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Molecular Characterization of Multidrug-Resistant *Escherichia coli* Isolates from Irish Cattle Farms▽†

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This study describes the genotypic characteristics of a collection of 100 multidrug-resistant (MDR) *Escherichia coli* strains recovered from cattle and the farm environment in Ireland in 2007. The most prevalent antimicrobial resistance identified was to streptomycin (100%), followed by tetracycline (99%), sulfonamides (98%), ampicillin (82%), and neomycin (62%). Resistance was mediated predominantly by *strA-strB* (92%), *tetA* (67%), *sul2* (90%), bla_{TEM} (79%), and *aphA1* (63%) gene markers, respectively. Twenty-seven isolates harbored a class 1 integrase (*intI1*), while *qacEΔ1* and *sul1* markers were identified in 25 and 26 isolates, respectively. The variable regions of these integrons contained aminoglycoside, trimethoprim, and β-lactam resistance determinants (*aadA12*, *aadB-aadA1*, bla_{OXA-30}-*aadA1*, *dfrA1-aadA1*, *dfrA7*). Class 2 integrons were identified less frequently (4%) and contained the gene cassette array *dfrA1-sat1-aadA1*. Resistance to ampicillin, neomycin, streptomycin, sulfonamide, and tetracycline was associated with transferable high-molecular-weight plasmids, as demonstrated by conjugation assays. A panel of virulence markers was screened for by PCR, and genes identified included *vt1*, K5 in 2 isolates, *papC* in 10 isolates, and PAI IV_{536} in 37 isolates. MDR commensal *E. coli* isolates from Irish cattle displayed considerable diversity with respect to the genes identified. Our findings highlight the importance of the commensal microflora of food-producing animals as a reservoir of transferable MDR.

Growing evidence suggests that intensive agricultural and veterinary usage of antimicrobial compounds contributes to the emergence and dissemination of antimicrobial resistance (AMR) in bacteria derived from food-producing animals (23, 41). While antimicrobials are used to target pathogenic organisms, a simultaneous selection pressure is exerted on the commensal enterobacteria, encouraging the development and maintenance of AMR in these bacteria (2). In fact, persistence of resistance to antimicrobials has been shown to occur in the absence of selective pressure, highlighting the significance of not only the resistance determinants but also their associated genetic elements (3). The emergence of AMR in food-producing animals is of major public health significance arising from the risk of these bacteria entering the food chain (49). Thus, investigation of commensal bacteria is important in order to assess the extent of the drug resistance problem. Much of the current scientific literature describing AMR in commensal bacteria of food animal origin has concentrated on phenotypic resistance, a feature investigated principally within the framework of national resistance-monitoring programs (7, 16). Corresponding data on the genotypes and mechanisms of resistance involved are limited (24, 26). Horizontal gene transfer is acknowledged to be an important route of the dissemination of a variety of resistance-encoding genes. The main vehicles that facilitate these events include resistance plasmids, transposons, integrons, and bacteriophages. All contribute to the dissemination of resistance in different environments, including livestock, and frequently, the same genetic structures are found in ecologically unrelated bacteria, various species, and genera (8, 41, 46).

In this paper, we report on the geno- and phenotypic basis for multidrug resistance (MDR) in a collection of 100 *Escherichia coli* isolates recovered from cattle feces and environmental samples taken from Irish farms. The potential dissemination of the resistance markers was assessed *in vitro*.

MATERIALS AND METHODS

Collection of samples and isolation of *E. coli*. A collection of 100 *E. coli* isolates classified MDR (being resistant to 3 or more different classes of antimicrobial compounds) was obtained. All samples were collected from 21 dairy herds by veterinarians from three practices between March 2007 and June 2008 in the Cork region in Ireland. The MDR isolates obtained were isolated from eight of the dairy herds sampled in 2007. However, three more farms out of four sampled in 2007 were also positive for MDR, as were the majority of the farms sampled in 2008. The bacterial isolates originated from a range of animal and environmental samples (including calves, milk filters, overshoes, water samples, swabs of the calf pen, and feeding bucket) collected on two separate occasions. The only criterion for the selection of herds for the study was the fact that at the time of first sampling these herds contained a young calf (age, 6 days to 84 days) showing clinical signs of enteritis which necessitated examination by a veterinarian and the prescription of antimicrobial therapy. Fecal samples were inoculated onto MacConkey no. 2 agar (Oxoid, Basingstoke, United Kingdom) and incubated at 37°C for 24 h. Up to 5 colonies with a phenotypic appearance consistent with *E. coli* were subcultured to tryptone bile X-glucuronide agar (Cruinn Diagnostics Ltd., Dublin, Ireland) and incubated at 37°C for 24 h. Isolated blue-

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green single colonies were subcultured on blood agar medium (Cruinn Diagnostics Ltd.) and incubated at 37°C for 24 h. Water samples were filtered using a 45-μm-pore-size filter (Millipore, United Kingdom). The water filters, milk filters, swabs, and overshoes were each placed in 40 ml, 200 ml, 80 ml, and 200 ml of buffered peptone water (BPW; Merck, Darmstadt, Germany), respectively, and incubated overnight at 37°C for 24 h. A 10-μl loop of BPW from each sample was inoculated onto MacConkey no. 2 agar and incubated overnight at 37°C. Isolation was carried out as described for fecal samples above.

Resistance of isolates recovered from the sampling strategy described above against a panel of 19 antimicrobial agents was tested by disc diffusion. The following antimicrobial compounds were used: amikacin (Ak), 30 μg; amoxicillin-clavulanic acid (Amc), 20/10 μg; ampicillin (Amp), 10 μg; cefpirome (Cfp), 30 μg; cefpodoxime (Cpd), 10 μg; ceftiofur (Cfr), 30 μg; cephalothin (Cpl), 30 μg; chloramphenicol (C), 30 μg; ciprofloxacin (Cip), 5 μg; colistin (Ct), 25 μg; florfenicol (Ffc), 30 μg; furazolidone (Fr), 15 μg; gentamicin (Gm), 10 μg; nalidixic acid (Na), 30 μg; neomycin (Neo), 30 μg; streptomycin (S), 10 μg; sulfonamide compound (Su), 300 μg; tetracycline (Te), 30 μg; and trimethoprim (Tmp), 5 μg. Antibiotic-containing discs were supplied by Oxoid (Basingstoke, United Kingdom), except for furazolidone discs, which were supplied by Mast Group Ltd. (Bootle, United Kingdom). Clinical and Laboratory Standards Institute (CLSI) guidelines (12) were followed for all drugs except cefpirome, colistin, florfenicol, furazolidone, and neomycin, in which cases CLSI does not specify breakpoints for *E. coli*. Resistant zone diameters breakpoints adopted for these antimicrobials were the following: <15 mm for cefpirome, ≤14 mm for colistin, ≤14 mm for florfenicol, ≥12 mm for furazolidone, and ≤12 mm for neomycin. Zone diameters were measured using a Mastascan Elite system (Mast Group Ltd.). A quality control strain, *E. coli* ATCC 25922, was included in the testing.

DNA extraction and PCR detection of resistance genes, integrons, transposons, and virulence genes. DNA extraction was carried out using a Promega Wizard genomic DNA purification kit (Madison, WI), following the manufacturer's instructions. The concentration of DNA and its purity were assessed spectrophotometrically using a Nanodrop ND-1000 spectrophotometer (Thermoscientific, Wilmington, DE). The following resistance-encoding determinants were screened for as described previously (32): *aadA*, *aadB*, *strA-strB*, *aac(3)-IV*, *aphA1*, and *aphA2*, associated with aminoglycoside resistance; *ampC*, bla_{OXA}, bla_{SHV}, and bla_{TEM}, which encode β-lactamases; genes coding for chloramphenicol acetyltransferase (*cat1*) and the chloramphenicol (*cmlA*) and chloramphenicol/florfenicol (*floR*) efflux pump; *sul1*, *sul2*, and *sul3* sulfonamide resistance genes; and *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), and *tet*(G) tetracycline efflux pumps. Selected transposon-associated loci *tnpM* from Tn*21*, *tnpR* of Tn*1721*, and *tnpA* of Tn*3* were also analyzed by PCR using previously published primers (39, 43, 50). The presence of integrons was investigated by amplification of *intI1* and *intI2* integrase genes (derived from classes 1 and 2, respectively), *qacE*Δ*1*, and *sul1*, the right-sided conserved segments of class 1 integrons, together with their variable regions, as described previously (32). Detection of the 8 pathogenicity islands (PAIs), including PAI I_{536}, PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073}, PAI I_{J96}, and PAI II_{J96}, was performed using previously published PCR protocols (44). Serotype and virulence-associated genes included were *rfb* O157 and O111 (42), *wzx* O26 (15), *vt1* and *vt2* (22), *hly* and *eae* (42), *fli*C7 (21), CNF1 (5), *afa-draBC*, *papC*, and *sfa-focDE* (14), *iucD* (53), and K5 and *neuC* (27).

All isolates in the collection were assigned to phylogenetic groups, in which group A/B1 typically contained commensal isolates and group B2/D isolates were associated with virulence, using a triplex PCR protocol (11).

Sequencing of amplified gene cassettes. Gene cassettes from class 1 and 2 integrons were amplified with the primer pairs 5′CS/3′CS and hep74/hep51, respectively, and were subsequently purified from the agarose gel using a Promega Wizard PCR and a gel purification system. Recovered amplicons were commercially sequenced (Qiagen, Hilden, Germany). Assembly of the sequence data was performed using DNAStar software and the SeqMan application (DNAStar Inc., Madison, WI). Sequence similarity searches were carried out against sequences deposited in the current version of the GenBank database using the BLAST search tool (http://www.ncbi.nlm.nih.gov/BLAST). Sequence alignments were performed using the online ClustalW program available at the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw).

Plasmid purification. Plasmids were extracted from 2-ml overnight cultures grown in Luria-Bertani (LB) broth (Difco Laboratories, Becton Dickinson, Sparks, MD) using a QuickGene plasmid kit S (Fuji, Tokyo, Japan) according to the manufacturer's instructions. Plasmid sizes were estimated by comparison with previously characterized plasmids from the reference strains *E. coli* V517 and *E. coli* 39R861, isolated under identical conditions (37).

Conjugation mating experiments. Conjugation mating experiments were performed with plasmid-less recipient strain *E. coli* 26R793 strain (Rif^r, *lac* negative) in order to assess the transfer of the associated resistance markers. Seventeen representative MDR *E. coli* isolates from the collection were selected for study and were investigated on the basis of the presence of heterogeneous plasmid profiles. Briefly, single colonies of the donor and recipient were inoculated in LB broth (Difco Laboratories) and grown overnight at 37°C. Subsequently, equal volumes of the donor and recipient cultures were mixed and incubated overnight at 37°C without shaking. Serial dilutions were then plated on MacConkey agar (Oxoid) selection plates supplemented with a combination of 100 μg/ml rifampin (Sigma-Aldrich, Ireland) and one of each of the following antimicrobial compounds: ampicillin sodium salt (50 μg/ml), chloramphenicol (20 μg/ml), nalidixic acid (50 μg/ml), streptomycin (50 μg/ml), tetracycline (30 μg/ml), or trimethoprim (50 μg/ml). All antibiotic powders were purchased from Sigma-Aldrich. Three colonies from each resultant selection plate were examined and phenotypically confirmed to have newly acquired AMR by using the disc diffusion technique. Plasmids were then extracted from the confirmed isolates, and PCR was performed to identify the resistance genes acquired by the transconjugants. This was done for each of 3 transconjugants possessing the same resistance profile derived from each donor.

Plasmid incompatibility typing. Plasmids were assigned to incompatibility groups on the basis of the presence of specific replicon sequences identified by PCR. A modification of the method previously published by Carattoli et al. (9), using a panel of 3 multiplex PCRs, was applied (28).

RESULTS

MDR profiles. Table 1 in the supplemental material presents all results in detail. All isolates in this study collection were resistant to between 3 and 10 antimicrobial compounds, with 18 different resistance profiles recognized. The distribution of resistance was as follows: amikacin (Ak), 0%; amoxicillin-clavulanic acid (Amc), 3%; ampicillin (Amp), 82%; cefpirome (Cfp), 0%; cefpodoxime (Cpd), 2%; ceftiofur (Cfr), 0%; cephalothin (Cpl), 5%; chloramphenicol (C), 9%; ciprofloxacin (Cip), 0%; colistin (Ct), 0%; florfenicol (Ffc), 4%; furazolidone (Fr), 2%; gentamicin (Gm), 2%; nalidixic acid (Na), 2%; neomycin (N), 62%; streptomycin (S), 100%; sulfonamide (Su), 98%; tetracycline (Te), 99%; and trimethoprim (Tmp), 22%. The most frequent resistance profile identified was AmpNSSuTe, present in 44 of 100 isolates (44%), followed by SSuTe (13%), AmpSSuTe (10%), AmpNSSuTeTmp (9%), AmpCNSSuTeTmp (4%), and AmpSSuTeTmp (3%). Each of the following 5 resistance patterns was identified in 2 isolates: AmcAmpCpdCplNaSSuTeTmp, AmpCFfcSSuTeTmp, AmpGmNSSuTeTmp, CFfcSSuTe, and FrSSuTe. In single isolates, the resistance profiles observed included AmcAmpNSSuTe, AmpCplNSSu, AmpCplNSSuTe, AmpCSSuTe, AmpSTe, AmpSSu, and CplSSuTe.

Phylogenetic groups. Most of the isolates investigated could be assigned to phylogenetic groups A and B1, with 52 assigned to group A and 38 assigned to group B1. Ten isolates were assigned to the D and B2 phylogenetic groups, with 8 and 2 members in each of those groups, respectively.

Identification of virulence genes and pathogenicity islands. The majority of the virulence determinants, pathogenicity islands, or typical markers of pathogenic *E. coli*, including *afa-draBC*, *iucD*, *sfa-focDE*, *neuC*, *sfaS*, *vt2*, *hly*, *eae*, *fliC7*, *CNF1*, *rfb* O157 and O111, and *wzx* O26, were absent in the collection. The *papC*-encoding gene and PAI IV_{536} were identified in 10 and 37 isolates, respectively. The gene encoding verocytotoxin 1 (*vt1*) was identified in a single isolate, and the K5 marker was identified in 2 isolates.

Frequency of AMR genes. Table S1 in the supplemental material details the resistance genotypes from the isolates in

TABLE 1. Frequency of the antimicrobial resistance genes in 100 MDR isolates of *Escherichia coli* from cattle

Antibiotic class (% resistant)	Gene	% of isolates
Aminoglycosides (100)	*aac(3)-IV*	0
	aadA	19
	aadB	1
	aphA1	63
	aphA2	0
	strA-strB	92
β-Lactams (82)	bla_{CARB}	0
	bla_{TEM}	79
	bla_{SHV}	0
	bla_{OXA}	2
Phenicols (9)	*cat1*	5
	cmlA	0
	floR	4
Sulfonamides (98)	*sul1*	26
	sul2	90
	sul3	0
Tetracycline (99)	*tet*(A)	67
	tet(B)	31
	tet(C)	0
	tet(D)	0
	tet(E)	0
	tet(G)	0

the collection. A summary of the frequency of these resistance markers is presented in Table 1. Genotypes were generally consistent with the phenotypes observed, with occasional discrepancies noted. With regard to aminoglycoside resistance, the *strA-strB* streptomycin resistance gene pair was the most common determinant found in 92 isolates ($n = 92$), followed by *aphA1* ($n = 63$), *aadA* ($n = 19$), and *aadB* ($n = 1$). No *aphA2* or *aac(3)-IV* genes were identified. One isolate (denoted C10.602 in Table S1 in the supplemental material) possessed none of the aminoglycoside resistance markers, despite being phenotypically resistant. The predominant β-lactamase gene was bla_{TEM} ($n = 79$), followed by bla_{OXA} ($n = 2$). None of the isolates were positive for the bla_{SHV} or bla_{CARB} genes. Among 9 chloramphenicol-resistant strains detected, 5 carried the *cat1* gene, encoding a chloramphenicol acetyltransferase, while a further 4 isolates were resistant to florfenicol and possessed the *floR*-encoding chloramphenicol/florfenicol efflux pump. The *cmlA* marker was not detected in the collection. With regard to sulfonamide resistance determinants, *sul2* had the widest distribution ($n = 90$), followed by *sul1* ($n = 26$), while *sul3* was not detected. The only 2 tetracycline resistance determinants identified among the 99 tetracycline-resistant isolates, were *tet*(A), present in 66 isolates, and *tet*(B), present in 31 isolates. Nine isolates harbored both *tet*(A) and *tet*(B).

Distribution of integrons, gene cassettes, and transposon markers. The genetic markers associated with class 1 integrons, *intI1*, *qacEΔ1*, and *sul1*, were detected in 27, 25, and 26 strains, respectively. Twenty-five of these were positive for all 3 of these markers. All but one (isolate number C19.203; see Table S1 in the supplemental material) yielded an amplicon by PCR targeting the conserved flanking segments. Two of the *intI1*-positive isolates (C2.401 and C4.301) did not carry *qacEΔ1* or *sul1* and did not contain a variable gene cassette region. Gene cassettes from these class 1 integrons (26 in total) had the following sizes: 800 bp ($n = 9$), 1 kb ($n = 9$), 1.5 kb ($n = 3$), 2 kb ($n = 2$), and 2.7 kb ($n = 1$). None of the isolates contained multiple integrons.

Sequencing of the 800-bp class 1 gene cassette revealed the presence of a single open reading frame encoding a *dfrA7* dihydrofolate reductase gene that showed 100% sequence identity with the sequence of a class 1 integron identified in *E. coli* strain KD8 (GenBank accession no. EU339236). An *aadA12* determinant, encoding aminoglycoside adenyltransferase, was identified within the 1-kb amplicon and showed 100% sequence similarity with the sequence reported previously to be a part of a class 1 integron found in *E. coli* (GenBank accession no. FJ855128.1). The 1.6-kb variable region exhibited 99% nucleotide sequence identity with the sequence of a class 1 integron containing *dfrA1-aadA1* gene cassettes (GenBank accession no. EF527229). The *aadB-aadA1* gene arrangement identical to the one deposited previously in GenBank (accession no. AM932676) was identified in the 2.7-kb variable region. A schematic illustrating the genetic organization of these cassettes is shown in Fig. 1.

Fewer isolates ($n = 4$) carried class 2 integrons, as determined by amplification of the integrase gene (*int2*). All possessed gene cassettes of approximately 2 kb with the gene array *dfrA1-sat1-aadA1*, the sequence of which was 100% homologous at the nucleotide sequence level with that of Tn*7* (GenBank accession no. AB188272).

Genes indicative of the presence of some transposons were present in 34% ($n = 34$) of the collection. Among the 100

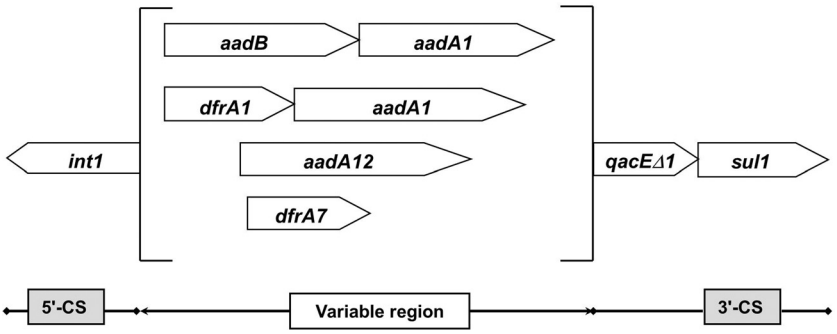

FIG. 1. Class 1 gene cassette repertoire identified in MDR isolates of *E. coli* from cattle and their environment. CS, conserved sequence.

TABLE 2. Transfer of resistance phenotypes and genotypes of representative MDR isolates of *Escherichia coli* from cattle

Donor	Donor resistance profile	Resistance markers transferred to *E. coli* 26R793 genes[b]	Size of plasmid(s) acquired (kb)[a]	Incompatibility type
C5.101	AmpNSSuTe	**AmpNSSuTe/*aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)**	60 + 150 (3)	IncB/O, IncP, IncFrep
C2.401	AmcAmpCpdCplNaSSuTeTmp	AmpSSuTmp/*intI1*-Tn*21*-Tn*1721*-*bla*$_{TEM}$-*strA-strB-sul2*	150 (3)	IncFIB
C6.201	AmpNSSuTe	**AmpNSSuTe/*aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)**	2 + 4 + 7 + 150 (2)	IncB/O, IncP
			2 + 5 + 9 + 150 (1)	IncB/O, IncP
C4.102	AmpNSSuTe	**AmpNSSuTe/*aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)**	150 (3)	IncB/O, IncP
C8.801	AmpNSSuTe	AmpNSSuTe/*aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(B)	150 (3)	IncFIB
C8.803	AmpNSSuTe	**AmpNSSuTe/*aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)**	150 (3)	IncB/O
C8.401	AmpGmNSSuTeTmp	**AmpNSSuTe/*aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)**	150 (2)	IncB/O, IncP
			60 + 150 (1)	IncB/O, IncP, IncFrep
C15.101	AmpNSSuTe	**AmpNSSuTe/*aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)**	150	IncB/O, IncP
C24.201	AmpSSuTeTmp	AmpSSuTeTmp/*intI1*-800-bp GC[c](1)-*qacEΔ1-sul1*-Tn*21-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)	150	IncB/O, IncP
C19.202	AmpNSSuTeTmp	AmpNSSuTeTmp/*intI1-800-bp* GC(1)-*qacEΔ1-sul1*-Tn*21-aphA1-bla*$_{TEM}$-*strA-strB-sul2-tet*(A)	7 + 150 (2)	IncB/O, IncP
			150 (1)	IncB/O, IncP

[a] Numbers in parentheses relate to the numbers of transconjugants with each plasmid profile. Mating-out assays were performed on two separate occasions.
[b] The predominant transferable phenotype-genotype is indicated in boldface font. (1) indicates the presence of a class 1 integron type.
[c] GC, gene cassette.

isolates, 27 (27%) tested positive for the *tnpM* gene of Tn*21*. All Tn*21* carriers also harbored *intI1*, indicating that both truncated ($n = 2$) and complete ($n = 25$) class 1 integrons were associated with the Tn*21*-like elements. The *tnpR* gene of the Tn*1721* transposon associated with tetracycline resistance was identified in 11 isolates (11%), 10 of which also carried *tet*(A). Fifteen isolates also carried a Tn*3* marker, *tnpA*, and all of these were positive for *bla*$_{TEM}$.

Plasmid profiles. Overall, 34 different plasmid profiles were observed. Seventeen isolates (17%) were devoid of plasmids. The majority of the isolates ($n = 83$; 83%) carried multiple plasmids ranging in size from approximately 2 kb to more than 150 kb. Seventy-nine isolates contained plasmids of more than 50 kb, and some contained several large plasmids (data not shown).

Horizontal transfer of antimicrobial resistance, associated genes, and plasmids. Resistance marker transfer by conjugation was observed for 10 of 17 isolates that were subjected to conjugation assays. In all cases, the MDR phenotype could be transferred regardless of the antimicrobial(s) used for selection. The transconjugants derived from the same donor strains were identical with regard to their resistance patterns. Seven of the donor strains transferred resistance to ampicillin, neomycin, streptomycin, sulfonamide, and tetracycline (AmpNSSuTe), while single isolates transferred the following resistance phenotypes: AmpSSuTmp, AmpSSuTeTmp, and AmpNSSuTeTmp. Three transconjugants derived from each donor strain were subjected to genotypic analysis. Plasmid profiling demonstrated that the recipient acquired high-molecular-weight plasmids of approximately 150 kb in all cases. In some cases, multiple plasmids were acquired (Table 2). The plasmids present in the transconjugants were assigned to the following incompatibility groups: IncB/O, IncFIB, IncFrep, and IncP. The characteristics of the donors and their corresponding transconjugants are listed in Table 2.

DISCUSSION

In this study, genetic determinants implicated in MDR were identified, and these provided important clues about their potential for horizontal transmission. Notably, the predominant MDR phenotype (AmpNSSuTe) and the corresponding genotype [*aphA1 bla*$_{TEM}$ *strA-strB sul2 tet*(*A*)] were similar to those observed earlier by Guerra and coworkers (24) in *E. coli* O111 isolates recovered from humans and cattle. Resistance to streptomycin, sulfonamide, and tetracycline among *E. coli* isolates associated with cattle is well documented (24, 38). The prevalent MDR profile included resistance to antimicrobials commonly used in cattle, possibly reflecting selective pressures exerted during animal production, a view that was expressed previously by other investigators (38). Resistance levels to newer, more valuable antimicrobial compounds, such as fluoroquinolones and expanded-spectrum cephalosporins, were low in this study. This was a positive finding, but the number of farms sampled in the study was small and may not be representative of all Irish dairy farms.

Class 1 and class 2 integrons in the collection were found to contain trimethoprim (*dfr*) and streptomycin (*aad*) resistance-encoding genes, which are frequently reported in *E. coli* isolates recovered from various sources, including human, animal, and environmental samples. Their extensive dissemination could be attributed to their association with integrons and plasmids (13, 31, 30, 51).

In this study collection, we observed a remarkably high frequency of *strA-strB* determinants, encoding two enzymes, both of which are required for high-level streptomycin resistance (10). Consistent with other studies, *strA-strB*-mediated resistance is common and has been widely reported both in the environment and in farm animals, including cattle (19). Of the 16 *strA-strB*-positive isolates, 10 transferred these markers, suggesting the association of these genes with conjugative or mobilizable plasmids (Table 2). The *strA-strB* gene pair can be

found within Tn*5393*, carried by large self-transmissible plasmids (35). Further investigation is required to clarify whether the *strA-strB* genes predominating in this collection are linked with a similar transposon.

The *aadA* streptomycin resistance gene, conferring streptomycin and spectinomycin resistance, was identified in 19 isolates (19%), all of which also carried either class 1 integrons (15 isolates, with gene cassettes of 1, 1.3, 2, and 2.7 kb) or class 2 integrons (4 isolates, with gene cassettes of 2 kb). The association of both classes of integrons with *aadA* genes has been demonstrated in numerous studies, for instance, as a part of the Tn*7*-like class 2 integron gene array *dfrA1-sat1-aadA1* (30, 51, 52). The *aadB* marker, encoding resistance to gentamicin, kanamycin, and tobramycin, was identified in a single isolate as part of a 2.7-kb variable region within a class 1 integron along with *aadA1* (1%) in a previously reported gene arrangement, *aadB-aadA1* (30, 52). The *aphA1* gene marker was identified in the majority of neomycin-resistant isolates. This finding is consistent with other reports that noted *aphA1* to be the predominant neomycin/kanamycin resistance determinant (40, 47). On occasion, resistance genes were detected in isolates classified susceptible by phenotyping, an observation that was not unique to this study (6, 34, 36).

Narrow-spectrum TEM-1 is common in animals, and consequently, high rates of ampicillin-resistant, bla_{TEM}-positive isolates are to be expected (38). The bla_{OXA} genes, encoding class D β-lactamases, had a low occurrence (2%). It is interesting to note that class 1 integrons containing the bla_{OXA}-*aadA* genes in a classical head-to-tail arrangement have previously been identified in salmonellae from both food and human sources and, sporadically, in *E. coli* isolates from humans, suggesting that food-producing animals might be a likely source of these genes (1, 18). Our findings support this hypothesis. Overall, resistance to chloramphenicol in the collection was low (9%) and was for the most part mediated by *cat1*, responsible for enzymatic resistance, and *floR*, encoding a florfenicol/chloramphenicol transporter. This low occurrence could perhaps be due to the association of *cat1* and *floR* genes with nonconjugative plasmids or their chromosomal location since no transfer of resistance to chloramphenicol or florfenicol was observed. *sul2*, present in 90% of the isolates, has been reported to be the most frequent mechanism of resistance to sulfonamides in human clinical *E. coli* isolates (20, 33). The presence of *sul1* (26%), which was almost exclusively accompanied by both the *intI1* and *qacEΔ1* genes, was indicative of the class 1 integrons containing both conserved segments (46).

The *tet*(A) marker was the predominant tetracycline resistance gene identified in the collection (67%), followed by *tet*(B) (31%). Both of these determinants were among the most widespread *tet* genes found in enterobacteria, and their occurrence was within the range reported by other investigators (36, 48). We demonstrated that some of the isolates could transfer the *tet* gene by conjugation (Table 2). This observation is in agreement with that from another study that reported that *tet* genes encoding efflux pumps tend to be associated with large conjugative plasmids of various incompatibility types (29).

Conjugative transfer of plasmids belonging to incompatibility groups IncP, IncB/O, and, less frequently, IncFIB and IncFrep (Table 2) was observed. This feature is interesting, given that both IncB/O and IncFIB plasmids were previously found to be relatively common among avian pathogenic *E. coli* (APEC) isolates as well as in both human commensal and uropathogenic *E. coli* (UPEC) isolates (28). Plasmids of the IncP group are recognized to be a significant antibiotic resistance reservoir due to their self-transmissibility and their broad host range (45). In the present study, IncP replicons in the transconjugants were always accompanied by IncB/O (Table 2). It is noteworthy that the plasmids identified here as vehicles of transferable MDR resistance did not belong to incompatibility groups considered epidemic, such as IncFII, IncA/C, IncL/M, or IncI1, which are ubiquitous in various geographical regions and bacterial hosts (8).

With regard to the phylogenetic groups, phylogenetic groups B2 and D are considered to be more likely to carry pathogenicity-associated genes, while groups A and B1 are classified nonpathogenic commensal strains (11, 13). In our study, most of the isolates belonged to groups A and B1 (52 and 38%, respectively), in agreement with findings from other surveys showing that bovine *E. coli* isolates belong predominantly to lineages A and B1 (25). In this study, 8 isolates were classified in group D, and it is noteworthy that 5 of these were cultured from clinically affected calves.

papC, an adhesin associated with extraintestinal pathogenicity and an increased capacity to colonize the intestine, was identified in 10 isolates. Also, a single isolate (C19.202) possessed *vt1*, and 2 more isolates (C10.802 and C10.602) contained K5 (capsular polysaccharide). The presence of a single verocytotoxigenic *E. coli* (VTEC) isolate in this collection is somewhat surprising since VTEC isolates are considered to be less likely to demonstrate resistance (4).

PAI IV_{536}, also known as a high-pathogenicity island encoding a siderophore system, yersiniabactin, in uropathogenic *E. coli*, was identified in 37 isolates. Consistent with our study, surveys performed previously have shown that PAI I_{536} to PAI III_{536} are usually absent in intestinal pathogenic isolates and are probably unique to extraintestinal pathogenic *E. coli* (ExPEC) strains. In contrast, PAI IV_{536} was found in 17 to 28% of the intestinal pathogenic strains and 67 to 92% of ExPEC strains (17) and 38% of commensal *E. coli* strains (44). In this context, the 37% occurrence of this pathogenicity island in commensal *E. coli* isolates originating from animals is not unusual. Nevertheless, the genes present therein contribute to the virulence genotype of presumptively harmless strains.

Conclusion. We reported on a comprehensive study of the molecular basis for MDR in bovine *E. coli* isolates recovered from Irish farms. Emergence of resistance in the food chain is an important public health concern. Our data support previous findings that the intestinal microflora of cattle can serve as an important reservoir of diverse resistance determinants frequently associated with mobile genetic elements, such as transposons, integrons, and conjugative plasmids, contributing collectively to their evolution and dissemination. We observed that cotransfer of antimicrobial resistance is a common mechanism, indicating that further spread of these genes is possible. Considering the direct links with humans, surveillance of resistance in food-producing animals is essential.

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