antibiotics sulfamethoxazole, tetracycline and trimethoprim. None of the isolates were resistant to the early quinolone antibiotic nalidixic acid, but one isolate was resistant to the fluoroquinolone antibiotic ciprofloxacin, ciprofloxacin resistance in the absence of nalidixic acid resistance suggesting transferable fluoroquinolone resistance, but this was not confirmed. The only other resistances seen were to chloramphenicol and gentamicin for some isolates.

Overall, results showed about 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. None of these isolates were resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin. These results compare favourable with previous results for beef and pork from published studies in other European countries that used similar methodology. Comparison with results from other European countries should be possible late in 2016 when EFSA are expected to publish the results for all reporting European countries in the EU Summary Report on Antimicrobial Resistance for 2015.

3. Glossary

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

AmpC enzyme – Enzyme conferring AmpC type resistance

AMR – Antimicrobial resistance

APHA – Animal and Plant Health Agency

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

CRL– Community Reference Laboratory

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

MIC – Minimum Inhibitory Concentration

MS – Member States

NUTS - Nomenclature of Units for Territorial Statistics

QC – Quality control

SOP – Standard Operating Procedure

4. Materials and Methods

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

These SOPs are:-

- Isolation of background (indicator commensal) and antibiotic resistant *Enterobacteriaceae* from meats and caecal contents according to CRL, EU and / or APHA protocols (CBU 0278).
- Microbank -70ºC Bacterial Storage System (CBU0155).
- Storage of *Salmonella* and *E. coli* Day Cultures (CBU0093).
- Identification of Bacteria by MALDI ToF (BAC 0334).
- Minimum Inhibitory Concentration (MIC) – The Sensititre Method (BA0604).

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

- **EU method** - Isolation of ESBL, AMPC and carbapenemase producing *E. coli* from fresh meat - October 2015
- **EU method** - Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AMPC producing *E. coli* in meat and animals - November 2015

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

In brief, 25 grams of meat sample collected, transported and stored under conditions as stipulated by the EU protocols, was enriched in BPW at 37 ± 1ºC for 18-22 hours. The BPW was then used to inoculate (10µl) a MacConkey agar plate containing 1 mg/L cefotaxime (MCA-CTX). All MCA-CTX plates were QC tested prior to use, according to the EU method, outlined also in the SOP.

MCA-CTX plates were incubated for 18-22 hours at 44 ± 0.5 °C before checking for lactose fermenting colonies which were assumed to be presumptive AmpC / EBSL *E. coli*. A single colony was plated to a new MCA-CTX plate to ensure purity prior to storage and further tests.

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

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 presumptive AMPC / EBSL *E. coli* prior to further tests.

Isolates will be stored for up to five years to comply with EU requirements. Isolates were stored in duplicate, on Dorset egg slopes, and in "bead" culture (frozen in cryogenic material at -70ºC).

In brief, for Dorset egg slopes, a small amount of purified bacterial culture was aseptically transferred using a 10 µl loop from the second agar plate to the Dorset egg slope, which was then stored at room temperature. For "beads," a larger amount of purified bacterial culture was aseptically transferred using a 10 µl 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 - 70ºC.

Identification of Bacteria by MALDI ToF

Identification of bacterial by MALDI ToF was performed as described in our in-house SOP and based on that previously described.[2]

In brief, isolates prior to MALDI ToF were grown on blood agar, and 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 (BA0604), based on EN ISO 20776-1:2006.

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

The presence of carbapenemase producing strains, extended spectrum beta lactamase producers (ESBL) or AmpC enzyme producers was determined initially by assessing isolate MIC's against the microbiological breakpoints for meropenem, cefotaxime and ceftazidime. Any isolates showing a meropenem MIC's greater than 0.125mg/l, cefotaxime MIC's greater than 0.25mg/l or ceftazidime MIC's greater than 0.5mg/l were tested against a further panel of antimicrobials containing imipenem, ertapenem, temocillin, cefoxitin, cefepime, cefotaxime / clavulanate and ceftazidime / clavulanate, meroropenem, cefotaxime and

clavulanate. Consequently some isolates may 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 (ceftaxime, ceftazidime of meropenem) included in the first panel of antimicrobials.

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

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

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.

5. Results

General considerations

An excellent collaborative partnership was built up with the company contracted by FSA to supply the meat samples (HallMark Veterinary and Compliance Services), who came to visit the APHA at the start of the sampling year. Communication between the two organisations and all other aspects of the partnership were excellent.

With the exception of 15 meat samples (most of which arrived in the first 3 months), all meat samples arrived within the correct temperature range, as stipulated by the EU requirements.

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.[1] Table 1 of the FSA report has been reproduced in the main in this report (Table 1) for convenience (with agreement from HallMark).

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

Details of the samples positive for presumptive AmpC / ESBL *E. coli* on MacConkey agar + 1 mg/L cefotaxime are shown in Table 2, 3 and 4. These tables only show isolates that were confirmed as *E. coli* using MALDI ToF.

Two of the beef samples and six of the pork samples yielded growth on the selective agar, representing 0.64% (95% confidence interval 0.08% to 2.30%) of the beef samples and 1.9% (95% confidence interval 0.71% to 4.14%) of the pork samples. The resistance phenotype derived from the MIC data is given in Tables 2, 3 and 4.

Two of the isolates (one from beef and one from pork) had an AmpC phenotype, whilst all the others had an ESBL phenotype.

The percentages of beef and pork samples therefore that were positive for ESBL phenotype *E. coli* were 0.32% (95% confidence interval 0.01% to 1.77%) and 1.6% (95% confidence interval 0.52% to 3.7%) respectively. The percentages of beef and pork samples therefore that were positive for AmpC phenotype *E. coli* were 0.32% (95% confidence interval 0.01% to 1.77%) and 0.32% (95% confidence interval 0.01% to 1.77%) respectively.

MIC results

The summary interpretation of MIC results for *E. coli* isolates from MacConkey agar + 1 mg/L cefotaxime for the eight positive samples is shown in Table 2. The patterns of resistance were used to determine isolates as having an AmpC or ESBL phenotype, as shown in Table 3 with sample details. The individual MIC results for each strain tested, and interpretation of MICs is shown in Table 4.

None of the isolates were resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin (Tables 2 and 4). As would be expected, all isolates were resistant to the beta-lactam antibiotic ampicillin (Tables 2 and 4).

All of the isolates designated as ESBLs were resistant to the cephalosporin antibiotics cefepime, cefotaxime and ceftazidime, but were sensitive to the cephalosporin antibiotic cefoxitin and the combinations cefotaxime and clavulanate or ceftazidime and clavulanate showed synergy against these isolates (Table 4).

Conversely, the isolates designated as having an AmpC phenotype were resistant to cefoxitin and there was no synergy shown with clavulanate and the cephalosporin antibiotics ceftazidime and cefotaxime (Table 4).

Several of the isolates were resistant to the older antibiotics sulfamethoxazole, tetracycline and trimethoprim (Tables 2 and 4). None of the isolates were resistant to the early quinolone antibiotic nalidixic acid, but one isolate was resistant to the fluoroquinolone antibiotic ciprofloxacin (Tables 2 and 4). Nalidixic acid susceptible, but resistance to ciprofloxacin is the typical pattern for the transferable fluoroquinolone resistance genes (qnr genes), so from the phenotype a transferable fluoroquinolone resistance mechanism is inferred.

The only other resistances seen were to chloramphenicol and gentamicin (Tables 2 and 4).

6. Tables

Table 1. HallMark summary of sampling strategy (over temperature samples have been exluded from this table).

Summary of sampling strategy	Comments and deviations
Selection of NUTS3 regions (n=80) Using the 2011 census data, 80 NUTS3 regions were selected so that they covered all 4 countries in the UK, and comprise at least 80% of the UK population.	After receiving the EC agreement that NUTS2 sampling locations were acceptable, 5 far outlying NUTS3 locations were replaced with another NUTS3 area in the same NUTS2 regions (same first 4 digits code) wherever possible. If there were no other region available, then one of the adjacent NUTS-3 regions was used.
Sample Numbers The number of samples allocated in each NUTS3 area was proportional to the population size. To account for potential loss of samples, missing data etc an additional 5% of samples was planned in the sampling scheme = 316 beef and 316 pork samples.	**No deviation**
The number of samples to be collected for each food group (includes 5% contingency) England - 264 beef Scotland - 26 beef Wales - 15 beef Northern Ireland - 11 beef **Total = 316** England - 264 pork Scotland - 26 pork Wales - 15 pork Northern Ireland - 11 pork **Total = 316** **Total for both food groups: 632**	**Achieved number of samples** England – 264 beef Scotland - 22 beef (2 unavailable and 2 unassayable) Wales – 15 beef Northern Ireland – 11 beef **Total = 312** England - 263 pork (1 unassayable) Scotland - 23 pork(3 unavailable) Wales – 15 pork Northern Ireland – 11 **Total = 312** **Total for both food groups: 624**
Samples were collected quarterly (averaging 79 pork and 79 beef) during 1 week per month to ensure an even distribution.	**No deviations**
Food Categories Only fresh meat cuts were collected. Processed meat, minced meat, joints or meat with added herbs/spices etc was all excluded from sampling. Each sample was randomly assigned to a cut category (105/106 samples in each category).	**No deviations**
Target numbers for Meat Cuts Beef steak expensive (£2 and over): 106 Beef steak less expensive (£1.99 and under): 105 Other sliced/diced Beef: 105 Pork Fillets and Steaks: 105 Pork chops: 106 Other diced/sliced pork: 105	**Numbers sampled** Beef steak expensive: 101 (4 unavailable/1 unassayable) Beef steak less expensive: 108 Other sliced/diced Beef: 103 (1 unavailable/1 unassayable) Pork Fillets and Steaks: 104 (1 unassayable) Pork chops: 106 Other diced/sliced pork: 102 (3 unavailable)

Table 1. HallMark summary of sampling strategy (cont.)

Selection of Retailers All samples were collected from the five major supermarket chains, the eight largest convenience store groups and other convenience stores (smaller retailers not included in the named list). This was based on the market share data of meat sale spending (according to 2012 UK spending on meat and fish by type of retailers in the Mintel 2013 UK report). Meat samples were randomly and proportionally allocated to these retailer groups based on the market share data. Large retailers: 81% market share, 515 samples Convenience Stores: 19% market share, 117 samples (81 samples from named convenience stores and 36 from 'Other convenience stores')	**No deviations** Achieved Large Retailer samples: 510 Achieved Convenience store samples: 114 (72 samples from named convenience stores and 42 from 'Other convenience stores') Finding Convenience Stores which sell fresh meat was challenging and very time consuming. Most convenience stores did not sell fresh meat. (Some stock pre-prepared pork (eg sausages), beef (eg mince) or chicken pieces). In August 15 FSA agreed that samples could be bought over the butcher's counter from farm shops to ensure the fulfilment of the targeted sample numbers.
Selection of purchase points within supermarket chains and within a NUTS3 region Within a NUTS3 region, according to availability of the specified retail chains, surveyors were assigned specific retail outlet addresses.	**No deviations**
Regional Variations of Retailer availability Given that some smaller retailers were regional and might not operate in the selected NUTS3 areas, HallMark may have needed to swap the pre-assigned retailers between NUTS3 regions in some circumstances. To be done minimising the impact and maintaining the market share %.	**No deviations**
Selection of specific products within each meat category Surveyors were to freely select a sample from the randomly assigned cut category and from the assigned retailer outlet.	**No deviations**

Table 2. Summary of resistance phenotypes from meats, and resistances to antibiotics tested

Antibiotic	No. resistant[a] / No. tested			
	Beef ESBL	**Beef AmpC**	**Pork ESBL**	**Pork AmpC**
Ampicillin	1/1	1/1	5/5	1/1
Azithromycin	0/1	0/1	0/5	0/1
Cefepime	1/1	0/1	5/5	1/1
Cefotaxime	1/1	1/1	5/5	1/1
Cefoxitin	0/1	1/1	0/5	1/1
Ceftazidime	1/1	1/1	5/5	1/1
Chloramphenicol	0/1	1/1	1/5	0/1
Ciprofloxacin	0/1	0/1	1/5	0/1
Colistin	0/1	0/1	0/5	0/1
Ertapenem	0/1	0/1	0/5	0/1
Gentamicin	0/1	0/1	2/5	0/1
Imipenem	0/1	0/1	0/5	0/1
Meropenem	0/1	0/1	0/5	0/1
Naladixic Acid	0/1	0/1	0/5	0/1
Sulfamethoxazole	1/1	1/1	4/5	0/1
Temocillin	0/1	0/1	0/5	0/1
Tetracycline	1/1	0/1	4/5	1/1
Tigecycline	0/1	0/1	0/5	0/1
Trimethorpim	1/1	0/1	2/5	0/1

Orange highlight denotes the four different cephalosporin antibiotics which were tested.
Grey highlight denotes the three carbapenem antibiotics ertapenem, imipenem and meropenem and colistin (all last resort antibiotics).

a – Microbiologically resistant using using EUCAST ECOFFS.

Table 3. Summary of samples positive for AMPC or ESBL phenotype *E. coli* from MacConkey agar + 1 mg/L cefotaxime

Sample ID	Date sent	Meat type	Meat cut	Brand	Retail store	Purchase area	Country of origin	Resistance phenotype
1418110	03-02-2015	Pork	Diced leg	A	A	Berkshire	UK	ESBL
1563564	03-11-2015	Pork	Diced	B	B	Northern Ireland	UK	ESBL
1563609	12-10-2015	Pork	Diced ribs	C	C	Calderdale and Kirklees	UK	ESBL
1563635	13-10-2015	Pork	Steaks	D	D	West Sussex	UK	ESBL
1563636	13-10-2015	Beef	Steaks	D	D	West Sussex	UK	ESBL
1563817	07-07-2015	Pork	Loin steaks	E	E	Birmingham	UK	ESBL
1613145	10-06-2015	Beef	Steak	E	E	North and North East Lincolnshire	UK	AMPC
1613950	25-02-2015	Pork	Ribs	A	F	Greater Manchester South	UK	AMPC

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

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1418110	Ampicillin	>	64	R
	Azithromycin		8	S
Meat type Pork	Cefepime		4	R
	Cefotaxime		32	R
	Cefotaxime / Clavulanate	<=	0.06	Synergy
Meat cut Diced / leg	Cefoxitin		4	S
	Ceftazidime		8	R
	Ceftazidime / Clavulanate		0.25	Synergy
Brand A	Chloramphenicol	<=	8	S
	Ciprofloxacin	<=	0.015	S
Retail store A	Colistin	<=	1	S
	Ertapenem	<=	0.015	S
	Gentamicin	<=	0.5	S
Purchase area Berkshire	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid	<=	4	S
Country of origin UK	Sulfamethoxazole	<=	8	S
	Temocillin		16	S
	Tetracycline		64	R
ESBL phenotype	Tigecycline	<=	0.25	S
	Trimethoprim	<=	0.25	S

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1563564	Ampicillin	>	64	R
	Azithromycin		4	S
Meat type Pork	Cefepime		8	R
	Cefotaxime		16	R
	Cefotaxime / Clavulanate	<=	0.06	Synergy
Meat cut Diced	Cefoxitin		2	S
	Ceftazidime		1	R
	Ceftazidime / Clavulanate	<=	0.12	Synergy
Brand B	Chloramphenicol		32	R
	Ciprofloxacin	<=	0.015	S
Retail store B	Colistin	<=	1	S
	Ertapenem	<=	0.015	S
	Gentamicin		8	R
Purchase area Northern Ireland	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid	<=	4	S
Country of origin UK	Sulfamethoxazole	>	1024	R
	Temocillin		4	S
	Tetracycline		64	R
ESBL phenotype	Tigecycline	<=	0.25	S
	Trimethoprim	>	32	R

Table 4. (cont.)

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1563609	Ampicillin	>	64	R
	Azithromycin		4	S
Meat type Pork	Cefepime		8	R
	Cefotaxime		32	R
	Cefotaxime / Clavulanate	<=	0.06	Synergy
Meat cut Diced / Ribs	Cefoxitin		8	S
	Ceftazidime		2	R
	Ceftazidime / Clavulanate		0.25	Synergy
Brand C	Chloramphenicol	<=	8	S
	Ciprofloxacin	<=	0.015	S
Retail store C	Colistin	<=	1	S
	Ertapenem		0.03	S
	Gentamicin	<=	0.5	S
Purchase area Calderdale and Kirklees	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid	<=	4	S
	Sulfamethoxazole	>	1024	R
Country of origin UK	Temocillin		8	S
	Tetracycline		64	R
	Tigecycline	<=	0.25	S
ESBL phenotype	Trimethoprim		0.5	S

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1563635	Ampicillin	>	64	R
	Azithromycin		4	S
Meat type Pork	Cefepime		16	R
	Cefotaxime		64	R
	Cefotaxime / Clavulanate		0.12	Synergy
Meat cut Steaks	Cefoxitin		8	S
	Ceftazidime		4	R
	Ceftazidime / Clavulanate	<=	0.12	Synergy
Brand D	Chloramphenicol	<=	8	S
	Ciprofloxacin		0.25	R
Retail store D	Colistin	<=	1	S
	Ertapenem	<=	0.015	S
	Gentamicin		16	R
Purchase area West Sussex	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid		8	S
Country of origin UK	Sulfamethoxazole	>	1024	R
	Temocillin		4	S
	Tetracycline	>	64	R
ESBL phenotype	Tigecycline	<=	0.25	S
	Trimethoprim	<=	0.25	S

Table 4. (cont.)

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1563636	Ampicillin	>	64	R
	Azithromycin		4	S
Meat type Beef	Cefepime		4	R
	Cefotaxime		32	R
	Cefotaxime / Clavulanate	<=	0.06	Synergy
Meat cut Steaks	Cefoxitin		4	S
	Ceftazidime		1	R
	Ceftazidime / Clavulanate	<=	0.12	Synergy
Brand D	Chloramphenicol	<=	8	S
	Ciprofloxacin	<=	0.015	S
Retail store D	Colistin	<=	1	S
	Ertapenem	<=	0.015	S
	Gentamicin	<=	0.5	S
Purchase area West Sussex	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid	<=	4	S
Country of origin UK	Sulfamethoxazole	>	1024	R
	Temocillin		2	S
	Tetracycline	>	64	R
ESBL phenotype	Tigecycline	<=	0.25	S
	Trimethoprim	>	32	R

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1563817	Ampicillin	>	64	R
	Azithromycin		4	S
Meat type Pork	Cefepime		2	R
	Cefotaxime		8	R
	Cefotaxime / Clavulanate	<=	0.06	Synergy
Meat cut Loin steaks	Cefoxitin		4	S
	Ceftazidime		1	R
	Ceftazidime / Clavulanate	<=	0.12	Synergy
Brand E	Chloramphenicol	<=	8	S
	Ciprofloxacin	<=	0.015	S
Retail store E	Colistin	<=	1	S
	Ertapenem	<=	0.015	S
	Gentamicin	<=	0.5	S
Purchase area Birmingham	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid	<=	4	S
Country of origin UK	Sulfamethoxazole	>	1024	R
	Temocillin		8	S
	Tetracycline	<=	2	S
ESBL phenotype	Tigecycline	<=	0.25	S
	Trimethoprim	>	32	R

Table 4. (cont.)

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1613145	Ampicillin	>	64	R
	Azithromycin		4	S
Meat type Beef	Cefepime		0.12	S
	Cefotaxime		1	R
	Cefotaxime / Clavulanate		1	No synergy
Meat cut Steak	Cefoxitin		32	R
	Ceftazidime		2	R
	Ceftazidime / Clavulanate		2	No synergy
Brand E	Chloramphenicol		128	R
	Ciprofloxacin	<=	0.015	S
Retail store E	Colistin	<=	1	S
	Ertapenem	<=	0.015	S
	Gentamicin	<=	0.5	S
Purchase area Lincolnshire	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid	<=	4	S
Country of origin UK	Sulfamethoxazole	>	1024	R
	Temocillin		4	S
	Tetracycline	<=	2	S
AmpC phenotype	Tigecycline	<=	0.25	S
	Trimethoprim	<=	0.25	S

Isolate details	Antibiotic	Indicator	MIC (µg/ml)	Interpretation of MIC[a]
Sample ID 1613950	Ampicillin	>	64	R
	Azithromycin		4	S
Meat type Pork	Cefepime		0.5	R
	Cefotaxime		4	R
	Cefotaxime / Clavulanate		2	No synergy
Meat cut Ribs	Cefoxitin		32	R
	Ceftazidime		8	R
	Ceftazidime / Clavulanate		4	No synergy
Brand A	Chloramphenicol	<=	8	S
	Ciprofloxacin	<=	0.015	S
Retail store F	Colistin	<=	1	S
	Ertapenem		0.03	S
	Gentamicin	<=	0.5	S
Purchase area Manchester	Imipenem	<=	0.12	S
	Meropenem	<=	0.03	S
	Naladixic Acid	<=	4	S
Country of origin UK	Sulfamethoxazole	<=	8	S
	Temocillin		4	S
	Tetracycline	>	64	R
AmpC phenotype	Tigecycline	<=	0.25	S
	Trimethoprim	<=	0.25	S

Orange highlight denotes four different cephalosporin antibiotics. 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.
R- resistant, S – sensitive.

a – Microbiologically resistant or sensitive using using EUCAST ECOFFS.

7. Discussion

There are many different studies that have shown that ESBL-producing *E. coli* can be detected on raw poultry meat.[3; 4; 5] For example, in the Netherlands, one study showed that 94% of chicken meat samples were positive for ESBL-producing *E. coli*.[6] Another study showed that 60% of 120 chicken meat samples purchased in 2012 in Germany were positive for mainly CTX-M-1 ESBL-producing Enterobacteriaceae.[7]

There are fewer studies that have looked for AMPC / ESBL-producing *E. coli* in beef and pork. However, in one study, 20% of minced beef from Austria were positive for mainly CTX-M-1 ESBL-producing *E. coli*,[8] and in a Danish study, 1.2% of 173 pork samples contained AmpC or ESBL phenotype *E. coli*.[9]

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 as many as 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.[10] Conversely, a 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.* [11]

One of the problems of comparing results from different studies is that each study may use a slightly different enrichment technique and / or final isolation agar, and as such results are not truly comparable. For current and on-going EU studies however, all participants are using identical methodologies and sample sizes, so results will be comparable across Member States.

In this study, 2 and 6 (0.64% and 1.92%) of beef and pork samples respectively were positive for ESBL phenotype *E. coli*. These results are similar to previous unpublished observations for beef and pork respectively from the UK in 2013-14, bearing in mind there were slight differences between the two studies. These results also compare favourable with those obtained in some previous studies in other European countries. [8; 11]

In previous work it was also found that numbers of beef and pork samples contaminated with detectable levels of *E. coli* were much lower than the numbers of chicken samples contaminated with *E. coli*, and counts of *E. coli* on chicken meat tended to be higher than for

beef and pork (unpublished observations). This may at least partly explain why fewer beef and pork samples tend to be positive for AmpC / ESBL producing *E. coli* when compared to chicken samples.

Whilst a total of eight samples out of 624 tested in this study were positive for AmpC / ESBL producing *E. coli*, none of these isolates were resistant to colistin, or to any of the three carbapenem antibiotics tested.

Isolates as expected were resistant to ampicillin. Considering the isolates with an ESBL phenotype, most had higher MIC values for cefotaxime than for ceftazidime, suggesting that they were mainly cefotaximases. Isolates were also mainly resistant to the older antibiotics such as sulfamethoxazole, tetracycline and trimethoprim. With the exception of a few isolates that were resistant to choramphenoicol, ciprofloxacin or gentamicin, isolates were sensitive to other antibiotic tested.

It has been suggested 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.[11]

However, although for example, cephalosporin antibiotics have not been used in poultry in Denmark for more than 10 years it has been considered that the high prevalence of ESBL/AmpC-producing bacterial detected in Danish broiler meat might be caused by practices upstream in the production pyramid, since the breeding company supplying birds until recently used cephalosporin antibiotics as a prophylactic measure. [11]

In pigs, a study has shown that the use of ceftiofur and cefquinome can exert selective pressure for ESBL *E. coli*, [12] whilst another study showed reduction of ESBL *E. coli* in pigs following introduction of voluntary restrictions on cephalosporin use. [13]

In conclusion, the results of the first year of EU monitoring retail beef and pork for AmpC and ESBL producing *E. coli* in the UK show only a very low level of ~ 1 to 2% of samples were contaminated with these organisms following examination using sensitive detection

methods. None of these isolates were resistant to the last resort carbapenem antibiotics ertapenem, imipenem and meropenem or to colistin

8. Conclusions

- Only ~ 1 to 2% of beef and pork samples were positive for AMPC or ESBL-producuing *E. coli.*
- None of the eight positive isolates were resistant to colistin or the carbapenems imipenem, ertapenem or meropenem.

9. References

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