SHORT REPORT
Cross-sectional survey of antibiotic resistance in *Escherichia coli* isolated from diseased farm livestock in England and Wales

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SUMMARY

Between 2005 and 2007, *E. coli* obtained from clinical diagnostic submissions from cattle, goats, pigs and sheep to government laboratories in England and Wales were tested for sensitivity to 16 antimicrobials. Resistance was most commonly observed against ampicillin, streptomycin, sulphonamides and tetracyclines. Resistance levels varied significantly between species, with isolates from cattle frequently showing the highest levels. Verocytotoxigenic *E. coli* (VTEC) expressed less resistance than non-VTEC. Only 19·3% of non-VTEC and 43·5% of VTEC were susceptible to all antimicrobials, while 47·1% and 30·4%, respectively, were resistant to ⩾5 antimicrobials. The resistance phenotype SSuT was commonly observed, and isolates resistant to third-generation cephalosporins were also identified. We recommend judicious antimicrobial usage in the livestock industry in order to preserve efficacy.

Antimicrobial agents are widely used for the treatment, prophylaxis and metaphylaxis of bacterial disease in farm animals. Antimicrobial usage can promote the amplification of resistant strains in microorganisms present in the intestinal tract, whether these are the direct target of treatment or members of the commensal gut flora. Resistance genes can also be transferred between bacterial species via plasmids, transposons and integrons [1] and resistant zoonotic bacteria may be acquired by humans via the food chain or through contact with infected animals, their faeces or contaminated environments. If antibiotics become ineffective and there are fewer treatment options available, there may be adverse consequences; these include prolonged illness, greater risk of mortality, and increased costs associated with veterinary and/or human infections, as well as a reduced ability to contain the spread of veterinary bacterial diseases and protect animal welfare. To aid the reduction of antimicrobial usage, the use of antibiotics as growth promoters in livestock feed was banned within the European Union (EU) in 2006.

Escherichia coli is often used as an indicator of resistance levels in Gram-negative bacteria, as most livestock species asymptomatically carry the bacterium as a commensal organism in their intestine. *E. coli* also often acquires conjugative plasmids

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carrying resistance genes, which facilitate the spread of resistance to other enteric bacteria [1]. A wide diversity of strains of *E. coli* can exist in the intestine of mammals and birds and these strains can change over time [2]. While the majority of *E. coli* may be harmless commensals under normal circumstances, some strains are of public or animal health relevance and verocytotoxin-producing *E. coli* (VTEC) have emerged as a major public health concern. VTEC produce potent verotoxins (VT1 and/or VT2) that inhibit protein synthesis in target cells, while intimin, an outer membrane protein encoded by the *eae* gene, facilitates intimate attachment to intestinal epithelial cells thereby increasing disease severity [3]. VTEC infection in people can result in a broad spectrum of clinical manifestations ranging from non-specific diarrhoea through to haemolytic uraemic syndrome (HUS). The serotypes of VTEC most commonly associated with human disease appear to vary in different parts of the world but O157 is the most common cause of human VTEC infections in the UK [3]. VTEC have been isolated from a range of animals but cattle are considered their most important reservoir [3]. The majority of VTEC infections in animals are asymptomatic.

In England and Wales, the VTEC status of animals has usually only been determined when conducting specific surveys to determine prevalence or when investigating the epidemiology of human cases where zoonotic transmission is suspected. In order to provide more information concerning the prevalence of VTEC in diseased livestock in England and Wales, the Animal and Plant Health Agency (APHA) established a cross-sectional study. This report summarizes the results of the antimicrobial susceptibility testing; an additional description of the non-antimicrobial characteristics of an overlapping subset of samples was published previously [4]. The objectives were to evaluate the overall prevalence and profiles of antimicrobial resistance, and to investigate any association with virulence factors, *E. coli* serotypes, or livestock species.

Between May 2005 and December 2007, clinical diagnostic material from diseased cattle, goats, pigs and sheep was submitted by private veterinary surgeons to APHA regional laboratories and surveillance centres in England and Wales. Necropsy examinations and other diagnostic tests were performed as considered appropriate for the disease under investigation. *E. coli* isolates from non-selective culture plates (usually blood agar and MacConkey) were included in the study when they were associated with disease outbreaks ($\geqslant 2$ cases on a premises), where there was no obvious alternative diagnosis to *E. coli* infection, and when they originated from a predefined set of diagnostic scenarios: colisepticaemia; attaching and effacing activity as determined by histopathology; haemorrhagic diarrhoea in animals aged $\leqslant 12$ months; non-haemorrhagic diarrhoea in pigs aged $\leqslant 3$ months and in other animals aged $\leqslant 1$ month; β-haemolytic *E. coli* colonies in pigs and/or OK-serotyped *E. coli* isolates from pigs including cases of porcine bowel oedema. To avoid geographical or species bias, regional laboratories were allocated a quarterly target number of isolates from each species based on relative annual throughput in the previous year.

Isolates were submitted for serotyping by microagglutination, and examined for virulence factor genes *vtx1*, *vtx2* and *eae* by multiplex PCR. For this report, isolates were considered to be VTEC if they were serologically confirmed and had the *eae* gene and one or both *vtx* genes. All other isolates are termed 'non-VTEC'. Isolates were tested against 16 antimicrobial disks according to the disc diffusion method of the British Society for Chemotherapy (BSAC): amikacin (AK) 30 µg, amoxicillin/clavulanic acid (AMC) 30 µg, ampicillin (AMP) 10 µg, apramycin (APR) 15 µg, cefotaxime (CTX) 30 µg, cefuroxime (CXM) 30 µg, chloramphenicol (C) 10 µg, ciprofloxacin (CIP) 1 µg, furazolidone (FR) 15 µg, gentamicin (CN) 10 µg, nalidixic acid (NA) 30 µg, neomycin (N) 10 µg, streptomycin (S) 10 µg, sulphonamides (S3) 300 µg, tetracycline (TE) 10 µg and trimethoprim/sulphamethoxazole (SXT) 25 µg. Where available, BSAC breakpoints were used, with intermediate categories considered as resistant. A breakpoint of resistance $\leqslant 13$ mm was used for NA and for antimicrobials for which BSAC breakpoints were not available for the disk concentration used (APR, FR, N, S3, TE). The minimum inhibitory concentration (MIC) corresponding to the historical veterinary zone size breakpoint of $\leqslant 13$ mm, derived from studies of zone size obtained in the BSAC disk diffusion method *vs.* MIC, has been provisionally established for APR (zone diameter $\leqslant 13$ mm corresponding to a breakpoint of resistant >32 mg/l), N (zone diameter $\leqslant 13$ mm corresponding to a breakpoint of resistant >8 mg/l) and TE (zone diameter $\leqslant 13$ mm corresponding to a breakpoint of resistant >8 mg/l) [5].

Data were recorded in MS Access (Microsoft Corp., USA) and checked for inconsistencies.

Descriptive analyses were conducted in MS Excel (Microsoft). Associations were investigated using Pearson's χ^2 test in Stata statistical software release 10 (Stata Corp, USA) with differences considered statistically significant at $P < 0.05$. Variation in the levels of resistance per calendar year was investigated using χ^2 tests for trend. Differences in age distributions of the source livestock populations were compared using Kruskal–Wallis or Rank Sum tests. Logistic regression models were used to investigate any trends in resistance according to age in months. A principal-component factor analysis was completed in Stata to identify clusters of drugs against which *E. coli* tended to show resistance concurrently. Factors with eigenvalues >1 were maintained in the analysis (Kaiser criterion), and factor loadings were rotated using the varimax (orthogonal) method to identify the antibiotics encompassed by each factor.

In total, 1022 diagnostic *E. coli* isolates were identified and fulfilled the criteria described above from almost 16 000 carcases and 240 000 other samples. Of these, 853 were tested for the presence of *vtx* and *eae* genes and the full panel of antimicrobials. These originated from cattle ($n = 534$), pigs ($n = 205$), sheep ($n = 101$) and goats ($n = 13$). The most common presenting clinical sign was diarrhoea (62·7%) followed by found dead (11·7%) and malaise (6·7%). Only 23 VTEC isolates were identified, all of which were from cattle, accounting for 4·3% of isolates from this species. This is lower than the prevalence in previous abattoir-based surveys in the UK [6, 7]. Possible explanations include the differences in sampling methodology (since abattoir-based surveys have employed immunomagnetic separation for *E. coli* O157), as well as the potential influences of concurrent disease, the particular organs sampled and differences in the ages of animals sampled. Previous abattoir-based surveys have been performed on animals at slaughter, whereas the diagnostic samples tested here include a large number of samples from young animals.

The highest levels of resistance were observed against AMP, S, S3 and TE, although resistance against SXT was also common in non-VTEC from goats and pigs (Table 1). Other studies have also reported high levels of resistance to these antimicrobials [1, 8]. This is consistent with the fact that tetracyclines, β-lactams and trimethoprim/sulphonamides account for the majority of therapeutic antimicrobials sold for veterinary use [5] and with the observation that class 1 integrons, which are common in *E. coli*, frequently carry resistance to sulphonamides [9] as well as other resistance genes.

Third-generation cephalosporins and fluoroquinolones are critically important antimicrobials in human medicine. None of the VTEC isolates or non-VTEC from goats or sheep were resistant to CTX or CIP. However, the non-VTEC isolates from pigs and cattle expressed low levels of resistance against CTX (0·5–3·1%). Furthermore, 6·3% of porcine isolates and 14·3% of bovine isolates were resistant to CIP. All non-VTEC from sheep were susceptible to NA whereas 4·4-17·4% of isolates from other livestock species tested resistant. The resistance levels observed have potential implications for veterinary medicine where related antimicrobials are used for treatment.

None of the VTEC isolates or non-VTEC isolates from goats expressed resistance to C whereas a relatively large proportion (19·8-43·4%) from the other animals tested resistant. Resistance to N ranged between 15·4-33·1%, whereas resistance against CN and FR was low in all species; in all but one case being ⩽5%. Over a quarter of cattle non-VTEC isolates were resistant to AMC compared to ⩽10% from other species, while APR resistance was ⩽5% in all species apart from pigs. No isolates were resistant to AK.

AK, CTX, CIP, CN and NA are not authorized for use in food-producing animals in England and Wales, although other aminoglycosides, third-generation cephalosporins and fluoroquinolones are authorized. The relatively high prevalence (>10%) of resistance to C in cattle, pigs and sheep and the occurrence of resistance to FR are of interest because these compounds were formerly permitted for animal treatment in the EU but are no longer permitted for use in food-producing animals.

With regard to the critically important human medicines, 3·1% of non-VTEC from cattle and 0·5% from pigs were resistant to CTX coinciding with the emergence of resistance related to extended-spectrum β-lactamases (ESBLs) which has occurred over this period [10]. Sales of β-lactams have increased over the last decade [5] and greater consumption of third- and fourth-generation cephalosporins could potentiate further increases in CTX resistance.

Including both VTEC and non-VTEC isolates, there was evidence of a statistically significant increasing trend in the proportion of isolates from cattle expressing resistance to AMC (19·7%, 21·7%, 31·3% in 2005, 2006, 2007, respectively; $P = 0.01$), AMP (59·0%, 69·4%, 72·0%; $P = 0.03$), C (29·9%, 43·0%,

Table 1. *Number of antimicrobial resistant verocytotoxigenic* E. coli *(VTEC) and non-VTEC isolates*

Antibiotic*	VTEC isolates (all cattle) (N = 23) n (%)	Non-VTEC isolates, n (%)				
		Cattle (N = 511)	Goats (N = 13)	Pigs (N = 205)	Sheep (N = 101)	Total (N = 830)
AK	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
AMC	1 (4·4)	130 (25·4)	0 (0·0)	7 (3·4)	9 (8·9)	146 (17·6)
AMP	8 (34·8)	355 (69·5)	7 (53·9)	89 (43·4)	38 (37·6)	489 (58·9)
APR	0 (0·0)	23 (4·5)	0 (0·0)	25 (12·2)	0 (0·0)	48 (5·8)
C	0 (0·0)	222 (43·4)	0 (0·0)	44 (21·5)	20 (19·8)	286 (34·5)
CIP	0 (0·0)	73 (14·3)	0 (0·0)	13 (6·3)	0 (0·0)	86 (10·4)
CN	0 (0·0)	13 (2·5)	0 (0·0)	9 (4·4)	1 (1·0)	23 (2·8)
CTX	0 (0·0)	16 (3·1)	0 (0·0)	1 (0·5)	0 (0·0)	17 (2·0)
CXM	0 (0·0)	8 (1·6)	0 (0·0)	1 (0·5)	0 (0·0)	9 (1·1)
FR	1 (4·4)	13 (2·5)	0 (0·0)	12 (5·9)	0 (0·0)	25 (3·0)
N	5 (21·7)	169 (33·1)	2 (15·4)	36 (17·6)	21 (20·8)	228 (27·5)
NA	1 (4·4)	89 (17·4)	1 (7·7)	30 (14·6)	0 (0·0)	120 (14·5)
S	10 (43·5)	248 (48·5)	8 (61·5)	80 (39·0)	32 (31·7)	368 (44·3)
S3	12 (52·2)	376 (73·6)	10 (76·9)	141 (68·8)	49 (48·5)	576 (69·4)
SXT	3 (13·0)	186 (36·4)	9 (69·2)	91 (44·4)	17 (16·8)	303 (36·5)
TE	11 (47·8)	361 (70·7)	10 (76·9)	165 (80·5)	57 (56·4)	593 (71·4)

* AK, Amikacin; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; APR, apramycin; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamicin; CTX, cefotaxime; CXM, cefuroxime; FR, furazolidone; N, neomycin; NA, nalidixic acid; S, streptomycin; S3, sulphonamides; SXT, trimethoprim/sulphamethoxazole; TE, tetracycline.

47·3%; $P = 0.004$), CTX (1·7%, 0·4%, 7·1%; $P = 0.002$) and CXM (0·0%, 0·4%, 3·9%; $P = 0.004$). The non-VTEC from pigs showed a statistically significant increasing trend in resistance to CIP (2·8%, 5·1%, 12·7%; $P = 0.03$) and NA (8·3%, 15·4%, 21·8%; $P = 0.03$) but a decreasing trend in resistance to N (25·0%, 15·4%, 10·9%; $P = 0.03$). However, all of these results must be treated with caution given the limited time period during which this study was conducted and the restricted sample size per species. There were insufficient isolates from goats and sheep, particularly from 2005, to investigate any trends for those species.

The bovine isolates (both VTEC and non-VTEC) originated from animals with a median age of 7 days [interquartile range (IQR) 3-16·5] with just over 90% coming from calves aged <1 year. The median ages of the pigs, sheep and goats from which the non-VTEC were isolated were 28 days (IQR 7-56), 17·5 days (IQR 2·5-61) and 7 days (IQR 3-63), respectively. There were no significant differences in the median age of each species over time or between the cattle from which VTEC and non-VTEC were isolated. As the majority of isolates were taken from immature animals it was difficult to accurately assess any changes in resistance with age although there was a significant declining trend in the proportion of bovine isolates resistant to AMC ($P = 0.002$), AMP ($P = 0.006$), C ($P = 0.02$), S3 ($P = 0.008$) and TE ($P = 0.005$), and in the proportion of ovine isolates resistant to C ($P = 0.04$) and TE ($P = 0.04$). A similar trend has been observed in cattle elsewhere [1, 11] and could be related to ruminal development, changes in diet, antimicrobial usage and/or housing, or some form of fitness advantage for resistant strains in calves [11].

Besides AK, the non-VTEC isolates originating from goats were fully susceptible to a further eight antimicrobials, and non-VTEC from sheep to another six (Table 1). In contrast, non-VTEC of bovine and porcine origin expressed resistance to some extent to all other drugs. Isolates from cattle had the highest level of resistance for eight antimicrobials (AMC, AMP, C, CIP, CTX, CXM, N, NA), from pigs for four (APR, CN, FR, TE), and goats for three (S, S3, SXT). Some previous studies have shown pigs to have the highest resistance [1], and to be responsible for the most sales of veterinary antimicrobials [5]; however, the population sampled is likely to be important in influencing the results and in this study a large number of isolates from neonatal calves was included.

The VTEC isolates were fully susceptible to seven antimicrobials (Table 1). The proportion displaying

resistance against each drug tended to be lower than in non-VTEC. However, there was only strong evidence of a significant difference for AMP ($P = 0.02$), C ($P = 0.001$), SXT ($P = 0.02$) and TE ($P = 0.01$), and weak evidence for AMC ($P = 0.10$), CIP ($P = 0.10$) and S3 ($P = 0.08$). When restricted to only cattle non-VTEC isolates, there was strong evidence for all seven: AMC ($P = 0.02$), AMP ($P \leqslant 0.001$), C ($P \leqslant 0.001$), CIP ($P = 0.05$), S3 ($P = 0.02$), SXT ($P = 0.02$), TE ($P = 0.02$). The association with NA resistance also improved but remained weak ($P = 0.10$). It is possible that a larger sample of VTEC isolates would identify significant differences for other antimicrobials. The resistance levels themselves appear to be higher than those in VTEC O157 from cattle in previous GB abattoir studies [6, 7] and VTEC non-O157 from Spanish cattle [8] although this could be attributable, at least in part, to differences in study populations and methodology.

Thirteen (56.5%) VTEC isolates were resistant to $\geqslant 1$ antimicrobial, compared to 80.7% of non-VTEC (cattle 81.4%, goats 76.9%, pigs 90.2%, sheep 57.4%). This provides strong evidence of a difference between VTEC and non-VTEC ($P = 0.004$), as well as between the non-VTEC isolates recovered from different livestock ($P \leqslant 0.001$). Our results complement those of Bettelheim *et al.* [12] who also found that a greater proportion of commensal, non-VTEC expressed resistance than VTEC, and that more commensal *E. coli* from pigs expressed resistance compared to cattle or sheep.

Seven (30.4%) VTEC isolates were resistant to $\geqslant 5$ antimicrobials. This compares with 47.0% of non-VTEC (cattle 54.2%, goats 53.8%, pigs 37.1%, sheep 29.7%). Although there was no statistical evidence of any difference between VTEC and non-VTEC as a whole ($P = 0.12$), a significant difference was found when comparing VTEC against only the cattle non-VTEC isolates ($P = 0.03$) and also between the non-VTEC from different livestock ($P \leqslant 0.001$). Five of the VTEC isolates resistant to $\geqslant 5$ antimicrobials belonged to serotype O26; the others were O103 and O118. Of the bovine non-VTEC, the most common serotypes resistant to $\geqslant 5$ antimicrobials were O101 ($n = 69$), O9 ($n = 25$), O8 ($n = 22$), O153 ($n = 11$) and O73 ($n = 10$). In goats, only three of the seven isolates resistant to $\geqslant 5$ antimicrobials were typed; these were O103, O139 and O9. In pigs, the three most frequent serotypes were O149 ($n = 12$), O147 ($n = 8$) and O2 ($n = 5$). In sheep, O101 was isolated from a third of the isolates ($n = 10$) and O9 from four.

Twenty-one (2.5%) non-VTEC isolates were resistant to $\geqslant 10$ antimicrobials. These originated from cattle (3.7%) and pigs (1.0%). The cattle isolates belonged to serotypes O9 ($n = 3$), O101 ($n = 3$), O1 ($n = 2$), O21 ($n = 1$), O33 ($n = 1$), O86 ($n = 1$), O89 ($n = 1$) and O153 ($n = 1$); all others were untyped. The two porcine isolates were O159 and O26. The maximum number of drugs against which a single isolate showed resistance was 12, and originated from cattle. The maximum number of drugs against which goat isolates were resistant was five, and for sheep it was eight. For VTEC isolates, the maximum was multiple resistance to six antimicrobials.

Class 1 integrons are common in *E. coli* and frequently carry resistance to sulphonamides with other resistances [9]. Therefore, as in previous studies, the resistance phenotype SSuT, with or without other resistances, was commonly observed: 39.1% of VTEC expressed this profile, as well as 40.9% of bovine non-VTEC, 61.5% of caprine isolates, 34.1% of porcine isolates, and 28.7% of ovine isolates. This renders an overall total of 38.1% in non-VTEC, indicating no major difference to VTEC but, again, a significant difference between livestock species ($P = 0.02$).

Four isolates were co-resistant to AMP plus CTX without concurrent AMC resistance suggesting possible ESBL production. All were non-VTEC from cattle and were from samples submitted in each of the years. ESBL *E. coli* were first isolated from animals in the UK in 2004 on a Welsh dairy farm and there have since been a number of further cases [13]. Thirteen isolates expressed resistance to AMP, CTX and AMC indicating potential AmpC production. Again, all were non-VTEC, one was of porcine origin and the others were from cattle. All thirteen isolates were from samples examined in 2007.

The most common resistance profile in VTEC isolates was AMP-N-S-S3-TE (13.0%). This profile, without other resistances, was identified in 3.7% of non-VTEC overall and 3.5% of cattle non-VTEC isolates. Two (8.7%) VTEC isolates had the resistance profile S3-TE, compared to 2.9% and 1.0% of the total and cattle non-VTEC isolates, respectively. All other resistance profiles occurred in only single VTEC isolates. Plasmid analysis was not performed as part of this study, but is likely to have influenced the occurrence of the observed patterns of resistance.

The most common resistance profiles in cattle non-VTEC were: AMP-C-S3-TE (4.5%), AMC-AMP-C-S3-TE (3.7%) and AMP-N-S-S3-TE (3.5%).

In goats, AMP-S-S3-SXT-TE was identified in 30·8% of isolates and S-S3-SXT-TE in two isolates; four other profiles were each identified in single isolates. The three most common resistance profiles in pigs were TE (9·8%), AMP-S-S3-SXT-TE (9·3%) and S3-TE (4·4%), and in sheep were S3-TE (9·9%), AMP-N-S-S3-SXT-TE (5·9%), AMP-N-S-S3-TE (4·9%) and AMC-AMP-C-S3-TE (4·9%).

Five antibiotic resistance profiles were identified in the principal-component factor analysis. None of these profiles were found in the goat isolates. The antibiotic profile accounting for the most variance (eigenvalue 3·974) was AMP-N-S-S3-SXT-TE. This profile was isolated in 11·8% of cattle E. coli isolates (both VTEC and non-VTEC isolates), 6·3% of pig isolates and 12·0% of sheep isolates, rendering an overall prevalence of 10·3%. There was only weak evidence that the prevalence varied between species ($P = 0.09$). The profile CIP-NA (eigenvalue 1·695) was isolated in 13·5% of bovine isolates and 6·3% of porcine isolates but no ovine isolates (overall prevalence 10·0%). There was very strong evidence that this profile differed in prevalence between species ($P \leqslant 0.001$). CTX-CXM (eigenvalue 1·618) was found in 1·1% of cattle isolates and 0·5% of pig isolates but no sheep isolates (overall prevalence 0·8%), but there was no evidence of any significant variation between species ($P = 0.61$). The differences are interesting because mutational resistance can occur to NA and CIP, whereas for the other resistances, specific enzymatic mechanisms of resistance are usually involved. AMC-C (eigenvalue 1·376) was isolated from 17·2% of cattle isolates, 2·0% of pig isolates and 9·0% of sheep isolates (overall prevalence 12·3%). There was very strong evidence that its occurrence varied significantly between species ($P \leqslant 0.001$). The final profile, APR-CN-FR (eigenvalue 1·060), was only found in one cattle isolate (0·2%).

The present study provides a useful insight into the occurrence of antimicrobial resistance in commensal and pathogenic E. coli in samples from diseased farm animals. The representativeness of the overall study population was previously discussed by Hutchinson et al. [4]. The exclusion of a small number of isolates for the analysis herein did not affect the regional distribution. The mean cattle herd size also remained comparable to that quoted by Hutchinson et al.; however, the mean sheep flock size increased to 297·5 and the mean pig herd size to 279·2 indicating a possible bias towards larger sheep flocks and smaller pig herds in the present study compared to the national average. The moderate sample size in the present study, particularly for VTEC and goat isolates, inevitably raises concerns in relation to the amount of variation which may occur within the study population and the degree to which this could be assessed. Furthermore, all isolates were from clinical cases precluding extrapolation to the healthy livestock population in England and Wales. Although it is best practice to sample animals prior to treatment with antimicrobials, the animals in this study may have been exposed to antimicrobials as a result of previous treatment. The study population included a significant proportion of isolates from neonates and young animals; a class of animals often reared in particular locations on farms where terminal hygiene and disinfection as well as management (e.g. 'all-in/all-out') procedures will have been important in influencing the occurrence and persistence of resistance. These factors probably account for the higher levels of resistance identified relative to previous studies involving healthy animals, although methodological differences make direct comparisons between studies difficult.

The resistance observed in this study in E. coli from animals with clinical disease has veterinary implications, constitutes a reservoir of antimicrobial resistance genes and has some public health relevance where it occurs in human pathogens. VTEC with multiple resistance could possess a selective advantage over other bacteria colonizing the gastrointestinal tract of animals if they are treated with antibiotics, allowing them to selectively multiply within the animals' intestines, leading to greater shedding that in turn might lead to an increased risk of contamination of animal food products with VTEC and increased likelihood of an outbreak. However, VTEC isolates usually showed less resistance than non-VTEC isolates in this study.

Over recent decades, there has been a gradual increase in the proportion of invasive E. coli isolated from humans that are resistant to ampicillin, amoxicillin, cefotaxime, ceftazidime, ciprofloxacin and gentamicin [14]. The extent to which food-producing animals contribute to the overall levels of antimicrobial resistance in humans remains unquantified; antimicrobials are used in both people and animals and exchange of resistant strains between these ecological niches (in either direction) is clearly possible. Prudent use of antimicrobial medicines in all sectors should be strongly encouraged to minimize the emergence, spread and persistence of resistant bacteria.

Many countries, including the UK, have developed strategies for monitoring resistance in farm animals and for promoting responsible use of antimicrobials by farmers [15]. To preserve the efficacy of antimicrobials, prudent use must be maintained and efforts made to prevent the spread of resistant *E. coli* through high standards of sanitation and hygiene.

ACKNOWLEDGEMENTS

DECLARATION OF INTEREST

None.

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