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Heterogeneity among Virulence and Antimicrobial Resistance Gene Profiles of Extraintestinal *Escherichia coli* Isolates of Animal and Human Origin

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) isolates collected from different infected animals and from human patients with extraintestinal infections in 2001 were characterized for their phenotypic and genotypic antimicrobial resistance profiles, genotypes, and key virulence factors. Among the 10 antimicrobial agents tested, resistance to ampicillin, tetracycline, and sulfonamides was most frequent. Multiresistant strains were found in both the animal and the human groups of isolates. Resistance gene distribution was assessed by colony hybridization. Similar antibiotic resistance patterns could be observed in the animal and the human isolates. Although some resistance genes, such as bla_{TEM}, *sulI*, and *sulII*, were equally represented in the animal and human ExPEC isolates, differences in the distributions of tetracycline [*tet*(D)], chloramphenicol (*catI*, *catIII*, and *floR*), and trimethoprim (*dhfrI*, *dhfrV*, *dhfrVII*, and *dhfrXIII*) resistance genes were observed between the animal and the human isolates. Approximately one-third of the ExPEC isolates possessed a class 1 integron. The four major different variable regions of the class 1 integron contained aminoglycoside (*aadA1*, *aadA2*, *aadA5*, and *aadA6*) and/or trimethoprim (*dhfrIb*, *dhfrXII*, and *dhfrXVII*) resistance genes. The ExPEC strains belonged to different phylogenetic groups, depending on their host origin. Strains isolated from animal tissues belonged to either a commensal group (group A or B1) or a virulent group (group B2 or D), while the majority of the human isolates belonged to a virulent group (group B2 or D). Although the limited number of isolates evaluated in the present study prevents firm epidemiological conclusions from being made, on a more global scale, these data demonstrate that extraintestinal isolates of *E. coli* can possess relatively distinct intra- and intergroup resistance gene profiles, with animal isolates presenting a more heterogeneous group than human isolates.

Escherichia coli is an important cause of disease in animals and humans worldwide (43). Strains of this bacterium can be classified into three major groups: commensal, intestinal pathogenic (enteric or diarrheagenic), and extraintestinal pathogenic *E. coli* (ExPEC) strains. ExPEC infections are common in both animals and humans and can involve almost any organ or anatomical site (41). Typical extraintestinal infections include urinary tract infections, meningitis, diverse intra-abdominal infections, pneumonia, soft tissue infections, intravascular device-associated infections, and osteomyelitis (41). ExPEC harbors specific virulence factors, such as adhesins, fimbriae, hemolysin, and aerobactin, which participate in the pathogenesis of the bacterium (13).

Although antimicrobial therapy is an important tool for the treatment of these infections, resistance to antimicrobials is widespread and of great concern in veterinary medicine (35, 51). Indeed, a close association exists between the use of antimicrobial agents for the treatment of infections in animals and the levels of resistance observed (1, 46). From a human health perspective, the direct impact of the antimicrobial resistance that has evolved from the use of antimicrobials for the treatment of infections in animals is not clear. Since the antimicrobial classes routinely used for the treatment of infections in humans are also used in animals either for therapy and prevention or as a growth promotion factor, it is difficult to show the relative contributions of an animal-derived resistant strain—or more specifically, the antimicrobial resistance genes—in human *E. coli* disease, and vice versa.

The potential for resistance gene transmission has been demonstrated in cases in which an antimicrobial agent is used for animals but not for humans. For example, after the introduction of streptothricin for growth promotion in the 1980s, *E. coli* isolates harboring plasmid-mediated resistance appeared and were isolated from pigs as well as from the pig farmers, including their families (19). Another example is the emergence of apramycin resistance after its introduction in veterinary medicine. The direct transfer of plasmids mediating apramycin resistance from *E. coli* isolates from pigs to *E. coli* isolates from humans has been observed (20, 23). Many antimicrobial resistance and virulence genes are located on plasmids and transposons, which enables their transfer among a variety of bacterial species. Genes for resistance to antibiotics that are or that have been used only in animals (i.e., nourseo-

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thricin, apramycin, etc.) were found soon after their introduction and were found not only among the bacteria isolated from animals but also in the human commensal flora. This was found among zoonotic pathogens, like salmonellae, as well as strictly human pathogens, like shigellae. This makes it clear not only that the clonal spread of resistant strains occurs but also that the transfer of resistance genes between human and animal bacteria occurs. It has been suggested that food animals or pets may act as potential reservoirs for transmission of *E. coli* infections, as virulent ExPEC strains from animals and humans have been shown to share some phylogenetic, pathotypic, and genotypic similarities (1, 17, 54). More specifically, the findings of some studies support the hypothesis that ExPEC isolates represent populations that contain members of certain clones or clonal groups that can cause infections in pets, food animals, and humans (1, 26, 53). Indeed, it was shown that transmission of resistant *E. coli* clones from poultry to humans commonly occurs (25, 53).

This study examines the antimicrobial susceptibilities of animal and human ExPEC isolates, the carriage of resistance genes by these isolates, and the phylogenetic relatedness of animal and human ExPEC isolates.

MATERIALS AND METHODS

Bacterial isolates and growth conditions. In this study, only isolates showing resistance to at least one antimicrobial were selected. Among the ExPEC strains isolated in 2001 from different infected animal tissues (lung, heart, liver, kidney, brain, and other), only a small number ($n = 39$) showed resistance to at least one antimicrobial and were kept for this study. ExPEC strains were isolated at the Faculté de Médecine Vétérinaire in Saint-Hyacinthe, Quebec, Canada, from swine (40%), chicken (20%), cattle (20%), and pets (20%). Seventy resistant *E. coli* ExPEC isolates derived from patients with urinary tract infections and collected in 2001 at Honoré-Mercier Hospital in Saint-Hyacinthe were analyzed in this study. Reference strain *E. coli* ATCC 25922 was used to assess sensitivity to each lot of antimicrobials by the disk diffusion method. The bacterial isolates, kept at −80°C in tryptic soy broth medium containing 10% (vol/vol) glycerol, were cultured on tryptic soy agar supplemented with 5% (vol/vol) sheep blood.

The 32 isolates used as positive controls for amplification of DNA hybridization probes were obtained from different laboratories (Table 1). These isolates were kept at −80°C as frozen stocks in tryptic soy broth medium containing 10% (vol/vol) glycerol and were propagated on Luria-Bertani broth or agar containing one of the following antimicrobials at the indicated concentrations: ampicillin (50 μg/ml), gentamicin (30 μg/ml), kanamycin (50 μg/ml), tetracycline (10 μg/ml), chloramphenicol (10 μg/ml), trimethoprim (10 μg/ml), and sulfamethazine (200 μg/ml).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was carried out for all isolates by the disk diffusion method on Mueller-Hinton agar as the culture medium, according to NCCLS standards (55). The antimicrobial agents used in this study were the beta-lactams ampicillin (10 μg), ceftiofur (30 μg), and cefotaxime (30 μg); the aminoglycosides gentamicin (10 μg), kanamycin (30 μg), and neomycin (30 μg); tetracycline (30 μg); chloramphenicol (30 μg); trimethoprim (5 μg); and sulfaminoxazole (250 μg). The antimicrobial breakpoints for all antimicrobials except neomycin were taken from NCCLS recommendations; the breakpoints used for neomycin were those recommended by the manufacturer.

PCR oligonucleotide primers and amplification. Antibiotic resistance gene primers were designed with the software program Prime (Genetics Computer Group, Madison, Wis.), as described previously (33). The virulence genes normally associated with ExPEC strains, *afaC*/*afaD*, *afaD8*, *sfaDE*, *papC*, *hlyA*, and *iucD*, were amplified with the primer sequences described elsewhere (18, 29, 31, 57) (Table 1). Primers were synthesized with a DNA synthesizer (Biocorp Inc., Montreal, Québec, Canada). Primers located in the 3′ conserved segment of the class 1 integron, characterized by the *qacEΔ1* and *sulI* genes, were used to determine the presence of the integron, as described by Sandvang and Aarestrup (44) (Table 1).

PCR amplifications were performed with 5 μl of supernatant from a bacterial culture that had been boiled in water for 10 min (11). The PCR mixture (total volume, 50 μl) included 29.6 μl of H_2O, 5 μl of 10× PCR buffer (Amersham Pharmacia Biotech Inc., Piscataway, N.J.), 5 μl of 2 mM deoxynucleoside triphosphates, 1 U of *Taq* polymerase (Amersham Pharmacia Biotech Inc.), and 25 pmol of each primer. DNA amplification was carried out with a GeneAmp PCR system 9700 instrument (Perkin-Elmer, Foster City, Calif.) by using the following conditions: 5 min at 94°C, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min. The size and purity of the PCR product from a sample of 3 μl was verified by gel electrophoresis (1.2% [wt/vol] agarose in 1× Tris-acetate-EDTA buffer).

All isolates containing the class 1 integron 3′ conserved segment were further investigated by a second PCR amplification of a variable region (VR) within the integron by using the primers described by Sandvang and Aarestrup (44) (Table 1). The amplification conditions for these primers were as follows: 5 min at 94°C, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 5 min.

The different ExpEC phylogenetic groups were determined by the PCR-based method described by Clermont et al. (9) (Table 1). The data from the three amplifications resulted in an assignment of the following isolates to the indicated phylogenetic groups: *chuA*- and TspE4.C2-negative isolates, group A; *chuA*-negative and TspE4.C2-positive isolates, group B1; *chuA*- and *yjaA*-positive isolates, group B2; *chuA*-positive and *yjaA*-negative isolates, group D (9, 12).

DNA sequencing. The amplified products were purified with a QIAquick PCR purification kit (Qiagen Inc., Mississauga, Ontario, Canada) or a QIAquick gel extraction purification kit (Qiagen Inc.). Their sequences were confirmed with a dRhodamine Terminator Cycle Sequencing Ready reaction kit and a model 377 DNA sequencer (Applied Biosystems, Foster City, Calif.). Sequences were submitted to the National Center for Biotechnology Information (Bethesda, Md.) for comparison with sequences in the GenBank database by use of the BLAST program. Multiple DNA alignments were performed by using the CLUSTALW program (52).

Colony hybridization. The amplicons were labeled with [α-^{32}P]CTP by using a DNA labeling beads kit (Amersham Pharmacia Biotech Inc.). Colony hybridizations were performed on membranes, as described previously (16).

Statistical methods. Comparisons of the associations between resistance genes, class 1 integrons, and virulence genes in animal and human ExPEC isolates were performed separately by using Pearson's chi-square exact test (SAS software, version 8.2; SAS Institute, Inc., Cary, N.C.), as described previously (33). Statistical significance was set at a P value of <0.05. An association between two genes can be either positive, indicating that the genes are found together, or negative, indicating that the genes are not found together.

RESULTS

Antimicrobial resistance phenotype characteristics. One hundred nine isolates (39 isolates of animal origin and 70 isolates of human origin) were selected and characterized for their phenotypes of antimicrobial resistance to 10 commonly used antimicrobials by a disk diffusion method. The percentage of *E. coli* isolates resistant to the different antimicrobials is presented in Table 2. Isolates showing a zone of inhibition at the limit (the zone between resistance and susceptibility) were seen only with cephalosporins (ceftiofur and cefotaxime) and aminoglycosides. These were classified as resistant in the tabulation of the prevalence data.

Similar patterns of resistance to the different antimicrobial agents between isolates of animal or human origin were observed for 6 of the 10 antimicrobials tested. More animal-derived ExPEC isolates were resistant to cephalosporins, neomycin, kanamycin, and tetracycline. Fifty-one percent of the animal isolates were resistant to at least four antimicrobials; and 43 percent had a profile of resistance to ampicillin, tetracycline, trimethoprim, and sulfonamides (data not shown). Only one isolate (from a pet) was multiresistant. The majority of isolates showed only one phenotype of resistance to either ampicillin or tetracycline.

Globally, the majority of human isolates were resistant to ampicillin, with none showing resistance to cephalosporins. Twenty-three percent of the human isolates were resistant to

TABLE 1. PCR primers used for antimicrobial resistance and virulence gene amplifications

Antimicrobial family, virulence genes, or genetic group	Genetic marker	PCR primer sequence (5′–3′)		Amplicon size (bp)	GenBank accession no.	Source of DNA
		Forward	Reverse			
Beta-lactams	bla_{TEM}	GAGTATTCAACATTTTCGT	ACCAATGCTTAATCAGTGA	857	AF309824	R. C. Levesque
	bla_{SHV}	TCGCCTGTGTATTATCTCCC	CGCAGATAAATCACCACAATG	768	AF148850	R. C. Levesque
	bla_{OXA-1}	GCAGCGCCAGTGCATCAAC	CCGCATCAAATGCCATAAGTG	198	AJ238349	Pasteur Institute
	bla_{OXA-7}	AGTTCTCTGCCGAAGCC	TCTCAACCCAACCAACCC	591	X75562	R. C. Levesque
	bla_{PSE-4}	CTGCTCGTATAGGTGTTTCC	TCGCATCATTTCGCTCTTC	705	J05162	R. C. Levesque
	$bla_{CTX-M-3}$	AATCACTGCGTCAGTTCAC	TTTATCCCCCACAACCCAG	701	X92506	A. Huletsky
Aminoglycosides	*ant(2″)-Ia* (*aadB*)[a]	TCCAGAACCTTGACCGAAC	GCAAGACCTCAACCTTTTCC	700	X04555	R. C. Levesque
	aac(3)-IIa (*aacC2*)	CGGAAGGCAATAACGGAG	TCGAACAGGTAGCACTGAG	740	X54723	D. Sandvang
	aac(3)-IV	GTGTGCTGCTGGTCCACAGC	AGTTGACCCAGGGCTGTCGC	627	X01385	J. Harel
	aph(3′)-Ia (*aphA1*)	ATGGGCTCGCGATAATGTC	CTCACCGAGGCAGTTCCAT	600	M18329	J. Harel
	aph(3′)-IIa (*aphA2*)	GAACAAGATGGATTGCACGC	GCTCTTCAGCAATATCACGG	680	V00618	J. Harel
Tetracycline	*tet*(A)	GTGAAACCCAACATACCCC	GAAGGCAAGCAGGATGTAG	888	X00006	J. Harel
	tet(B)	CCTTATCATGCCAGTCTTGC	ACTGCCGTTTTTTCGCC	774	J01830	J. Harel
	tet(C)	ACTTGGAGCCACTATCGAC	CTACAATCCATGCCAACCC	881	J01749	J. Harel
	tet(D)	TGGGCAGATGGTCAGATAAG	CAGCACACCCTGTAGTTTTC	827	X65876	S. B. Levy
	tet(E)	TTAATGGCAACAGCCAGC	TCCATACCCATCCATTCCAC	853	L06940	M. C. Roberts
	tet(Y)	ACCGCACTCATTGTTGTC	TTCCAAGCAGCAACACAC	823	AF070999	M. C. Roberts
Phenicols	*catI*	AGTTGCTCAATGTACCTATAACC	TTGTAATTCATTAAGCATTCTGCC	547	M62822	J. Harel
	catII	ACACTTTGCCCTTTATCGTC	TGAAAGCCATCACATACTGC	543	X53796	Pasteur Institute
	catIII	TTCGCCGTGAGCATTTTG	TCGGATGAGTATGGGCAAC	286	X07848	I. A. Murray
	floR	CGCCGTCATTCCTCACCTTC	GATCACGGGCCACGCTGTGTC	215	AF252855	D. G. White
Trimethoprim	*dhfrI*	AAGAATGGAGTTATCGGGAATG	GGGTAAAAACTGGCCTAAAATTG	391	X00926	J. Harel
	dhfrV	CTGCAAAAGCGAAAAACGG	AGCAATAGTTAATGTTTGAGCTAAAG	432	X12868	O. Sköld
	dhfrVII	GGTAATGGCCCTGATATCCC	TGTAGATTTGACCGCCACC	265	X58425	O. Sköld
	dhfrIX	TCTAAACATGATTGTCGCTGTC	TTGTTTTCAGTAATGGTCGGG	462	X57730	C. Wallen
	dhfrXIII	CAGGTGAGCAGAAGATTTTT	CCTCAAAGGTTTGATGTACC	294	Z50802	P. V. Adrian
Sulfonamides	*sulI*	TTCGGCATTCTGAATCTCAC	ATGATCTAACCCTCGGTCTC	822	X12869	R. C. Levesque
	sulII	CGGCATCGTCAACATAACC	GTGTGCGGATGAAGTCAG	722	M36657	J. Harel
Virulence genes	*afaC/afaD*	CAT CAA GCT GTT TGT TCG TCC GCC G	GCT GGG CAG CAA ACT GAT AAC TCT	793	X76688	J. Harel
	afaD8	GTT GAA CTG AGT CTT AAT ACC AGT	TGA GCA TTC TCC GCT AAC TGA TAA T	351	AF072900	J. Harel
	sfaDE	CTC CGG AGA ACT GGG TGC ATC TTA C	CGG AGG AGT AAT TAC AAA CCT GGC	408	X16664	J. Harel
	papC	GAC GGC TGT ACT GCA GGG TGT GGC G	ATA TCC TTT CTG CAG GGA TGC AAT A	318	X61239	J. Harel
	hlyA	TGT TGA AAG ATC AGT CCT CA	CTG CGT AGA TAT TGG CTG AG	500	M10133	J. Harel
	iucD	AAG TGT CGA TTT TAT TGG TGT A	CCA TCC GAT GTC AGT TTT CTG	778	M18968	J. Harel
Class 1 integron	*qacEΔ1-sulI*	ATCGCAATAGTTGGCGAAGT	GCAAGGCGGAAACCCGCGCC	797	X12870	J. Harel
	VR	GGCATCCAAGCAGCAAGC	AAGCAGACTTGACCTGAT	Variable	X12870	
Phylogenetic groups	*chuA*	GAC GAA CCA ACG GTC AGG AT	TGC CGC CAG TAC CAA AGA CA	279	AF280396	
	yjaA	TGA AGT GTC AGG AGA CGC TG	ATG GAG AAT GCG TTC CTC AAC	211	NC_000913	
	TspE4.C2	GAG TAA TGT CGG GGC ATT CA	CGC GCC AAC AAA GTA TTA CG	152	AF222188	

[a] Alternative nomenclatures are presented in parentheses.

TABLE 2. Number of antimicrobial-resistant animal and human isolates

Antimicrobial[b]	No. (%) of resistant isolates[a]	
	Animal isolates (n = 39)	Human isolates (n = 70)
AMP	28 (72)	54 (77)
CTX, XLN	7 (18)	0 (0)
GEN	6 (15)	9 (13)
NEO	7 (18)	4 (6)
KAN	8 (21)	7 (10)
TET	32 (82)	35 (50)
CHL	8 (21)	17 (24)
TMP	16 (41)	27 (38)
SUL	30 (77)	48 (68)

[a] As determined by the disk diffusion method.
[b] AMP, ampicillin; CTX, cefotaxime; XLN, ceftiofur; GEN, gentamicin; NEO, neomycin; KAN, kanamycin; CHL, chloramphenicol; TET, tetracycline; TMP, trimethoprim; SUL, sulfonamides.

the following four antimicrobials: ampicillin, tetracycline, trimethoprim, and sulfonamides (data not shown). Fifty percent of the isolates were resistant to more than three of the antimicrobials tested (data not shown).

Distribution of resistance genes. The choice of the resistance genes studied was based on their high frequency of occurrence in resistant *E. coli* strains (7, 8, 21, 33, 34, 36, 56). Accordingly, 28 genes encoding resistance to antimicrobials belonging to six antimicrobial families (beta-lactams, aminoglycosides, tetracycline, phenicols, trimethoprim, and sulfonamides) were chosen to determine their distributions in animal- and human-derived ExPEC isolates (Table 2).

(i) Beta-lactams. The DNA hybridization probes derived from the bla_{TEM} and bla_{SHV} sequences detect all known variants within the corresponding bla_{TEM} and bla_{SHV} gene families. The $bla_{\text{OXA-7}}$-specific probe detects bla_{OXA} variants such as $bla_{\text{OXA-10}}$ to $bla_{\text{OXA-14}}$, $bla_{\text{OXA-16}}$ to $bla_{\text{OXA-19}}$, $bla_{\text{OXA-28}}$, $bla_{\text{OXA-31}}$, and $bla_{\text{OXA-35}}$ (with which it has 80 to 96% similarities) and $bla_{\text{PSE-2}}$ (with which it has 96.3% similarity). To further discriminate variants among the $bla_{\text{OXA-7}}$ hybridization-positive isolates, a PCR amplification was undertaken with the $bla_{\text{OXA-7}}$ specific primers. All except four animal isolates and seven human isolates were positive for at least one of the genes for which tests were conducted (data not shown).

More human beta-lactam-resistant isolates (88%) than animal beta-lactam-resistant isolates (68%) were positive with the bla_{TEM}-specific probe (Table 3). Twelve percent of ampicillin-resistant human *E. coli* isolates also possessed a bla_{SHV} gene, whereas none of the animal isolates were bla_{SHV} positive. Three animal isolates but no human isolates possessed the $bla_{\text{OXA-1}}$ gene. No isolates hybridized to the probes specific for $bla_{\text{CTX-M-3}}$, $bla_{\text{PSE-4}}$, and $bla_{\text{OXA-7}}$. Eleven of the 82 isolates resistant to ampicillin (8 animal isolates and 3 human isolates) did not possess one of the beta-lactam genes for which tests were conducted. The gene bla_{TEM} was found in one cephalosporin-resistant animal ExPEC isolate (data not shown).

(ii) Aminoglycosides. Of the five aminoglycoside resistance genes for which tests were conducted, *ant(2″)-Ia*, *aac (3)-IV*, and *aph(3′)-IIa* were not found among any resistant isolate tested. The *aph(3′)-Ia* gene, which results in the kanamycin and neomycin resistance phenotype, was detected twice as frequently in animal isolates resistant to these two antimicrobials than in human isolates with the same resistance pattern (Table 3). The *aac(3)-IIa* resistance gene was found in both groups of isolates, but at a low frequency (in only 4 of the 15 gentamicin-resistant isolates).

TABLE 3. Hybridization of antimicrobial-resistant isolates with the resistance gene-specific probes tested and the associated resistance phenotype[a]

Antimicrobial agent	Genetic marker probe	No. (%[b]) of positive isolates by origin	
		Animal isolates	Human isolates
Ampicillin	bla_{TEM}	19 (68)	50 (88)
	bla_{SHV}	0 (0)	7 (12)
	$bla_{\text{OXA-1}}$	3 (11)	0 (0)
Gentamicin	*aac(3)-IIa*	1 (17)	3 (33)
Kanamycin	*aph(3′)-Ia*	6 (86)	2 (50)
Neomycin	*aph(3′)-Ia*	8 (100)	5 (71)
Tetracycline	*tet*(A)	17 (53)	15 (44)
	tet(B)	14 (44)	18 (53)
	tet(D)	0 (0)	2 (6)
Chloramphenicol	*catI*	1 (13)	14 (82)
	catIII	1 (13)	0 (0)
	floR	3 (38)	0 (0)
Trimethoprim	*dhfrI*	4 (25)	10 (37)
	dhfrV	6 (38)	1 (4)
	dhfrVII	0 (0)	9 (33)
	dhfrXIII	6 (38)	1 (4)
Sulfonamides	*sulI*	14 (50)	33 (69)
	sulII	18 (67)	29 (60)

[a] Negative results by hybridization with probes specific for $bla_{\text{OXA-7}}$, $bla_{\text{PSE-4}}$, $bla_{\text{CTX-M-3}}$, *aac(3)-IV*, *ant(2″)-Ia*, *tet(C)*, *tet(E)*, *tet(Y)*, *catII*, and *dhfrIX*.
[b] Percentage of probe-positive isolates among the resistant isolates.

(iii) Tetracyclines. Of the six tetracycline resistance genes for which tests were conducted, only *tet*(A), *tet*(B), and *tet*(D) were found among the isolates (Table 3). Since *tet*(A) and *tet*(C) are closely related (more the 80% sequence identity), PCR with *tet* gene-specific primers further confirmed the absence of the *tet*(C) gene, with the *tet*(A) gene being found in 75% of the isolates. The *tet*(D) gene was found only in the human isolates. Three animal isolates and one human isolate resistant to tetracycline were *tet* negative by hybridization.

(iv) Phenicols. Of the four chloramphenicol resistance genes for which tests were conducted, only *catI*, *catIII*, and *floR* were detected. Among the eight chloramphenicol-resistant isolates from animals, the most frequent phenicol resistance gene was *floR* (38%) (Table 3). The *catI* gene was found in one isolate, while the *catIII* gene was found in a separate isolate. Among the 17 human isolates, the most frequent chloramphenicol resistance gene was *catI* (82%). Two animal isolates and three human isolates resistant to chloramphenicol were negative with the chloramphenicol resistance gene-specific probes. One of 55 chloramphenicol-susceptible human isolates was *catI* positive.

(v) Trimethoprim. In this study, the trimethoprim resistance phenotype was associated with four genes: *dhfrI*, *dhfrV*, *dhfr-*

TABLE 4. Distribution of class 1 integron among the animal and human ExPEC strains

Isolate source	Total no. of strains	No. (%) of class 1 integron-positive strains	No. (%) of class 1 integron-positive strains by the following length of VR:				
			0.5 kb	1.0 kb	1.6 kb	1.7 kb	1.8 kb
Animal	39	13 (33)	1 (7)	8 (62)	3 (23)	1 (8)	0 (0)
Human	70	22 (31)	1 (4)	13 (59)	6 (28)	0 (0)	2 (9)
Total	109	35 (32)	2 (6)	21 (60)	9 (26)	1 (3)	2 (5)

VII, and *dhfrXIII* (Table 3). Whereas the *dhfrI* gene was common in the different groups of isolates, *dhfrVII* was found exclusively among the human isolates and *dhfrV* and *dhfrXIII* were primarily found among the animal isolates. Among the resistant isolates from animals, three were negative for the genes for which tests were conducted and one trimethoprim-susceptible isolate was positive for *dhfrXII*. Among the 27 resistant isolates from humans, 7 were negative for all trimethoprim resistance gene-specific probes.

(vi) Sulfonamides. The *sulI* and *sulII* genes were largely present among the animal and human isolates. Thirty-seven percent of the sulfonamide-resistant animal isolates had both *sulI* and *sulII*. Four of the 30 resistant animal isolates were *sul* negative, whereas 2 of the 22 susceptible human isolates were *sulI* positive.

Identification and characterization of integrons. The class 1 integron is characterized by the *qacEΔ1* and *sulI* genes at its 3′ conserved segment. These genes impart resistance to disinfectants and sulfonamides, respectively (38). PCR analyses of these genes were used to identify isolates containing the 3′ conserved region. Of the 109 isolates, 57 (52%) amplified a fragment from within this conserved region, as determined by PCR (Table 4). A similar percentage of class 1 integron-positive isolates was observed among the animal and the human groups of isolates. Two of the class 1 integron-positive animal isolates were *sulI* negative.

The VR of class 1 integron-positive isolates was characterized with primers Fint1 and Rint1. Among the 57 class 1 integron-positive isolates, five distinct amplicons of 0.5, 1.0, 1.6, 1.7, and 1.8 kb were obtained. Two representatives with each VR length except 1.7 kb were sequenced. Only a single isolate had a VR length of 1.7 kb, and it was also sequenced. Both VRs of the same length contained the same gene cassettes with more than 95% similarity. The most frequently amplified VR fragments (i.e., 1.0 kb) in animal and human isolates (58 and 59%, respectively) contained the *ant(3″)-Ia* (*aadA1*) gene cassette (GenBank accession no. X12870), which encodes streptomycin and spectinomycin resistance. In 21% of the animal isolates and 28% of the human isolates the VR was 1.6 kb and contained two gene cassettes, *dhfrIb* (GenBank accession no. AF393510), which encodes trimethoprim resistance, and *ant(3″)-If* (*aadA6*) (GenBank accession no AF140629), which encodes resistance to streptomycin and spectinomycin. Only one animal isolate had a VR length of 1.7 kb. This VR possessed two gene cassettes: *dhfrXVII*, which confers resistance to trimethoprim, and *ant(3″)-Ie* (*aadA5*), which confers resistance to streptomycin and spectinomycin (GenBank accession no. AF169041). Finally, the 1.8-kb VR fragments possessed two gene cassettes, *dhfrXII* and *ant(3″)-If* (GenBank accession nos. Z21672 and AF284063, respectively), which confer resistance to trimethoprim and to streptomycin and spectinomycin, respectively. This VR also possessed one open reading frame, named *orfF*.

Distribution of virulence genes. Adhesin-encoding operons, toxins, and iron acquisition systems have been shown to be prevalent in ExPEC strains. A subset of these known virulence factors representing various adhesins (*afaC*/*afaD*, *afaD8*, *sfaDE*, and *papC*), a hemolytic toxin (*hlyA*), and an iron uptake system (*iucD*) were selected; and the distributions of these genes are summarized in Table 5. More human isolates than animal isolates were positive for these genes. *iucD* was the virulence gene detected the most frequently (77%) among the animal isolates. *sfaDE*, *papC*, *hlyA*, and *iucD* were present in six of eight pet isolates (data not shown). Only 8% of animal isolates were negative for all six virulence genes, and 23% of the animal isolates had more than two of them.

Association between resistance genes. Statistically significant ($P < 0.05$) associations between the occurrence of individual resistance and virulence genes among the whole collection of animal and human ExPEC isolates were detected (Table 6 and 7). Within both groups of isolates, animal and human, the association of *sulI* with the class 1 integron was clear. Several different gene associations were evident when the ExPEC isolates between the animal and human groups were compared. For example, the positive association of bla_{TEM} and *dhfrV* genes and the negative association of *tet*(A) and *tet*(B) genes were observed only among the animal isolates. Moreover, some associations were observed only among human isolates, such as the positive association of bla_{TEM} with *sulII* and the positive association of *sulI* with *tet*(A). Additionally, associations of *hlyA* with *dhfrI* and of *iucD* with *sulII* were found only among the isolates of human origin (data not shown).

Phylogenetic grouping of results. PCR-based phylogenetic analyses (9) have suggested that *E. coli* can be divided into four main phylogenetic groups (groups A, B1, B2, and D). In general, highly virulent ExPEC strains belong to groups B2 and D, whereas commensal strains and some strains with lesser viru-

TABLE 5. Hybridization of animal and human ExPEC isolates with virulence probes

Virulence marker probe	No. (%) of positive isolates by isolate origin	
	Animal	Human
afaC/*afaD*	1 (2)	7 (10)
afaD8	5 (13)	1 (1)
sfaDE	10 (26)	27 (38)
papC	19 (49)	37 (53)
hlyA	9 (23)	34 (48)
iucD	30 (77)	34 (48)

TABLE 6. Association between antimicrobial resistance genes and class 1 integron among ExPEC strains from animals

Genetic marker	Association for the following antimicrobial resistance genes, class 1 integron, and virulence genes[a]:									
	bla$_{TEM}$	*floR*	*aph(3′)-la*	*dhfrI*	*dhfrV*	*dhfrXIII*	*sulI*	*sulII*	*tet*(A)	*tet*(B)
floR	−									
aph(3′)-Ia	−	+								
dhfrI	−	−	−							
dhfrV	++	−	−	−						
dhfrXIII	−	−	−	−	−					
sulI	−	−	−	−	−	−				
sulII	−	−	++	−	+	−	−			
tet(A)	+	−	−	−	−	−	−	−		
tet(B)	−	−	−	−	−	−	−	−	(+)	
int1	−	−	−	−	−	−	+++	−	+	−

[a] Only antimicrobial resistance genes that exhibited an association with another gene at the $P < 0.05$ level are shown. The levels of significance of the association (as assessed by the chi-square exact test) were as follows: −, $P > 0.05$; +, $0.05 \geq P \geq 0.01$; ++, $0.01 \geq P \geq 0.001$; +++, $0.001 \geq P$. Parentheses indicate negative associations.

lence belong to groups A and B1. Our phylogenetic analysis showed that 46% of the isolates from animal tissue belonged to phylogenetic group A, 20% belonged to D, 20% belonged to B2, and 15% belonged to B1. Among the animal isolates, isolates of phylogenetic groups A and B1 were mostly porcine isolates (56 and 83%, respectively) (data not shown). Most pet isolates (88%) belonged to phylogenetic group B2, whereas 63% of avian isolates were in group D.

Approximately half of the human isolates (54%) belonged to phylogenetic group B2, 23% belonged to group D, and 19% belonged to group A; but only 4% belonged to group B1. Human isolates mostly belonged to either group B2 (45%) or group D (45%); small proportions belonged to groups A and B1 (7 and 3%, respectively). Most animal isolates (71%) belonging to group B2 contained the virulence genes *sfaDE*, *papC*, and *hlyA*; among the human isolates the proportion was only 39%. Human isolates belonging to group A contained fewer virulence genes than the other human isolates.

DISCUSSION

E. coli causes diverse extraintestinal infections in animals and humans and is the most common cause of urinary tract infections, community-acquired bacteremia, and sepsis (41). Treatment for ExPEC infections commonly includes broad-spectrum antimicrobials (4). In the present study, animal and human ExPEC isolates were analyzed for the distributions of related acquired antimicrobial resistance genes, their integron profiles, and their phylogenetic groups.

Our study demonstrates that similar antibiotic resistance patterns could be observed in animal and human isolates. Most ExPEC isolates were resistant to ampicillin, tetracycline, and sulfonamides. It is noteworthy that although ampicillin and sulfonamides are old antimicrobials, they are still widely used. When the fact that tetracycline is used less in humans than in animals is considered, the relatively high rates of tetracycline resistance among human isolates was unexpected (Table 2). A similar observation can be made for kanamycin resistance. Although this antimicrobial agent is more widely used for human therapy than for animal therapy, more animal isolates exhibited resistance. The latter observation can be explained by the fact that the aminoglycoside resistance gene *aph(3′)-Ia*, which was found in the majority of animal isolates, can hydrolyze both kanamycin and neomycin. Therefore, the selective pressure exerted by the use of neomycin in animals, particularly pigs, would have simultaneously selected for neomycin- and kanamycin-resistant strains.

Many animal and human ExPEC isolates exhibited multiresistance profiles. This can be explained by the coselection of resistance genes by the use of a different antimicrobial (2). For example, it was shown that the use of injectable oxytetracycline

TABLE 7. Association between antimicrobial resistance genes and class 1 integron among the ExPEC strains isolated from humans

Genetic marker	Association for the following antimicrobial resistance genes, class 1 integron, and virulence genes[a]:										
	bla$_{TEM}$	*bla*$_{SHV}$	*catI*	*aac(3)-II*	*dhfrI*	*dhfrVII*	*sulI*	*sulII*	*tet*(A)	*tet*(B)	*tet*(D)
bla$_{SHV}$	−										
catI	−	−									
aac(3)-Ila	−	−	−								
dhfrI	−	−	−	−							
dhfrVII	−	−	−	++	−						
sulI	−	−	−	−	−	+					
sulII	+++	−	−	−	−	−	−				
tet(A)	−	−	−	−	−	−	++	−			
tet(B)	−	−	++	−	−	−	−	++	−		
tet(D)	−	++	−	−	+	−	−		−	−	
int1	−	−	−	−	−	+	+++	−	+	−	−

[a] Only antimicrobial resistance genes that exhibited an association with another gene at the $P < 0.05$ level are shown. The levels of significance of the association (as assessed by the chi-square exact test) were as follows: −, $P > 0.05$; +, $0.05 \geq P \geq 0.01$; ++, $0.01 \geq P \geq 0.001$; +++, $0.001 \geq P$.

in cattle receiving chlortetracycline in their feed was associated with increases in the rates of chloramphenicol and sulfisoxazole resistance (37).

Although ExPEC isolates from animals and humans could not be discriminated on the basis of their phenotypic patterns of antimicrobial resistance, this did not extend to their genotypic resistance patterns. For example, among the ampicillin-resistant strains, the $bla_{\text{OXA-1}}$ gene was found only in animal isolates, whereas the bla_{SHV}-type determinant was found only in human isolates. When the fact that cephalosporins are used more in human medicine than in animal medicine is considered, it was interesting that resistance to ceftiofur, a cephalosporin used in food-producing animals in Canada and the United States, was found only among animal isolates. The resistance of animal isolates to ceftiofur was shown to be associated with acquired beta-lactam resistance genes other than those for which tests were conducted in our study, such as the cephamycinase bla_{CMY} genes that were found in *E. coli* isolates showing a ceftiofur-ceftriazone resistance phenotype or a mutation in the chromosomal *ampC* gene (58). Aminoglycoside resistance gene *aph(3′)-Ia* was present in kanamycin-resistant human and animal ExPEC isolates, but it appeared that the kanamycin resistance in the human isolates negative for the resistance genes for which tests were conducted could be due to other resistance genes, such as *aac(3)-III* or *aph(3′)-VI*, as described in other studies (47). Despite the ban on the use of chloramphenicol in food animals in Canada since 1980 (15), chloramphenicol resistance was observed in animal isolates. This was also reported in various animal species by others (30, 50). In those studies, resistance to chloramphenicol was closely associated with the presence of the *catI* gene. In another study by Bischoff et al. (5), only 4 of 48 chloramphenicol-resistant isolates harbored a *catII* gene; *cmlA*, a relatively unknown gene, was responsible for the resistance of the other isolates. The *floR* gene is responsible for cross-resistance between chloramphenicol and florfenicol; the latter is a veterinary molecule not used in human medicine (46). Other studies have reported on the presence of *floR* in a large number of *E. coli* isolates recovered from chickens and cattle (10, 28, 56). The distribution of chloramphenicol resistance genes in our study was different from those published elsewhere. Chloramphenicol resistance among our human isolates was primarily due to the *catI* gene, while among the animal isolates it was due to *floR*. Finally, the *dhfrVII* gene, which was the trimethoprim resistance gene most frequently found among human isolates, was absent from the animal isolates; on the other hand, the *dhfrV* and *dhfrXIII* genes, the most frequent trimethoprim resistance genes detected in the animal isolates, were found in only one human isolate.

Nevertheless, some resistance genes, such as the bla_{TEM}-type beta-lactamase gene and *sulI* and *sulII*, were equally represented in animal and human ExPEC isolates. In our study and in other studies (6, 14), the most frequent type of beta-lactam resistance in animal and human ExPEC isolates was due to a bla_{TEM}-type beta-lactamase. These studies show that mutations in the *ampC* operon are another important cause of ampicillin resistance in *E. coli*.

Resistance genes can be associated with mobile DNA plasmids, transposons, and integrons, which are known to facilitate their distribution (22, 49). More than half of the isolates tested (52%) possessed a class 1 integron. Four different integron classes have been characterized, with class 1 being the most common among clinical isolates (27, 42). Class 1 integrons possess two conserved segments separated by a VR which includes integrated antimicrobial resistance genes or gene cassettes with unknown functions (39). The most frequently amplified VR length among the integron-positive avian pathogenic *E. coli* isolates was 1.0 kb. The 1.0-kb VR was frequently detected in integron-positive ExPEC isolates. This is consistent with the findings of a study by Bass et al. (3), in which the most frequently amplified VR length among the integron-positive avian pathogenic *E. coli* isolates was 1.0 kb. Although multiple gene cassette insertions and more than 40 distinct gene cassettes have been found to be linked to integrons (39), only five different gene cassette sizes were detected among *E. coli* isolates in this and other studies (40, 45). The presence of integrons emphasizes their potential to contribute to the efficient spread of antibiotic resistance.

Despite this gene diversity, however, some interesting gene associations were observed. Similar to other studies, the *sulI* gene was associated with class 1 integrons (32, 38). The bla_{TEM} genes detected in animal isolates were associated with *dhfrV*, whereas in human ExPEC isolates, they were associated with the *sulII* gene. Gene association analysis is useful for obtaining an understanding of why certain genes are present in an organism after coselection or transmission. For example, because the association of bla_{TEM} genes with either *dhfrV* or *sulII* was dependent on the origin of the isolate, this suggests that animal and human isolates acquired sets of different genes.

Although the virulence gene characterization was not exhaustive, most *E. coli* isolates examined in this study possessed some of the virulence factors commonly found in extraintestinal strains (48). The representation of virulence markers in ExPEC isolates from animals was different from that in ExPEC isolates from humans. The human ExPEC isolates shared many traits with the pet ExPEC isolates tested. This commonality between pet and human ExPEC isolates was also demonstrated by Johnson et al. (24, 26).

The human and animal (but not pet) ExPEC isolates were phylogenetically different. In agreement with other studies, our human isolates primarily belonged to one of two virulence groups (group B2 or D) (9, 26). In contrast, the majority of animal isolates were determined to be in groups A and B1. Interestingly, the majority of the pet isolates belonged to phylogenetic virulence group B2.

The modest number of isolates used in this study precludes us from making highly specific conclusions from the comparison of animal and human EXPEC isolates; however, some general observations from our data can be made. For example, ExPEC isolates both within and between the animal and human groups can possess relatively distinct profiles. This suggests that the number and diversity of genes driving phenotypic resistance are dynamic and have evolved through selection by antimicrobial use.

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