FS900189: Surveillance of AMR in *Escherichia coli* on raw fresh beef and pork meat on retail sale in Northern Ireland in 2023.

Northern Ireland specific

The FSA commissioned an AMR survey in fresh retail meats in Northern Ireland from September 2023 to December 2027.

Last updated: 31 May 2024

Area of research interest: <u>Antimicrobial resistance</u>

Study duration: 2023-09-20

Planned completion: 31 December 2027

Project status: Ongoing

Project code: FS900189

Conducted by: HallMark Meat Hygiene Ltd trading as HallMark Veterinary and Compliance Services and Agri-Food and Biosciences Institute (AFBI).

Background

Antimicrobial resistant (AMR) microorganisms are increasingly being recognised as a global problem. Whilst 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 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 AMR gene reservoirs. 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 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.

One of the main objectives within the <u>FSA's Strategy for 2022-2027</u> is to ensure that 'food is safe'. Addressing the public health threat posed by AMR is a national strategic priority for the UK and led to the Government publishing both a <u>20-year vision of AMR</u> and a <u>5-year</u> (<u>2024-2029</u>) <u>AMR National Action Plan (NAP</u>). This survey will provide continued monitoring for *E. coli* from retail meats and fits into the <u>Northern Ireland AMR Action Plan</u>, which aims to promote relevant research and to ensure that policy and practice are informed by this. The continued surveillance of AMR bacteria in humans, environments and food producing animals is crucial to monitor and understand if these meats pose a risk to animal public health, in relation to AMR and will allow future monitoring of trends over time. The FSA are responsible for the monitoring and reporting to the European Union (EU) of AMR from fresh meats at retail sale in Northern Ireland (NI) under (EU) 2020/1729 and Article 9(1) of Directive 2003/99/EC.

Approach

The FSA commissioned an AMR survey in beef and pork in 2023, 2025 and 2027 and chicken and turkey meats in 2024 and 2026 collected at retail sale in NI under (EU) 2020/1729. The sampling methodology and analytical methods met EU specifications to enable data comparability.

This survey collected 96 pork and 98 beef fresh meat samples on retail sale in NI from September to December 2023. The prevalence of commensal E. coli was also tested from all 194 retail meat samples. Selective agars and biochemical testing were used to confirm the identity of all bacterial isolates as E. coli. Analysis involved the initial isolation and enrichment of *E. coli* from all meat samples, prior to testing for the production of Extended Spectrum Beta Lactamases (ESBLs), AmpC β-Lactamases (AmpC) and Carbapenemases (CP) enzymes. The AMR phenotype was determined using a standard microbroth dilution method with European Committee on Antimicrobial Susceptibility Testing (EUCAST) thresholds for resistance and concentration ranges for (up to 25) antimicrobial substances according to EU decision (EU) 2020/1729. Five confirmed AMR resistant *E. coli* isolates had Whole Genome Sequencing (WGS) performed on them, including In-sillicon Multi-locus sequence typing (MLST) and AMR analyses using draft genome assemblies according to EU guidelines. All genes and

plasmids of AMR and virulence interest had at least 95% identity. All DNA sequences were uploaded to the European Nucleotide Archive (ENA) browser: <u>PRJEB72200</u>.

Results

- No isolates were resistant to last line antibiotics, including colistin and carbapenems.
- The prevalence of AMR *E. coli* was low (3%) from raw fresh beef and pork samples.
- Five isolates had confirmed EBSL resistance, one of which also had additional confirmed AmpC resistance. All of these isolates had resistance to Ampicillin, Cefepime, Cefotaxime and Sulfamethoxaoles. The isolate with both ESBL & AmpC resistance also displayed Cefoxitin resistance.
- **Table 1** below details the identity of AMR genes, **Table 2** details plasmids of AMR interest, and **Table 3** details virulence, acid and metal genes of interest from all five AMR *E. coli* isolates.

Conclusion

In conclusion, this study indicated a very low prevalence of AMR *E. coli* resistance from 96 pork and 98 beef fresh meat samples on retail sale in NI from September to December 2023. As per the rest of the UK, this has shown relatively little variation in prevalence since 2015 (the start of the UK monitoring period). Therefore, the risk of acquiring AMR related infections through the handling and consumption of retail contaminated meats is very low, provided that good hygiene and cooking practices are followed when handling raw meats.

Acknowledgements

Many thanks to Robin Clifford (FSA) for sampling statistical advice, to HallMark Veterinary and Compliance services for providing the samples, to AFBI for lab analysis and results interpretation and for writing this report, and to the FSA for funding this study. Table 1: Antimicrobial resistant (AMR) resistance genes and AMR phenotype with National Center for Biotechnology Information (NCBI) Accession numbers from the five *Escherichia coli* isolates from 194 fresh retail beef and pork samples from Northern Ireland in 2023. The study number of isolate identity (ID), Multi-locus sequence Type Identity (MLST ID) and MLST lineage are also included.

Isolate	MSLT	MLST	AMR resistance gene	AMR phenotype	NCBI Accession
ID	ID	Lineage			Number
RM-NI-	23	B1	aadA1	Spectinomycin,	JX185132
131-				Streptomycin	
CTX-23			aadA1	Spectinomycin,	JQ414041
				Streptomycin	
			blaCTX-M-1	Amoxicillin, Ampicillin,	DQ915955
				Aztreonam, Cefepime,	
				Cefotaxime, Ceftazidime,	
				Ceftriaxone, Piperacillin,	
				Ticarcillin	
			sul1	Sulfamethoxazole	U12338
			tet(A)	Doxycycline, Tetracycline	AJ517790
RM-NI-	4981	А	aadA1	Spectinomycin,	JQ414041
159-				Streptomycin	
CTX-23			aadA2b	Spectinomycin,	D43625
				Streptomycin	
			aph(6)-Id	Streptomycin	M28829
			aph(3'')-Ib	Streptomycin	AF321551
			aadA5	Spectinomycin,	AF137361
				Streptomycin	

			aph(3')-la	Kanamycin, Neomycin,	V00359
				Neomycin, Kanamycin,	
				Lividomycin, Paromomycin,	
				Ribostamycin, Unknown	
				Aminoglycoside,	
				Kanamycin	
			blaCTX-M-15	Amoxicillin, Ampicillin,	AY044436
				Aztreonam, Cefepime,	
				Cefotaxime, Ceftazidime,	
				Ceftriaxone, Piperacillin,	
				Ticarcillin	
			blaTEM-1B	Amoxicillin, Ampicillin,	AY458016
				Cephalothin, Piperacillin,	
				Ticarcillin	
			cmlA1	Chloramphenicol	M64556
			sul3	Sulfamethoxazole	AJ459418
			sul2	Sulfamethoxazole	HQ840942
			tet(B)	Doxycycline, Tetracycline,	AF326777
				Minocycline	
			dfrA17	Trimethoprim	FJ460238
RM-NI-	2179	AxB1	aph(6)-Id	Streptomycin	M28829
181-			aph(3'')-lb	Streptomycin	AF321551
CTX-23			blaTEM-1B	Amoxicillin, Ampicillin,	AY458016
				Cephalothin, Piperacillin,	
				Ticarcillin	

			blaCTX-M-65	Amoxicillin, Ampicillin,	EF418608
				Aztreonam, Cefepime,	
				Cefotaxime, Ceftazidime,	
				Ceftriaxone, Piperacillin,	
				Ticarcillin	
			floR	Chloramphenicol,	AF118107
				Florfenicol	
			qnrS2	Ciprofloxacin	DQ485530
			sul2	Sulfamethoxazole	AY034138
			tet(A)	Doxycycline, Tetracycline	AF534183
RM-NI-	69	D	aph(6)-Id	Streptomycin	M28829
058-			aph(3'')-Ib	Streptomycin	AF321551
CTX-23			blaCTX-M-15	Amoxicillin, Ampicillin,	AY044436
				Aztreonam, Cefepime,	
				Cefotaxime, Ceftazidime,	
				Ceftriaxone, Piperacillin,	
				Ticarcillin	
			blaTEM-1B	Amoxicillin, Ampicillin,	AY458016
				Cephalothin, Piperacillin,	
				Ticarcillin	
			qnrS1	Ciprofloxacin	AB187515
			sul2	Sulfamethoxazole	AY034138
			tet(A)	Doxycycline, Tetracycline	AJ517790
			aph(6)-Id	Streptomycin	M28829
	328	None	dfrA14	Trimethoprim	KF921535

RM-NI-	aadA17	Spectinomycin,	FJ460181
094-		Streptomycin	
CTX-23:	blaTEM-52B	Amoxicillin, Ampicillin,	AF027199
		Aztreonam, Cefepime,	
		Cefotaxime, Ceftazidime,	
		Ceftriaxone, Piperacillin,	
		Ticarcillin	
	lnu(F)	Lincomycin	EU118119

Table 2: Identified Antimicrobial resistant (AMR) plasmids with National Center for Biotechnology Information (NCBI) Accession numbers present in the five *Escherichia coli* isolates isolated from 194 fresh retail beef and pork samples from Northern Ireland in 2023. The study number isolate identity (ID) and isolate Multi-locus sequence Type Identity (MLST ID) are also included.

Isolate ID	MLST ID	Plasmid	NCBI Accession Number
RM-NI-131-CTX-23	23	Col(MG828)	NC008486
		IncFIB(AP001918)	AP001918
		IncFIC(FII)	AP001918
		IncFII	AY458016
		Incl1-l(Alpha)	AP005147
		IncX1	EU370913
		IncX4	CP002895
RM-NI-159-CTX-23	4981	Col(pHAD28)	KU674895
		Col8282	DQ995352
		IncFIB(AP001918)	AP001918
		IncFII(pAMA1167-NDM-5)	CP024805
		IncQ1	M28829
		p0111	AP010962
RM-NI-181-CTX-23	2179	IncFIB(AP001918)	AP001918
		IncFIC(FII)	AP001918
RM-NI-058-CTX-23	69	IncY	K02380
RM-NI-094-CTX-23	328	Col(MG828)	NC008486
		IncFII(29)	CP003035
		IncX1	EU370913

Table 3: Virulence, acid, and metal genes from the five *Escherichia coli* isolates from 194 fresh retail beef and pork samples from Northern Ireland in 2023. The study number isolate identity (ID), Multi-locus sequence Type Identity (MLST ID) and MLST lineage are also included.

Isolate	MSLT	MLST	Virulence genes	Acid genes	Metal genes
ID	ID	Lineage			
RM-NI- 131- CTX-23	23	B1	cvaC, espX1, fdeC, iroB, iroC ,iroD ,iroE ,iroN ,iss ,iucA ,iucB ,iucC ,iucD ,iutA ,lpfA , mchF, tsh, ybtP, ybtQ	merC, merP, merR, merT	emrE, qacEdelta1, ymgB
RM-NI- 159- CTX-23	4981	A	emrE, qacL	cvaC, espX1, fdeC, iroB, iroC, iroD, iroE, iroN, iss, iucA, iucB, iucC, iucD, iutA, mchF, ybtP, ybtQ	ymgB, merC, merP, merR, merT

RM-NI- 181- CTX-23	2179	AxB1	espX1, fdeC, iroB, iroC, iroD, iroE, iroN, iss, iucA, iucB, iucC, iucD, iutA, lpfA, ybtP, ybtQ	emrE	ymgB
RM-NI- 058- CTX-23	69	D	air, eilA, espX1, fdeC, iss, lpfA, ybtP, ybtQ	ymgB	arsD
RM-NI- 094- CTX-23	328	None	cif, eae, espA, espB, espF, espJ, espX1, fdeC, lpfA, nleA, nleB2, nleC, tir	ymgB	terD, terW, terZ

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