RESEARCH LETTER

A high prevalence of antimicrobial resistant *Escherichia coli* isolated from pigs and a low prevalence of antimicrobial resistant *E. coli* from cattle and sheep in Great Britain at slaughter

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Abstract

The incidence of antimicrobial resistance and expressed and unexpressed resistance genes among commensal *Escherichia coli* isolated from healthy farm animals at slaughter in Great Britain was investigated. The prevalence of antimicrobial resistance among the isolates varied according to the animal species; of 836 isolates from cattle tested only 5.7% were resistant to one or more antimicrobials, while only 3.0% of 836 isolates from sheep were resistant to one or more agents. However, 92.1% of 2480 isolates from pigs were resistant to at least one antimicrobial. Among isolates from pigs, resistance to some antimicrobials such as tetracycline (78.7%), sulphonamide (66.9%) and streptomycin (37.5%) was found to be common, but relatively rare to other agents such as amikacin (0.1%), ceftazidime (0.1%) and coamoxiclav (0.2%). The isolates had a diverse range of resistance gene profiles, with *tet*(B), *sul2* and *strAB* identified most frequently. Seven out of 615 isolates investigated carried unexpressed resistance genes. One trimethoprim-susceptible isolate carried a complete *dfrA17* gene but lacked a promoter for it. However, in the remaining six streptomycin-susceptible isolates, one of which carried *strAB* while the others carried *aadA*, no mutations or deletions in gene or promoter sequences were identified to account for susceptibility. The data indicate that antimicrobial resistance in *E. coli* of animal origin is due to a broad range of acquired genes.

Introduction

Historically, antimicrobials have been used in animal production for both therapeutic and growth promotion purposes. The European Union (EU) has gradually banned the use of all growth-promoting antimicrobials (Guardabassi & Courvalin, 2006). However, antimicrobials are still used as therapeutic agents in food production. In the United Kingdom (UK), veterinary antimicrobial use ranges between 440 and 480 tonnes annually, over 80% of which are used in food-producing animals. The tetracyclines account for approximately half of this amount, with significant use also recorded for trimethoprim/sulphonamides, β-lactams, aminoglycosides, macrolides and fluoroquinolones (http://www.vmd.gov.uk/Publications/Antibiotic/salesanti04.pdf).

Previous studies have demonstrated that antimicrobial resistance among *Escherichia coli* isolated from both healthy and infected farm animals is common (Wray *et al.*, 1991; Bywater *et al.*, 2004). A study conducted in the United Kingdom between 1986 and 1991 investigating *E. coli* sent to the Veterinary Laboratories Agency (Formerly the Central Veterinary Laboratory) found that 69.8–87.7% of isolates were resistant to at least one antimicrobial, depending on the source animal (Wray *et al.*, 1993). A study conducted in 1999–2000, comparing rates of resistance among *E. coli* isolated at slaughter from healthy animals, found a high prevalence of resistance (>40.0%) to four antimicrobials in isolates from chickens in the United Kingdom. However, among cattle isolates the prevalence of resistance was <10.0% to all nine antimicrobials tested (Bywater *et al.*, 2004).

In a recent study, it was shown that the expression of plasmid-borne antibiotic-resistance genes can be silenced in the pig gut, while intact resistance genes and their promoters are retained (Enne *et al.*, 2006). Such silent resistance genes pose a potential threat, as they are able to recover expression of resistance, and unawareness of them could hence result in a significant underestimation of the antibiotic resistance potential of a population (Enne *et al.*, 2006). Currently, very little is known about the frequency of unexpressed resistance genes among bacteria. In this study, the susceptibility of *E. coli* isolates collected from healthy farm animals at slaughter to 16 antimicrobials was examined, and the frequency of expressed and unexpressed copies of 11 resistance genes was determined among a subset of the isolates.

Materials and methods

Isolate collection

Samples were collected in 1999 from pigs, sheep and cattle at slaughter from 93 abattoirs of varying sizes distributed throughout Great Britain. Sampling was performed for 49 weeks, with no more than four samples taken from any one abattoir or more than one sample from the same farm of origin on any given day. Distal rectums and contents were collected from sheep and cattle, caecums were collected from pigs. In total 2509 pigs, 891 cattle and 973 sheep were sampled. *Escherichia coli* were isolated by inoculating a swab of rectal or caecal contents onto MacConkey agar followed by incubation at 37 °C for 18 h. Two colonies with typical *E. coli* morphology were selected at random and subjected to standard biochemical tests for identification (Sojka, 1965). One *E. coli* isolate per sample was included in the study. In total, this comprised 836 isolates from cattle, 836 isolates from sheep and 2480 isolates from pigs.

Susceptibility testing

All isolates were subjected to susceptibility testing by disc diffusion to amikacin (30 μg), amoxycillin/clavulanic acid (30 μg), ampicillin (10 μg), apramycin (15 μg), chloramphenicol (10 μg), ceftazidime (30 μg), cefoperazone (30 μg), gentamicin (10 μg), colistin (25 μg), cefotaxime (30 μg), furazolidinone (15 μg), neomycin (10 μg), nalidixic acid (30 μg), streptomycin (25 μg), sulphonamide compounds (300 μg), sulphamethoxazole/trimethoprim (25 μg) and tetracycline (10 μg) as described previously (Wray *et al.*, 1991; Andrews, 2001). *Escherichia coli* NCTC 10418 was used as a control for susceptibility testing. The susceptibilities of isolates that were suspected of carrying unexpressed resistance genes were confirmed by minimum inhibitory concentration (MIC) testing, using E-test strips (ABBiodisk, Solna, Sweden) according to the manufacturer's guidelines. The criteria used to interpret E-test results were those recommended by the British Society for Antimicrobial Chemotherapy (Andrews, 2001).

PCR and sequencing of antimicrobial resistance genes

PCR to detect 11 antimicrobial-resistance genes was performed on a random subset of the isolates, regardless of their susceptibility patterns. They comprised 615 isolates in total; 298 from cattle, 226 from sheep and 91 from pigs. The resistance genes investigated were bla_{TEM}, bla_{SHV}, $bla_{\mathrm{OXA-2}}$ and related genes ($bla_{\mathrm{OXA-2,\ 3,\ 15,\ 21,\ 32\ \&\ 46}}$), the *strAB* gene pair, *aadA1* and related genes (*aadA1*, 2, 3, 6, 7, 8, 10, 11, 12, 13 & 15), *sul1, sul2, tet*(A), *tet*(B), *dfrA12* and related genes (*dfrA12*, 7, 13, 21, 22 & 23) and *dfrA17*. Additionally, presence of the essential *rpsL* gene was investigated as a control for template integrity. All primer sequences are listed in Table 1. PCR was carried out using standard reagents and protocols as described previously (Enne *et al.*, 2006), with adjustment to the annealing temperature as appropriate for each primer pair (Table 1). Primers (Table 1) were also designed to amplify the complete ORFs and promoter regions of the potentially silent genes *sul2, tet*(B), *strA, strB, aadA1, aadA2* and *dfrA17*. The *tetR*(B) regulator of *tet*(B) was also amplified. The presence of class 1 and 2 integrons and their respective gene-cassette promoter regions in isolates with unexpressed *aadA* or *dfrA17* genes was determined. The distances of selected *aadA* genes cassettes from the integron promoter were determined by carrying out PCR amplification using primers targeting the 5′-conserved segment of the integron and the *aadA* gene. All PCR reactions included a negative control containing no template DNA and a positive control, consisting of template DNA from an *E. coli* isolate known to carry the gene in question. PCR products were visualized by UV illumination after gel electrophoresis on 1% agarose gels in Tris boric acid/EDTA buffer (pH 7.0) incorporating ethidium bromide.

Two randomly chosen PCR amplification products for each resistance gene were selected for DNA sequencing as a control for specificity of the primers. Additionally, for isolates with potentially silent resistance genes, all appropriate gene, promoter and integron amplicons were sequenced. PCR products were purified using a Qiaquick PCR purification kit (Qiagen, Crawley, UK) according to the manufacturer's instructions and sent for DNA sequencing at Advanced Biotechnology Centre, Imperial College, London, UK. Sequence analysis was carried out using the LASERGENE DNASTAR software package and comparisons to known sequences were carried out using BLAST. Integron DNA sequences from isolates P185.10.99.C2, P311.10.99.C3 and P187.11.99.C2 have been deposited to Genbank under accession numbers EF560797, EF560798 and EF560799, respectively.

Table 1. Sequences of primers used for PCR amplification

Primer	Sequence 5′–3′	Position (gene)	Annealing temperature (°C)	References
AADF*,†	CATTTGTACGGCTCCGCAGT	93–112 (*aadA1*)	55	This paper
AADR*,†	AGAATGTCATTGCGCTGCCA	352–333 (*aadA1*)	55	This paper
AADA1F‡	AACATCATGAGGGAAGCGGT	− 7 to 13 (*aadA1*)	50	This paper
AADA1R‡	ACTAACGCTTGAGTTAAGCC	851–832 (*aadA1*)	50	This paper
AADA2F‡	CATGAGGGTAGCGGTGACCA	− 13 to 7 (*aadA2*)	56	This paper
AADA2R‡	GTAGCGCGGTCGGCTTGAAC	812–793 (*aadA2*)	56	This paper
DFRA12F*	CGGGTTATTGGCAATGGTCC	52–71 (*dfrA12*)	53	This paper
DFRA12R*	CTTGAATGGTTTCGGTTGAG	451–432 (*dfrA12*)	53	This paper
DFRA17F*	AAGGTGAGCAACTACTCTTTAA	77–98 (*dfrA17*)	50	This paper
DFRA17R*	GGCATTATAGGGAATTTGATAT	395–374 (*dfrA17*)	50	This paper
DFRA17WHOLEF‡	CCATTAAGGGAGTTAAATTG	1–20 (*dfrA17*)	45	This paper
DFRA17WHOLER‡	CACAAAGACGCGACTTAAAAGC	551–530 (*dfrA17*)	45	This paper
INT1F‡	GCCGCCAATGCCTGACGATG	253–234 (*intI1*)	58	This paper
INT1R‡	CTGCCCTGCTGCGTAACATC	20–39 (*attI*)	58	This paper
INT2F‡	GTGAAACAGAATAAAACGCTTA	113–92 (*intI2*)	48	This paper
INT2R‡	CTGATGCTTACCGTTAATTAAT	− 34 to − 55 (*attI*)	48	This paper
OXA2F*	TTCAAGCCAAAGGCACGATAG	113–133 (bla_{OXA-2})	58	Steward *et al*. (2001)
OXA2R*	TCCGAGTTGACTGCCGGGTTG	815–795 (bla_{OXA-2})	58	Steward *et al*. (2001)
RPSLF*,†	CTCGCAAAGTTGCGAAAAGC	38–57 (*rpsL*)	58	Enne *et al*. (2006)
RPSLR*,†	TTCACGCCATACTTGGAACG	359–340 (*rpsL*)	58	Enne *et al*. (2006)
SHVF*	ATGCGTTATATTCGCCTGTG	1–20 (bla_{SHV})	55	This paper
SHVR*	AGCGTTGCCAGTGCTCGTAC	862–843 (bla_{SHV})	55	This paper
STRAF*,†	CAACTGGCAGGAGGAACA	207–225 (*strA*)	55	Livermore *et al*. (2001)
STRAR†	CGCAGATAGAAGGCAAGG	779–761 (*strA*)	55	Livermore *et al*. (2001)
STRPROF‡	GCACATTCGGGATATTTCTC	− 58 to − 39 (*strA*)	50	This paper
STRPROR‡	GTTCCTCCTGCCAGTTGATG	223–204 (*strA*)	50	This paper
STRA2F‡	TTGAATCGAACTAATATTTTTTT	1–23 (*strA*)	54	This paper
STRA2R‡	CAGGAAAAACAGGCGGCATG	808–827 (*strA*)	54	This paper
STRBR*,†	GGCATTGCTCATCATTTG	472–454 (*strB*)	55	Livermore *et al*. (2001)
STRB2F‡	ACGCCTTGCCTTCTATCTGC	− 45 to − 26 (*strB*)	53	This paper
STRB2R‡	CCAGGGGATAGGAGAAGTCG	864–845 (*strB*)	53	This paper
STRBRTF†	ATGTTCATGCCGCCTGTTTT	1–20 (*strB*)	55	This paper
SUL1F*	CCGATATTGCTGAGGCGGACT	337–357(*sul1*)	58	Livermore *et al*. (2001)
SUL1R*	CCAACGCCGACTTCAGCTT	603–585 (*sul1*)	58	Livermore *et al*. (2001)
SUL2F*	TCGTCAACATAACCTCGGACAG	29–50 (*sul2*)	55	Livermore *et al*. (2001)
SUL2R*	GTTGCGTTTGATACCGGCAC	507–488 (*sul2*)	55	Livermore *et al*. (2001)
SUL2PROF‡	TCACCGCAAACAGGTTACTC	− 93 to − 74 (*sul2*)	53	This paper
SUL2HALF1R‡	AAGAACGCCGCAATGTGATC	461–442 (*sul2*)	53	This paper
SUL2HALF2F‡	GCAATTGGCGAAATCATCTG	348–367 (*sul2*)	50	This paper
SUL2HALF2R‡	GAGAAATATCCCGAATGTGC	837–818 (*sul2*)	55	This paper
TEMF*	ATGAGTATTCAACATTTCCG	1–20 (bla_{TEM})	50	Livermore *et al*. (2001)
TEMR*	CCAATGCTTAATCAGTGACG	858–839 (bla_{TEM})	50	Livermore *et al*. (2001)
TETAF*	GTAATTCTGAGCACTGTCGC	24–43 [*tet*(A)]	55	Schmidt *et al*. (2001)
TETAR*	CTGCCTGGACAACATTGCTT	980–961 [*tet*(A)]	55	Schmidt *et al*. (2001)
TETBF*	CTCAGTATTCCAAGCCTTTG	770–789 [*tet*(B)]	53	Schmidt *et al*. (2001)
TETBR*	CTAAGCACTTGTCTCCTGTT	1205–1186 [*tet*(B)]	53	Schmidt *et al*. (2001)
TETBPROF‡	TACGGGTTGTTAAACCTTCG	84–65 [*tetR*(B)]	53	This paper
TETB1R‡	TAACCACAAGGAAAGCGACA	525–606 [*tet*(B)]	53	This paper
TETB2F‡	ATAGCGGGGCCTATTATTGG	426–445 [*tet*(B)]	53	This paper
TETB2R‡	CGTAAAAAATGCCCTCTTGG	1280–1261 [*tet*(B)]	53	This paper
TETRF‡	AACAGCGCATTAGAGCTGCT	33–52 [*tetR*(B)]	55	This paper
TETRR‡	AGATTGGAGTGAACGCCGTT	763–744 [*tetR*(B)]	55	This paper

*Primer used for initial detection of gene.

†Primer used for RT-PCR.

‡Primer used for amplification and DNA sequencing of entire gene and/or promoter region.

Reverse transcriptase (RT)-PCR to investigate potentially unexpressed genes

RNA was isolated from bacteria growing exponentially in nutrient broth using an Rneasy kit (Qiagen, Crawley, UK). Isolated RNA was treated with RNAse-free DNAse (Promega, Southampton, UK), and then RNA samples were adjusted to a concentration of $100\,ng\,\mu L^{-1}$ by adding molecular biology-grade water following measurement of $OD_{260\,nm}$. mRNA was detected by RT-PCR using a OneStep RT-PCR kit (Qiagen), using standard protocols as described previously, at annealing temperatures appropriate for each primer pair (Enne *et al.*, 2006). An RT-PCR reaction for the *rpsL* gene was performed on each RNA sample, either as a single reaction (*aadA*) or as part of a multiplex (*strAB*) to act as an internal control for the quality and quantity of mRNA. Primers used are listed in Table 1. RT-PCR products were separated on agarose gels and visualized as described for PCR reactions.

Results

Prevalence of antimicrobial resistance among *E. coli* isolated from healthy farm animals

The prevalence of resistance to each individual antimicrobial, per animal species, is shown in Table 2. Marked differences are apparent between the three species of farm animals studied. Resistance was rare among *E. coli* isolated from cattle and sheep; only 5.7% of isolates from cattle were resistant to at one least agent and 1.6% were multi-resistant (defined as resistance to three or more unrelated antimicrobials). Similarly, only 3.0% of isolates from sheep were resistant to one or more antimicrobials and 1.1% were multi-resistant. In contrast, 92.1% of isolates from pigs were resistant to at least one agent, while 62.8% were multi-resistant. The prevalence of resistance among the isolates also varied considerably according to the antimicrobial agent concerned. Whereas <1.0% of all isolates were resistant to some agents, such as ceftazidime, amikacin and nalidixic acid, prevalence of resistance to other agents such as tetracycline, sulphonamides and streptomycin was higher, particularly among isolates from pigs.

Considerable phenotypic diversity was observed among the resistant isolates. Most multi-resistant isolates were resistant to three, four or five different antimicrobials, but isolates that were resistant to as many as nine or 10 unrelated antimicrobials were identified. The most frequent resistance phenotypes observed are shown in Table 3. No single resistance phenotype was present in more than 5.2% of isolates.

Table 2. Prevalence of resistance among *Escherichia coli* isolates from three species of farm animal to 16 antimicrobials

	Frequency of resistance (%)		
Antimicrobial	Cattle ($n = 836$)	Sheep ($n = 836$)	Pigs ($n = 2480$)
Amikacin	0.0	0.0	0.1
Ampicillin	1.3	1.0	25.4
Apramycin	0.0	0.0	6.6
Cefoperazone	0.1	0.0	1.6
Ceftazidime	0.0	0.0	0.1
Chloramphenicol	0.1	0.1	20.8
Coamoxiclav	0.0	0.2	0.2
Colistin	0.2	0.0	34.1
Cotrimoxazole	0.6	0.4	30.8
Furazolidinone	0.0	0.0	3.7
Gentamicin	0.1	0.0	1.0
Nalidixic acid	0.1	0.0	0.6
Neomycin	0.6	0.6	21.5
Streptomycin	1.8	0.8	37.5
Sulphonamide	2.5	1.4	66.9
Tetracycline	4.1	2.5	78.7

Prevalence of antimicrobial resistance genes

A proportion of the isolates, 615 in total, was screened by PCR for the presence of a panel of antimicrobial resistance genes. To detect both expressed and unexpressed genes, all isolates were screened regardless of their antimicrobial susceptibility profile. More isolates from sheep and cattle were included than those from pigs, as they tended to be more susceptible, and may therefore have carried unexpressed resistance genes. The genes were chosen for investigation on the basis that they were expected to be prevalent among *E. coli* and because previous observations suggested that they may be unexpressed (Enne *et al.*, 2006; V.I. Enne, unpublished data). Two positive PCR products per gene were randomly selected for DNA sequencing in order to validate the specificity of the primers used. All DNA sequences obtained corresponded to their intended targets. The most common resistance genes were those encoding

Table 3. The most frequently observed resistance phenotypes among *Escherichia coli* isolated from farm animals

Resistance phenotype	Frequency (%) among all isolates ($n = 4152$)
TET	5.2
SUL TET	5.0
SUL SXT TET	3.1
STR SUL TET	2.0
CHL SUL TET	1.9
AMP STR SUL SXT TET	1.5
AMP SUL SXT TET	1.3
SUL	1.3
COL	1.1
STR SUL SXT TET	1.1

AMP, ampicillin; CHL, chloramphenicol; COL, colistin; STR, streptomycin; SUL, sulphonamide; SXT, cotrimoxazole; TET, tetracycline.

tetracycline, sulphonamide or streptomycin resistance. The resistance observed among the 615 isolates could be attributed to the presence of one or more of these resistance genes (Table 4). For example, of the 103- tetracycline resistant isolates tested, 18 carried *tet*(A), 58 carried *tet*(B), 19 carried both genes and eight carried neither. Among the 125 isolates that tested positive for carriage of at least one resistance gene, 59 different genotypes were observed, with individual isolates carrying anywhere between one and six different resistance genes. The most frequently observed genotype was carriage of *tet*(B) (19 isolates), followed by carriage of *strAB*, *sul2* and *tet*(B) (11 isolates) and *strAB* (7 isolates). The incidence of the other genotypes was low, with five or less isolates belonging to any one genotype.

Seven unexpressed resistance genes

Seven out of 615 isolates investigated carried unexpressed resistance genes (Table 5). One trimethoprim-susceptible isolate carried a complete *dfrA17* gene but lacked a promoter for it. However, in the remaining six streptomycin-susceptible isolates, one of which carried *strAB* while the others carried *aadA*, no mutations or deletions in gene or promoter sequences were identified to account for susceptibility. Additionally, four isolates were identified that carried incomplete antimicrobial resistance genes. One of these isolates had an incomplete *sul2* gene, one had an incomplete *tet*(B) gene and two had incomplete *strAB* genes (Table 5).

Discussion

This study demonstrates that the prevalence of antimicrobial resistance among *E. coli* from farm animals in Great Britain is variable depending on the animal species and antimicrobial in question. In some cases, a very high prevalence of resistance was observed, for example tetracycline and sulphonamide resistance among isolates from pigs, while in other cases such as nalidixic acid resistance among isolates from sheep or ceftazidime resistance among isolates from sheep and cattle, no resistance at all was detected (Table 2). Multi-resistance was considerably more prevalent among pig isolates than among sheep and cattle isolates. Such variation may reflect the differences in the amounts of therapeutic antimicrobials used in the husbandry of the three animal species studied. In 1999, 89 tonnes of therapeutic antimicrobial products aimed at pigs were sold in the UK, compared to 10 tonnes of products aimed at cattle and < 1 tonne of products aimed at sheep. The latest available figures demonstrate that therapeutic antimicrobial use in the United Kingdom has remained largely unchanged

Table 4. Genotypes of antimicrobial-resistant *Escherichia coli* isolates

Antimicrobial (n = number of resistant isolates)	Genotypes (n = frequency)
Tetracycline ($n = 103$)	*tet*(A) ($n = 18$), *tet*(B) ($n = 58$), *tet*(A)*tet*(B) ($n = 19$), other* ($n = 8$)
Sulphonamides ($n = 62$)	*sul1*($n = 7$), *sul2* ($n = 34$), *sul1 sul2* ($n = 10$), other* ($n = 11$)
Streptomycin ($n = 72$)	*aadA* ($n = 21$), *strAB* ($n = 26$), *aadA strAB* ($n = 18$), other* ($n = 7$)
Ampicillin ($n = 31$)	bla_{TEM} ($n = 20$), bla_{OXA-2} (n = 6), bla_{SHV} ($n = 0$), other* ($n = 5$)
Cotrimoxazole ($n = 27$)	*dfrA12* ($n = 7$), *dfrA17* ($n = 5$), other* ($n = 15$)

*Isolates were negative for all genes tested.

Table 5. Characteristics of *Escherichia coli* isolates that tested positive for resistance gene carriage by initial PCR assay but susceptible to the corresponding antimicrobial

Isolate	Source	MIC (μg mL^{-1}) (agent)*	Resistance gene detected	Explanation for susceptibility†
17.C342.5.99.1	Cattle	8 (STR)	*aadA1*	Unknown, promoter and gene wt
P187.11.99.C2	Pig	6 (STR)	*aadA1*	Unknown, promoter and gene wt
P502.10.99.C1	Pig	3 (STR)	*aadA1*	Unknown, promoter and gene wt
P185.10.99.C2	Pig	6 (STR)	*aadA2*	Unknown, promoter and gene wt
P311.10.99.C3	Pig	3 (STR)	*aadA2*	Unknown, promoter and gene wt
17.S251.2.99.2	Sheep	0.5 (TRM)	*dfrA17*	Promoter absent
17.C221.4.99.4	Cattle	2 (STR)	*strAB*	Part of *strB* missing
17.S420.2.99.4	Sheep	2 (STR)	*strAB*	Part of *strB* missing
17.S521.4.99.3	Sheep	8 (STR)	*strAB*	Unknown, promoter and genes wt
17.C524.4.99.3	Cattle	32 (SUL)	*sul2*	Part of *sul2* missing
17.C383.4.99.5	Cattle	3 (TET)	*tet*(B)	Part of *tet*(B) missing

*Resistance breakpoints used: STR $\geq$ 16, SUL $\geq$ 64, TET $\geq$ 16, TRM $\geq$ 4.

†wt, wild-type; indicates examined sequences were identical to those of isolates known to be resistant.

MIC, minimum inhibitory concentration; STR, streptomycin; SUL, sulphamethoxazole; TRM. trimethoprim; TET, tetracycline.

since 1999, and if anything, has slightly increased (http://www.vmd.gov.uk/Publications/Antibiotic/salesanti04.pdf).

A diverse range of resistance genes was detected among isolates included in this study. The resistance genes that were most common conferred resistance to antimicrobials to which resistance was prevalent, such as *tet*(B), *sul2* and *strAB*, which mediate resistance to tetracycline, sulphonamides and streptomycin, respectively. Many isolates had two different genes conferring resistance to the same antimicrobial and a high diversity of resistance genotypes was observed. Eight of the 103 tetracycline-resistant isolates investigated carried neither *tet*(A) nor *tet*(B). The remaining eight isolates may have carried one of the other six tetracycline efflux pumps present in *E. coli*, such as those encoded by *tet*(C) or *tet*(D) (Chopra & Robets, 2001). Trimethoprim was the only exception, where only 12 of the 27 resistant isolates investigated had *dfrA12*, *dfrA17* or related genes. Enterobacteria carry a diverse range of acquired trimethoprim-resistant dihydrofolate reductase genes, with the *dfrA* group comprising over 20 genes (Grape *et al.*, 2005) and the *dfrB* group comprising six genes (Levings *et al.*, 2006). The remaining resistant isolates may have carried any one or more of these genes.

This study found the incidence of unexpressed resistance genes to be low (~1%), although examples of such genes did occur. Isolates were identified that were not resistant despite the presence of apparently intact promoter and gene sequences. In all such cases the genes (*aadA* or *strAB*) normally confer resistance to streptomycin. As distal location from the integron promoter can result in the decreased expression of gene cassettes (Fluit & Schmitz, 1999), the distance of the *aadA* cassettes from the integron promoters in three isolates was examined. However, the distances from the integron promoters were not found to be unusually large, and similarly located gene cassettes are expressed in a variety of bacteria. All six isolates also expressed *aadA* or *strAB* mRNA (data not shown), and as such differed from previously described silent antibiotic resistance genes, which did not express resistance gene mRNA (Enne *et al.*, 2006). Others also have observed streptomycin susceptibility despite the presence of acquired streptomycin resistance genes (Randall *et al.*, 2004; Bischoff *et al.*, 2005; Sunde & Norstrom, 2005), not only in *E. coli* but also in *Salmonella enterica* (Randall *et al.*, 2004).

In conclusion, this study demonstrated that antimicrobial resistance is common among *E. coli* from healthy pigs in Great Britain, but rare among *E. coli* from sheep and cattle. Resistance phenotypes among *E. coli* of animal origin are extremely diverse and are mediated by a wide range of different resistance genes, suggesting the presence of a large population of resistant *E. coli*, particularly among pigs. This resistance is of concern as it can potentially spread to humans, either via direct colonization of the human gut by animal strains of *E. coli* or through transmission of resistance genes to resident bacteria in the human gut.

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