Antibiotic Resistant *Escherichia coli* in Uncooked Meat Purchased from Large Chain Grocery Stores and in Raw Dog Food Purchased From Pet Stores in the Same City.

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Abstract

Although previous studies have associated feeding dogs uncooked meat with their carriage and excretion of antibiotic resistant (ABR) *Escherichia coli*, this practice remains popular amongst dog owners in some countries, including the UK. Uncooked meat fed to dogs is purchased from stores selling meat primarily for human consumption, or it is a brand of commercial raw dog food (RDF), which is commonly sold frozen and thawed prior to feeding. We tested 58 packets of uncooked meat (beef, chicken, lamb and pork) purchased from large chain grocery stores (15 stores) and 15 packets of thawed, chicken-based RDF (11 brands) for the presence of ABR *E. coli*. All samples were purchased from the city of Bristol, UK. Among grocery store-purchased meat, chicken samples were significantly more likely to be positive for *E. coli* resistant to amoxicillin, spectinomycin, streptomycin, and the critically important antibiotics cefotaxime and ciprofloxacin, than other types of meat. When comparing grocery store chicken to chicken-based RDF we found no significant difference in sample-level positivity for resistant *E. coli*, which, for RDF was 80% (amoxicillin), 80% (spectinomycin), 87% (streptomycin), 27% (amoxicillin-clavulanate), 27% (cefotaxime) and 47% (ciprofloxacin). We conclude that despite it having been purchased frozen and thawed prior to testing according to the manufacturer's instructions, contamination of RDF with ABR *E. coli* is similar to that of uncooked meat primarily intended for human consumption after cooking, and so the same strict hygiene practices are advised when handling RDF as uncooked meat. Our findings provide a rationale to explain why feeding uncooked meat or RDF to dogs in the city of Bristol is associated with them excreting *E. coli* resistant to critically important antibiotics.

Introduction

Many food and health agencies promote safe handling and thorough cooking of meat to kill bacteria and reduce the risk of foodborne illness (FSA 2023, CDC 2023, WHO 2001). In this regard, *E. coli* is a commensal residing in the intestines of humans and other warm-blooded animals, but it is also an important acute and opportunistic pathogen (Kaper *et al* 2004). For example, pathogenic *E. coli* are the second most common causal species for food-borne diarrhea outbreaks worldwide and are responsible for 100% of haemolytic uraemic syndrome cases (Warmate & Onarinde 2023). In terms of opportunistic human infections derived from fecal colonizing flora, *E. coli* is also the leading cause of urinary tract infections, can cause respiratory tract infections and meningitis, and is one of the most common causes of potentially life threatening bloodstream infections (Jang *et al* 2017). Antibiotic resistant (ABR) opportunistic *E. coli* infections are commonly reported in both human and veterinary medicine, posing a serious threat to health (Poirel *et al* 2018).

Meat products become contaminated with bacteria, including *E. coli,* from faeces during slaughter and processing (FSA 2022). Faecal matter on feet, hide and from the intestines of animals can transfer onto meat during evisceration and processing in the abattoir. Cross-contamination can occur via workers, equipment, tools, surfaces and aerosols, and through direct contact between pieces of meat (FSA 2022). Regulations are in place to mitigate faecal contamination in the UK and meat sold for consumption by humans and domestic pets is regulated to ensure it falls within certain bacteriological limits. For commensal/opportunistic *E. coli*, these acceptable limits vary but can be hundreds of colony forming units per gram of meat. Hence, unhygienic practices whilst handling, storing and preparing uncooked meat in the home, and eating undercooked meat, may lead to unwanted ingestion of *E. coli* derived from

farmed animal feces. If these are ABR *E. coli*, there is the potential for this to increase the burden of ABR opportunistic infections in humans.

In addition to these relatively well understood risks associated with meat preparation and cooking when destined for human consumption, it has become apparent in recent years that such risks are amplified when people choose to feed domestic pets uncooked meat. For example, in a study of 600 adult dogs from the city of Bristol, UK, and surrounding counties, we found positive associations between dogs being fed uncooked meat and the likelihood that samples of feces from these dogs were positive for third generation cephalosporin resistant (3GC-R) or fluoroquinolone resistant (FQ-R) *E. coli* (Sealey *et al* 2022, Sealey *et al* 2023). These are considered critically important antibiotics by the World Health Organisation (WHO 2019). In a separate UK-wide study, we demonstrated that feeding uncooked meat was positively associated with the excretion of ABR *E. coli*, including FQ-R *E. coli* in dogs of less than 16 weeks of age (Mounsey et al, 2022). Other studies have also reported an association between excretion of ABR bacteria by dogs and them being fed uncooked meat (Groat *et al* 2022, Runesvard *et al* 2020, Lefebvre *et al* 2008). Therefore, it is clear that people who feed uncooked meat are not only exposed to the potential hazards of handling meat contaminated with ABR *E. coli* more often than those who do not chose this practice, but that they are further exposed by interactions with dogs more likely to excrete ABR *E. coli*. This so far unquantified risk potentially extends to other members of the household, and even outside the household, for example, affecting people that interact with environments contaminated with canine faeces (Schlesinger & Joffe 2011, Damborg *et al* 2016).

Though there is a body of evidence that feeding uncooked meat increases the risk of dogs encountering pathogenic and ABR bacteria, this practice remains popular

amongst dog owners in some countries (Morgan et al 2024, Davies et al 2019). A recent survey in the UK reported that around 50% of those who feed dogs uncooked meat prepare this food themselves, suggesting that the meat comes from sources primarily sold for human consumption after cooking (Morgan et al 2024). The remainder fed commercial raw dog food (RDF). There have been many studies worldwide showing that uncooked meat sold for human consumption is contaminated with ABR *E. coli*, including 3GC-R or FQ-R *E. coli* and some confirming this is also true for RDF (Lee et al 2023, Kim et al 2020, Davies *et al* 2019, Schlesinger & Joffe 2011). In the UK, a comprehensive study of uncooked meat for human consumption sold in large chain grocery stores identified that sample-level positivity for 3GC-R *E. coli* producing extended-spectrum β-lactamases (ESBLs; a common mechanism of 3GC resistance) was 65.4%, 2.5% and 1.9% among samples of uncooked chicken, pork and beef meat, respectively (Randall *et al* 2017). In another UK-based study (Morgan et al. 2024) 99% of RDF (n=110 packets, with meat from various animal sources) were positive for *E. coli*, of which 39% were positive for *E. coli* resistant to at least one class of antibiotic, including 3GC-R (16%) or FQs (8% of samples). A study in Italy investigating meat that was intended for human consumption but destined for RDF reported most poultry, pork, and beef packets tested positive for *E. coli* (100%, 100% and 93.3%, respectively), and identified 18.2% of *E. coli* were ESBL producers (Bacci *et al* 2019). In contrast, a study in the USA on chicken, turkey, beef, and pork retail meat reported 68%, 70%, 20% and 32% of samples tested positive for *E. coli*, 2% were positive for 3GC-R *E. coli*, being from poultry only (Lee et al 2023). The variation seen in levels of *E. coli* and ABR *E. coli* in meat from different studies could be due to a range of factors depending on country and/or how the studies were carried out, though most studies use a standardized method which uses 25 g of sample.

Due to the popularity of and potential ABR *E. coli* transmission risks associated with

feeding uncooked meat to dogs, and because we have identified associations between

this practice and the excretion of ABR *E. coli* by dogs within the city of Bristol, UK, our

aim was to compare sample-level positivity rates for *E. coli* resistant to a variety of

antibiotics in uncooked meat of different animal sources bought from large chain

grocery stores and that sold as RDF within Bristol.

Results

Sample-level positivity for ABR E. coli *in meat sold for human consumption*

Of 58 packets of meat sampled, purchased from large chain grocery stores across 5

postal districts with in the city of Bristol, 47 were positive for *E. coli* (81%). This was

highest for chicken (100%), being significantly higher (Fishers exact test $p < 0.05$) than

for pork, which had the lowest sample-level positivity for *E. coli* (69%) (**Table 1**). We

next tested samples for *E. coli* resistant to specific antibiotics or representatives of

antibiotic classes that have historically been and in some cases are currently used to

treat farmed animals: spectinomycin and streptomycin (both aminoglycosides), the β-

lactams amoxicillin, amoxicillin-clavulanate and cefotaxime (representative of the

3GCs) and ciprofloxacin (representative of FQs). Chicken meat samples from large

chain grocery stores were found to have the highest sample-level positivity for ABR *E.*

coli, being 100% for spectinomycin and streptomycin, 93% for amoxicillin, which were

significantly higher than found for these antibiotics in beef, lamb or pork (Fishers exact

test $p < 0.05$) (**Table 1, Table 2**). Resistance to amoxicillin-clavulanate was also

highest in chicken, 33%, however the difference was not significant compared with

other types of meat. Furthermore, 47% of chicken samples were positive for *E. coli*

resistant to critically important ciprofloxacin. Whilst FQ-R *E. coli* positivity was

observed in meat from all animal sources, lamb and pork had significantly lower

positivity rates than chicken (Fishers exact test p < 0.05). Positivity for FQ-R *E. coli* in

beef was not significantly lower than chicken (**Table 1, Table 2**). Seven percent of

chicken samples were positive for cefotaxime resistant (3GC-R) *E. coli*. Beef was the

only other type of meat positive for 3GC-R (13% of samples), not significantly different

from that found in chicken. We did not detect 3GC-R *E. coli* in samples of lamb or pork.

Sample-level positivity for streptomycin resistant *E. coli* was the most frequent

resistance type for beef and lamb, whereas pork samples had the same sample-level

positivity for these two agents and amoxicillin (38%) (**Table 1, Table 2**).

Prevalence of Resistant E. coli *in RDF*

We chose to sample RDF purchased at specialist pet stores from one protein source,

chicken, for comparison with chicken sold for human consumption, given that it had

the highest levels of sample-level positivity for *E. coli* resistant to all but one test

antibiotic. We found that 13 (87%) of RDF samples were positive for *E. coli*, which is

not significantly lower than that found for chicken meat sold at large chain grocery

stores (Fishers exact test p=0.48). Overall, we found no statistically significant

difference between sample-level positivity in RDF compared with uncooked chicken

meat purchased at large chain grocery stores for *E. coli* resistant to amoxicillin,

amoxicillin-clavulanate spectinomycin, streptomycin, cefotaxime and ciprofloxacin

(Fishers exact test p > 0.05, **Table 1, Table 3**).

Discussion

The first aim of this study was to test for ABR *E. coli* in samples of uncooked meat sold primarily for human consumption at large chain grocery stores, and also RDF sold by specialist pet stores in the same city, Bristol, UK. Secondly, to determine whether sample-level positivity for ABR *E. coli* differs between uncooked meat from different animal origins, and in the case of chicken meat, between meat sold at a large chain grocery stores, primarily for human consumption after cooking (but also fed to dogs without cooking), and RDF sold for consumption by dogs. This is the first study to compare ABR *E. coli* in meat sold for consumption to the local human and canine populations within a city or other confined geographical location. Of the different animal sources of meat sold primarily for human consumption, we found that chicken had greater sample-level positivity for ABR *E. coli* than beef, lamb or pork. But there was no significant difference between sample-level positivity for *E. coli* in chicken meat sold for human consumption and RDF stated as being from chicken. Whilst we tested for ABR *E. coli* in samples of packaged meat from large chain grocery stores rather than smaller butchers shops, it has previously been reported that there is no significant difference in *E. coli* counts for meat from stores packed in a modified atmosphere verse those freshly butchered (Diaz-Jimenez *et al* 2021). So we are reasonably confident that our survey is one representative of most meat sold for human consumption in our study region.

Our overall finding of little significant difference between sample-level positivity for resistant *E. coli* in chicken meat sold for human consumption and RDF is not surprising as both types of product sold in the UK are produced from the same source; RDF in the UK is regulated to 'category 3' classification, meat intended for human consumption but has been removed from production for reasons other than being a

public or animal health risk. For example, parts of slaughtered animals not popular for

human consumption are frequently used to make RDF (FSA 2024, EC 2018).

Reports concerning sample-level positivity for ABR in meat sold primarily for human

consumption vary between studies. In a large survey in the USA, 43% of retail meat

samples were contaminated with bacteria (Innes et al 2023), which is lower than

reported here. However, as we found, chicken meat was reported as having the

highest positivity for bacterial contamination (Innes et al 2023). More in keeping with

the results presented here, however, a Spanish survey testing chicken and turkey

meat products for *E. coli* found 92% of each were positive (Díaz-Jiménez et al 2021).

Sample-level positivity rates for ABR *E. coli* in chicken meat was 72%, 16%, 42%, and

70% for ampicillin, amoxicillin-clavulanate, cefotaxime and ciprofloxacin, respectively.

Resistance to critically important antibiotics was therefore more common than reported

here.

Our study was rather unusual in including the measurement of sample level positivity

for *E. coli* resistant to antibiotics only used to treat farmed animals (in the UK),

including streptomycin and spectinomycin. Many studies have only investigated ESBL

producing *E. coli* in uncooked meat because these cause resistance to the critically

important 3GCs. In a large UK study (Randall *et al* 2017), ESBL *E. coli* were found

considerably more often in samples of chicken meat than beef or pork (65%, vs ~2%),

85.6% of the ESBL positive *E. coli* from chicken meat carried resistance gene bla_{CTX-}

$_{M-1}$, which is found only rarely among ESBL *E. coli* from human infections, for example

in the city of Bristol (Findlay et al 2020). In comparison, in Singapore, ESBL-producing

E. coli were found in 51.2%, 26.9% and 7.3% of chicken, pork and beef meat samples

(Guo et al 2021). A UK-based study comparing UK-reared and imported uncooked

chicken products for FQ-R, ESBL-producing *E. coli*, found 2% and 37% of samples

were positive, respectively (Warren *et al* 2008). Another study found ESBL-producing *E. coli* from uncooked chicken imported into the UK was caused by CTX-M groups 2 and 8 (Dhanji et al 2010), which are very rare in clinical *E. coli* from the UK (Findlay et al., 2000).

For RDF, a study in the USA reported 10% of samples positive for ESBL-producing Enterobacterales, of which 76% were *E. coli*. Pork RDF had the highest sample-level positivity (38%) for ESBL-producers, followed by beef (10%), and chicken (7%) (Cole et al 2022). A recent survey in the UK on RDF report 14% of samples were positive for ESBL-producing *E. coli*, 47% of these positive samples were RDF stated as being made from offal/tripe, 33% from chicken, 17% beef, and 17% lamb, though it should be noted most samples were mixed protein sources with 67% of the offal based RDF contained chicken (Morgan *et al* 2024). Contrastingly, in Switzerland 61% RDF samples were positive for ESBL Enterobacterales, of which 85% were *E. coli*. Sample-level positivity for ESBL producers was highest in beef 87%, versus 62% and 17% in chicken and lamb, respectively (Nüesch-Inderbinen et al 2019). Interestingly, both Morgan *et al* (2024) and Nüesch-Inderbinen et al (2019) reported 76% and 73% of samples, respectively, did not meet the bacterial limit by UK and EU regulatory standards for RDF.

RDF sold in the UK is mostly stored frozen prior to preparation for consumption, and most owners believe that the freezing process kills bacteria (Morgan *et al* 2022). However, as is clearly evident from this study and others (Morgan *et al* 2024, Cole et al 2022, Davies *et al* 2019) ABR *E. coli* are present in defrosted RDF, which likely explains epidemiological findings that feeding RDF is associated with an increased likelihood that dogs excrete ABR bacteria (Groat *et al* 2022, Runesvard *et al* 2020, Davies et al 2019, Lefebvre *et al* 2008, Sealey *et al* 2022, Sealey *et al* 2023).

To conclude, uncooked meat contains ABR *E. coli*, including those resistant to critically

important antibiotics, whether they are intended for consumption by humans (once

cooked) or dogs (uncooked). Although strict regulations and procedures are in place

to reduce contamination with highly pathogenic bacteria such as *Salmonella* and

enteropathogenic *E. coli*, contamination with faecal commensals such as *E. coli*

cannot be avoided, and a certain amount of contamination with *E. coli* is accepted

within the regulations. This is because there is little acute threat to health, and because

practices of thoroughly cooking meat, decontaminating of surfaces that have come

into contact with uncooked meat and thoroughly washing hands after handling it will

minimise accidental ingestion of such ABR *E. coli*. However, the practice of feeding

RDF (or uncooked meat of any source) provides a strong One Health transmission

link for ABR *E. coli* between farmed and domestic animals because of the degree of

ABR *E. coli* contamination identified here and elsewhere. Enhanced hygiene practices

when serving RDF and interacting with dogs fed RDF are advised, since, whilst

commensal *E. coli* pose little acute health risk following ingestion, most urinary tract

and bloodstream *E. coli* infections are opportunistic, and come from commensal

bacteria carried in the intestines of the infected person, and so any ABR *E. coli* stably

entering the intestinal microbiome carries with it the risk of a resistant opportunistic

infection at some point in the future.

Methodology

Sample collection

We identified five sampling areas by postcode region (mean population per region is

approximately 25,000) within the city of Bristol. For each region, we chose three large

chain grocery stores to gain a representative sample of the meat available from such

stores in the city of Bristol. Four types of uncooked meat: beef, chicken, lamb and

pork, sold for human consumption after cooking were purchased from each store. The

cuts of meat were as follows; minced beef, minced lamb, chicken thighs and pork

chops. Samples were collected from all three stores in a postcode region, with each

region visited sequentially, one region per week, during October and November 2022.

A total of fifteen samples per type of meat were collected, except for pork where 13

samples were collected because there was no availability at the time of visiting two

stores. Samples were kept at 4°C and processed within the "use by" date.

RDF stores were identified within the City of Bristol. Using a convenience based

strategy, we purchased one of each brand of chicken-based RDF available in stores

at each visit. A total of 15 packets of RDF were collected in September 2023, covering

11 brands across five stores. As RDF is sold frozen, on the day of purchase RDF

samples were left at 4°C overnight to thaw and processed the next day.

Sample processing and microbiology

Under sterile conditions, packets were opened, and 200 g of meat was placed into a

stomacher bag (Stomacher® 400 Classic Standard Bag) with 200 mL of *E. coli*

enrichment broth (Oxoid LTD). Bags were sealed and placed into screw-top plastic

jars for incubation for 5 h, 37°C, with shaking at 180 rpm. The liquid culture was aliquot

into Eppendorf tubes and mixed with glycerol to achieve 25% v/v, and stored at -75°C.

Twenty microliters of each stock was then plated and spread onto Tryptone Bile X-

Glucuronide (TBX) agar to test for the presence of *E. coli*, and onto TBX with

antibiotics: amoxicillin (8 mg/L), spectinomycin (32 mg/L), streptomycin (32 mg/L),

amoxicillin-clavulanate (8 mg/L), cefotaxime (2 mg/L), and ciprofloxacin (0.5 mg/L)

according to EUCAST (2023) breakpoints to select for resistant *E. coli*. Plates were

incubated at 37°C for 18 h, and sample-level positivity was measured. For positivity,

at least one blue colony must have grown on the plate. Up to 10 colonies were replated

onto antibiotic-containing TBX agar to confirm resistance.

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Transparency declaration

M.B.A. is married to the owner of a veterinary practice that sells various mass-

manufactured dog foods amounting to a value less than 5% of total turnover.

Otherwise, the authors declare no competing interests.

Author Contributions

Conceived the Study: J.E.S., M.B.A.

Collection of Data: J.E.S.

Cleaning and Analysis of Data: J.E.S.

Initial Drafting of Manuscript: J.E.S., M.B.A.

Corrected and Approved Manuscript: All authors

 Table 1. Sample-level positivity for *E. coli* in meat sold primarily for human consumption at large chain grocery stores and in chicken-based RDF sold at specialist pet stores. Total samples tested in brackets.

	Samples positive for *E. coli* in									
Selection	**Beef (15)**		**Chicken (15)**		**Lamb (15)**		**Pork (13)**		**RDF (15)**	
TBX only	12	80%	15	100%	11	73%	9	69%	13	87%
AMX	5	33%	14	93%	5	33%	5	38%	12	80%
SPC	4	27%	15	100%	4	27%	5	38%	12	80%
STR	7	47%	15	100%	6	40%	5	38%	13	87%
AMC	2	13%	5	33%	3	20%	2	15%	4	27%
CTX	2	13%	1	7%	0	0%	0	0%	4	27%
CIP	2	13%	7	47%	1	7%	1	8%	7	47%

Table 2. Samples of meat sold for primarily for human consumption positive for *E. coli* on TBX without antibiotics and with the 6 test antibiotics.

		Positive for *E. coli* selected on					
SampleID	**TBX only**	**AMX**	**SPC**	**STR**	**AMC**	**CTX**	**CIP**
B1.A	+	+	+	+	+	+	-
B1.B	+	+	+	+	-	-	-
B1.C	+	+	-	+	-	-	+
B2.A	+	-	-	-	-	-	-
B2.B	+	+	-	+	-	-	+
B2.C	+	+	+	-	+	+	-
B3.A	-	-	-	-	-	-	-
B3.B	-	-	-	-	-	-	-
B3.C	+	-	+	+	-	-	-
B4.A	+	-	-	+	-	-	-
B4.B	+	-	-	+	-	-	-
B4.C	-	-	-	-	-	-	-
B5.A	+	-	-	-	-	-	-
B5.B	+	-	-	-	-	-	-
B5.C	+	-	-	-	-	-	-
C1.A	+	+	+	+	+	-	-
C1.B	+	+	+	+	+	-	+
C1.C	+	+	+	+	+	-	+
C2.A	+	+	+	+	-	-	+
C2.B	+	+	+	+	-	-	-

	1	2	3	4	5	6	7
C2.C	+	+	+	+	+	+	+
C3.A	+	+	+	+	-	-	-
C3.B	+	+	+	+	-	-	+
C3.C	+	+	+	+	+	-	+
C4.A	+	+	+	+	-	-	-
C4.B	+	+	+	+	-	-	-
C4.C	+	+	+	+	-	-	-
C5.A	+	+	+	+	-	-	+
C5.B	+	-	+	+	-	-	-
C5.C	+	+	+	+	+	-	-
L1.A	+	+	-	+	+	-	-
L1.B	+	+	+	+	+	-	-
L1.C	+	-	-	-	-	-	-
L2.A	+	-	+	+	-	-	-
L2.B	-	-	-	-	-	-	-
L2.C	+	+	+	+	+	-	+
L3.A	-	-	-	-	-	-	-
L3.B	+	+	-	+	-	-	-
L3.C	+	-	-	-	-	-	-
L4.A	+	-	-	-	-	-	-
L4.B	-	-	-	-	-	-	-
L4.C	+	+	+	+	-	-	-
L5.A	-	-	-	-	-	-	-
L5.B	+	-	-	-	-	-	-
L5.C	+	-	-	-	-	-	-
P1.C	+	+	+	-	+	-	-
P2.A	+	+	+	+	-	-	-
P2.B	-	-	-	+	-	-	-
P2.C	+	+	+	+	+	-	+
P3.A	-	-	-	-	-	-	-
P3.B	-	-	-	-	-	-	-
P3.C	-	-	-	-	-	-	-
P4.A	+	-	-	+	-	-	-
P4.B	+	+	+	-	-	-	-
P4.C	+	-	-	-	-	-	-
P5.A	+	+	+	+	-	-	-
P5.B	+	-	-	-	-	-	-
P5.C	+	-	-	-	-	-	-

Table 3. Sample positivity for *E. coli* in chicken-based RDF. Sample ID with symbols *, #, ^ denote multiple instances of the same brand of RDF.

Sample ID	Positive for *E. coli* in						
	TBX	**AMX**	**SPC**	**STR**	**AMC**	**CTX**	**CIP**
RDF1*	+	+	+	+	-	+	+
RDF2#	+	-	+	+	-	-	-
RDF3	+	+	+	+	-	-	-
RDF4	+	+	+	+	-	-	+
RDF5^	-	-	-	-	-	-	-
RDF6*	+	+	+	+	+	+	+
RDF7#	+	+	+	+	-	-	-
RDF8	-	-	-	-	-	-	-
RDF9	+	+	+	+	+	+	+
RDF10	+	+	+	+	-	-	+
RDF11*	+	+	+	+	+	+	+
RDF12	+	+	+	+	-	-	-
RDF13	+	+	+	+	-	-	-
RDF14^	+	+	-	+	-	-	-
RDF15	+	+	+	+	+	-	+
Positivity rate	13	12	12	13	4	4	7
(%)	87%	80%	80%	87%	27%	27%	47%

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