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Characterization of Multidrug-Resistant *Escherichia coli* Isolates Associated with Nosocomial Infections in Dogs

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Multidrug-resistant opportunistic pathogens have become endemic to the veterinary hospital environment. *Escherichia coli* isolates resistant to 12 antibiotics were isolated from two dogs that were housed in the intensive care unit at The University of Georgia Veterinary Teaching Hospital within 48 h of each other. Review of 21 retrospective and prospective hospital-acquired *E. coli* infections revealed that the isolates had similar antibiotic resistance profiles, characterized by resistance to most cephalosporins, β-lactams, and the β-lactamase inhibitor clavulanic acid as well as resistance to tetracycline, spectinomycin, sulfonamides, chloramphenicol, and gentamicin. *E. coli* isolates with similar resistance profiles were also isolated from the environment in the intensive care unit and surgery wards. Multiple *E. coli* genetic types were endemic to the hospital environment, with the pulsed-field gel electrophoresis fingerprint identified among *E. coli* isolates from diseased animals and the hospital environment matching. The extended-spectrum cephalosporin resistance in these nosocomial *E. coli* isolates was attributed to the cephamycinase-encoding gene, bla_{CMY2}. Chloramphenicol resistance was due in part to the dissemination of the florfenicol resistance gene, *flo*, among these isolates. Resistance encoded by both genes was self-transmissible. Although bla_{CMY2} and *flo* were common to the polyclonal, nosocomial *E. coli* isolates, there was considerable diversity in the genetic compositions of class 1 integrons, especially among isolates belonging to the same genetic type. Two or more integrons were generally present in these isolates. The gene cassettes present within each integron ranged in size from 0.6 to 2.4 kb, although a 1.7-kb gene cassette was the most prevalent. The 1.7-kb gene cassette contained spectinomycin resistance gene *aadA5* and trimethoprim resistance gene *dfrA17*.

One of the great challenges of practicing veterinary medicine and surgery in tertiary-care facilities in the postantibiotic era is multidrug-resistant nosocomial infections. Control of *Escherichia coli* infections in veterinary medicine has become especially problematic due to the emergence of multiple-antibiotic-resistant *E. coli* in food animals and companion animals (3, 5, 43, 44, 61, 64, 67).

Although there is considerable information concerning the epidemiology and ecology of hospital-acquired infections in human medicine (17), little is known about nosocomial illnesses in veterinary hospitals or clinics (15, 18, 19, 30, 31, 58, 59). Most of what is known about hospital-acquired infections in veterinary medicine is primarily limited to a few retrospective studies (19, 30, 31, 59).

During the past 2 years, several dogs developed postoperative surgical wound or lower urinary tract infections during their stay in The University of Georgia Small Animal Veterinary Teaching Hospital (UGA-SVTH). *E. coli* was often isolated from the site of infection. This organism is an important pathogen of companion animals (4, 46). These isolates were unusual in that they all had the same antibiotic susceptibility pattern. A dog with extensive bite wounds died suddenly from suspected septic shock. He was housed in the intensive care unit (ICU) that nine days earlier had contained a dog with a hospital-acquired *E. coli* infection of a surgical incision. In addition to an *Enterococcus* sp., *E. coli* was isolated from the bite wounds of the dog that died, and this isolate also had the same antibiotic resistance pattern as the *E. coli* isolate from the dog previously housed in the same unit. Was a single *E. coli* clone responsible for these infections, or was the dissemination of a common resistance plasmid in the *E. coli* population making the isolates more likely to cause disease due to their resistance to the antibiotics commonly prescribed in the teaching hospital? In an attempt to address this question, we typed *E. coli* isolates by ERIC PCR, pulsed-field gel electrophoresis (PFGE), and random amplified polymorphic DNA (RAPD) analysis. In this report, we identify several *E. coli* clones associated with nosocomial *E. coli* infections at our institution.

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MATERIALS AND METHODS

Bacterial strains. The present study included 21 bacterial isolates obtained from urine and wounds of dogs that were patients at UGA-SVTH, 1 isolate from a dog that had never been a patient at UGA-SVTH, and 12 environmental samples from the ICU and surgery wards of UGA-SVTH. Bacteriological examination of the clinical samples was carried out as follows; swabs were plated on blood agar (Difco, Sparks, Md.) and MacConkey agar (Difco) at 37°C overnight. The environmental samples were obtained with three sterile sponges (3 × 1 cm) soaked in sterile saline solution; after sampling the sponges were placed in EC broth (Difco), incubated at 45°C overnight, and then plated onto MacConkey agar plates containing 32 μg of chloramphenicol per ml. Chloramphenicol was chosen since resistance to this drug is rather unusual, especially in light of its limited use in veterinary and human medicine. *E. coli* isolates were identified by

standard procedures (9). All isolates were diluted in 15% glycerol and peptone water and stored at −70°C.

Antimicrobial susceptibility determination. Antimicrobial MICs for *E. coli* isolates were determined with the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) and were interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines for broth microdilution methods (39–41). Susceptibility testing with the Sensititre system was performed according to the instructions of the manufacturer. The following antimicrobials were assayed: amikacin, amoxicillin-clavulanic acid, ampicillin, cefazolin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, clindamycin, enrofloxacin, orbifloxacin, gentamicin, imipenem, sulfadimethoxine, tetracycline, ticarcillin, and trimethoprim-sulfamethoxazole. For each *E. coli* isolate, the disk diffusion method with florfenicol disks was performed by the method described by the NCCLS (39–41).

Characterization of class 1 integrons and antibiotic resistance genes associated with canine *E. coli* isolates. Multidrug-resistant *E. coli* isolates were screened by PCR for the florfenicol resistance gene (*flo*) (27), the extended-spectrum cephalosporinase gene (bla_{CMY2}) (67), and the class 1 integrase gene (*intI1*) (3). We characterized the gene cassette associated with the *aatI* integration site of class 1 integrons by PCR and sequenced the amplicon using primers specific for the conserved 5′ and 3′ sequences flanking the integration site (32). The transmissibility of extended-spectrum cephalosporin resistance was determined by bacterial conjugation with rifampin-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium as a recipient in filter matings (48). This recipient strain is sensitive to all antibiotics except rifampin. Transconjugants were selected by plating the isolates used in the filter matings on brilliant green agar with rifampin and ampicillin. The antibiotic susceptibilities of the *Salmonella* transconjugants were determined as described in the preceding section.

Plasmid DNA was isolated from *E. coli* by alkaline lysis with a plasmid DNA extraction kit (QIAprep Spin Miniprep Kit; Qiagen, Valencia, Calif.). *E. coli* V517 served as a positive control for plasmid extraction. This *E. coli* isolate contains plasmids of high, intermediate, and low molecular weight (MW) (33). Plasmid DNA (10 μl) was separated on a 0.8% agarose gel containing 1× TAE (Tris-acetate-EDTA) at 100 V for 1 h and was visualized by staining with ethidium bromide (0.2 μg/ml). A supercoiled DNA ladder (Promega, Madison, Wis.) was used as an MW standard for determination of the MW of the plasmid(s). Plasmid or megabase DNA (PFGE) from the gels was blotted onto nylon membranes with a Bio-Rad (Hercules, Calif.) Vacuum Blotter (61). DNA-DNA hybridizations were done by the procedure of Sambrook et al. (49), with a temperature of 68°C used for hybridizations and washes. The bla_{CMY2}-specific DNA probe was labeled with digoxigenin-labeled nucleotides by PCR (67), and membrane-bound probe was detected by the procedure of Bass et al. (3).

Molecular typing of clinical *E. coli* isolates. *E. coli* isolates were typed by RAPD analysis with primer 1290 (38) and by ERIC PCR (60). Whole-cell template was prepared for RAPD analysis and ERIC PCR by the protocol of Hilton et al. (23). RAPD analysis and ERIC PCR were performed with an Idaho Technologies (Idaho Falls, Idaho) Rapidcycler hot-air thermocycler (65). The conditions for RAPD analyses were those described for the procedure of Maurer et al. (38). The program parameters for ERIC PCR with the hot-air thermocycler were as follows: (i) 94°C for 15 s; (ii) 94°C for 0 s, 52°C for 0 s, and 72°C for 15 s (slope = 2.0) for 30 cycles; and (iii) final extension at 72°C for 4 min. DNA was separated on a 1.5% agarose gel containing 1× TAE at 100 V for 1 h and was visualized by ethidium bromide staining (0.2 μg/ml) (49). A 100-bp ladder (GIBCO/BRL, Gaithersburg, Md.) was used as an MW standard for determination of the MWs of the PCR products.

For the typing of the bacteria by PFGE, agarose-embedded bacterial genomic DNA was digested with 10 U of restriction enzyme *Xba*I or *Bln*I overnight at 37°C, and DNA fragments were separated by PFGE (2) in a 1% PFGE agarose gel (Bio-Rad) with a CHEF DR-II electrophoretic apparatus (Bio-Rad). Electrophoresis was for 25 h with a voltage of 200 V and a linearly ramped pulse time of 2 to 40 s (2). *Saccharomyces cerevisiae* chromosomes (Boehringer Mannheim, Indianapolis, Ind.) served as MW markers for PFGE. The restriction enzyme *Xba*I has proved useful in the typing of *E. coli* (2) and other gram-negative bacteria (1, 45) by PFGE. For isolates with indistinguishable *Xba*I PFGE DNA patterns, other molecular typing methods were introduced to discern more subtle genetic differences among isolates (45): RAPD analysis (23, 25, 26), a second PFGE with *Bln*I as the restriction enzyme (57), or ERIC PCR (12).

Nucleotide sequence accession number. The nucleotide sequences of PCR amplicons from canine *E. coli* isolates have been submitted to GenBank and given accession no. AF475279, AF475280, and AF475281.

RESULTS AND DISCUSSION

A 7-year-old male boxer was admitted to UGA-SVTH on 12 December 1999 for treatment of severe bite wounds to his neck, thorax, and abdomen inflicted during a dogfight. Five closed-suction surgical drains were implanted into the bite wounds after debridement and primary closure. Five days after admission to the hospital, the wounds were debrided a second time. Significant tissue necrosis had occurred. The dog died suddenly postoperatively from suspected septic shock. In addition to an *Enterococcus* sp., *E. coli* (clinical isolate 25055) was isolated from samples taken from the bite wounds during the second surgery (Tables 1 and 2). The organism was resistant to most cephalosporins, β-lactams and β-lactamase inhibitors, chloramphenicol, spectinomycin, tetracycline, gentamicin, and enrofloxacin, as determined by broth microdilution methods. The only drugs to which the isolate was susceptible were amikacin and imipenem. A similar multidrug-resistant *E. coli* isolate had been isolated from a dog that had developed a postoperative surgical wound infection 9 days earlier. Both animals had been housed in the ICU at UGA-SVTH within 48 h of each other.

A retrospective and prospective study of clinical submissions to The Athens Diagnostic Laboratory from UGA-SVTH identified 18 *E. coli* isolates from dogs with the same patterns of resistance to 12 antibiotics reported earlier by broth microdilution methods. In several of these cases, *E. coli* infection was associated with placement of a surgical wound drain or an indwelling urinary catheter. A thorough environmental sampling of the surgery rooms, surgery wards, and ICU for microorganisms was done. *E. coli* organisms were isolated from 42 of 55 locations surveyed. Twelve of these *E. coli* organisms exhibited the multidrug resistance profiles observed for clinical isolates obtained from materials submitted from the veterinary hospital. Are the multidrug-resistant *E. coli* infections observed in dogs admitted to UGA-SVTH attributed to a clone that is endemic to the hospital?

Genetic diversity in *E. coli* isolates associated with nosocomial infections in dogs. Nine distinct DNA patterns were observed among the 34 *E. coli* isolates typed by PFGE with *Xba*I (Table 1; Fig. 1A). Seven *E. coli* isolates could not be typed by PFGE due to problems with degradation of the genomic DNA during preparation of the agarose plugs. Other laboratories have noted similar problems with typing by PFGE (22, 35, 36). Isolates with seven or more different DNA fragments were assigned to a specific *Xba*I PFGE genetic type (types H to P), as recommended by Tenover et al. (56). Forty percent (7 of 18) of the clinical *E. coli* isolates were *Xba*I PFGE genetic type H. Half (6 of 12) of the environmental isolates were also *Xba*I PFGE DNA type H. Additional differences of two to three DNA fragments among the isolates of PFGE type H allowed further differentiation of these related *E. coli* isolates into subgroups H1.1 to H1.3. Using PFGE with a second restriction enzyme, *Bln*I, we were unable to distinguish among *E. coli* isolates that had earlier produced similar or identical patterns by PFGE with *Xba*I (Table 1; Fig. 1B). Seven distinct PFGE patterns (DNA patterns A to G) were observed with restriction enzyme *Bln*I. With this second restriction enzyme, isolates of *Xba*I PFGE type H could be further discriminated into three genetic types. By ERIC PCR and RAPD analysis with primer

TABLE 1. Bacterial strains

Isolate[a]	Date (mo/day/yr)	Source[b]	Sex[c]	Age (yr)	Site[d]	Genetic type[e]			
						PFGE		ERIC PCR	RAPD analysis
						*Bln*I	*Xba*I		
39737	4/10/00	Springer spaniel	F	12	Wound[s]	**A1**	**H1.1**	II	Q
22-2	8/22/00	Storage cart 2	NA	NA	NA	**A1**	**H1.1**	JJ	R
29	8/22/00	Cage no. 3	NA	NA	NA	**A1**	**H1.1**	II	R
34	8/22/00	Drain 3 runs	NA	NA	NA	**A1**	**H1.1**	II	R
53	8/22/00	ICU floor	NA	NA	NA	**A1**	**H1.1**	KK	R
56	8/22/00	ICU floor	NA	NA	NA	**A1**	**H1.1**	KK	R
22949	1/3/00	Labrador	M	14	Wound[s]	**A2.2**	**H1.2**	II	S
4479	8/3/00	Labrador	F	15	Bile	**A2.2**	**H1.2**	LL	T
38	8/22/00	Small hydrobath	NA	NA	NA	**A2.1**	**H1.3**	MM	U
4517A	8/6/98	Great Pyrenees	M	3	Wound[s]	**A2.3**	**I**	NN	V
4517B	8/6/98	Great Pyrenees	M	3	Wound[s]	**A2.3**	**I**	NN	V
22255	12/17/98	Mixed	M	?	Wound[s]	**A2.3**	**I**	OO	V
A1-72	7/5/00	Cocker spaniel	F	9	CSF	**B1.2**	**J**	PP	S
1745	7/19/00	Great Dane	M	10	Urine	**B1.2**	**J**	QQ	W
32	8/22/00	Drain 1 runs	NA	NA	NA	**B1.2**	**J**	RR	R
27315	1/31/00	Corgi	M	8	Urine	B1.1	K	KK	X
40362	4/12/99	Mixed	M	6	Urine[c]	B1.1	L	NN	Y
42614B	4/21/00	Boxer	F	4	Urine	D	M	SS	Z
1888	7/17/00	Samoyed	M	2	Wound[s]	**G**	**N**	JJ	AA
18813	10/31/00	Spitz	F	11	A. sac	**G**	**N**	JJ	AA
22559	12/22/99	Boxer	M	7	Wound[s]	**E**	**H1.1**	II	U
25055a	1/18/00	Labrador	M	14	Wound[s]	**E**	**H1.1**	II	U
25055b	1/18/00	Labrador	M	14	Wound[s]	**E**	**H1.1**	II	U
29610[f]	2/14/00	Mixed	F	11	Urine	**C**	**O**	OO	BB
12279	9/30/99	Mixed	F	12	Urine	**C**	**O**	TT	CC
21	8/22/00	Storage cart 1	NA	NA	NA	F	P	JJ	R
42614A	4/21/00	Boxer	F	4	Urine	B1.1	H1.2	II	Q
18397[f,g]	11/16/98	Saluki	F	9	RT	—[h]	—	UU	DD
23120[f,g]	12/12/00	Mixed	F	1	Urine	—	—	VV	EE
21411[f,g]	11/16/00	Pomeranian	F	8	Urine	—	—	VV	FF
37	8/22/00	Big hydrobath	NA	NA	NA	—	—	VV	GG
55-1	8/22/00	ICU floor	NA	NA	NA	—	—	WW	HH
55-2	8/22/00	ICU floor	NA	NA	NA	—	—	WW	HH
55-3	8/22/00	ICU floor	NA	NA	NA	—	—	LL	HH

[a] *E. coli* organisms were isolated from dogs that developed infections >48 h after admission to UGA-SVTH.
[b] All animals but the one from which isolate 29610 was obtained had been patients at UGA-SVTH.
[c] F, female; M, male; NA, not available.
[d] Site of infection was usually associated with an indwelling medical device. [s], surgical drain; [c], urinary catheter; CSF, cerebrospinal fluid; RT, reproductive tract; A. sac, anal sac.
[e] We chose PFGE, RAPD analysis, and ERIC PCR for typing of the *E. coli* isolates. For PFGE, the restriction enzymes *Xba*I and *Bln*I were selected for use for the typing of the bacterial isolates. RAPD analysis involved the use of a 10-mer oligonucleotide, primer 1290, as the typing primer. An alphabetical designation was assigned to each distinct DNA fingerprint. Letters highlighted in boldface identify isolates with the same pattern by PFGE with either the *Xba*I or *Bln*I restriction enzyme. Since no interpretative criteria have been developed for analysis of either RAPD or ERIC PCR patterns, DNA patterns with one or more band differences were given a different alphabetical designation.
[f] *E. coli* organisms isolated from samples submitted from regional veterinary practitioners to The Athens Diagnostic Laboratory for culture.
[g] Isolates obtained through submission by a clinician outside UGA-SVTH.
[h] —, isolates were recalcitrant to typing by PFGE.

1290, we could further discriminate several of these isolates, although in most cases we were able to identify only minor band differences in the patterns generated by either of the methods (Fig. 2 and 3). We identified the same *E. coli* genetic type among organisms isolated either from different animals admitted to the veterinary hospital (isolates 4517 and 22255, isolates 1888 and 18813, and isolates 22559 and 25055) or from a hospitalized animal and the veterinary clinic environment (isolates 29737, 29, and 34 and isolates 1745 and 32, respectively). Instead of identifying one multidrug-resistant clone that was endemic to the hospital environment, as has been reported for vancomycin-resistant enterococci (29), we identified several multidrug-resistant clones in the hospital environment. Others have made similar observations for gram-negative organisms that cause nosocomial infections in humans (21, 51, 53). Are we dealing with dissemination of a common plasmid in the hospital environment responsible for resistance to 11 antibiotics, including most of the broad-spectrum cephalosporins?

Antimicrobial susceptibility and drug resistance genes in nosocomial *E. coli* isolates associated with infections in dogs. Nosocomial *E. coli* isolates were resistant to most cephalosporins including ceftiofur, cephalothin, and ceftriaxone, as well as to the β-lactams ampicillin and amoxicillin and the β-lactamase inhibitor clavulanic acid. This broad spectrum of resistance to β-lactams, cephalosporins, and β-lactamase inhibitors is a feature common to the *ampC* class of cephamycinases (11). One particular *ampC*-like gene, bla_{CMY2}, was recently detected in ceftriaxone-resistant *E. coli* isolates of animal origin (67). We examined our canine isolates for the presence of this gene.

TABLE 2. Antibiotic resistance profiles of nosocomial *E. coli* isolates

Isolate	Genetic type[a]	Antibiotic resistance profile[b]	*intI*	Antibiotic resistance genes[c]				
				Class 1 integron		Cephamycinase		*flo*
				Cassette(s) size (kb)	Genes	bla_{CMY2}	Molecular size (kb) of fragment generated by PFGE[d]	
39737	1A	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri	+	**1.7**, 0.80	***aadA5, dfrA17***	+		−
22-2	1A	ESCp, Chl, Flq, Spc, Tet I, Sul, Tri, Flor	+	1.7, 0.90	***aadA5, dfrA17***	+		−
29	1A	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri	+	1.7, 0.60	***aadA5, dfrA17***	+	25	−
34	1A	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	1.7, 0.90	***aadA5, dfrA17***	+		−
53	1A	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri	+	1.5, 0.75		+		−
56	1A	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor I	+	1.5, 0.75		+		−
22949	1B	ESCp, Chl, Flq, Spc I, Tet, Gen, Sul, Tri, Flor	+	1.4, 0.70		+		+
4479	1B	ESCp, Chl, Flq, Spc I, Tet, Gen, Sul, Tri, Flor	+	1.3, 0.75		+		+
38	1B	ESCp, Chl, Flq, Spc I, Tet, Gen, Sul, Tri, Flor	+	1.2, 0.60		+	185	+
4517A	2	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	**1.7**, 0.75	***aadA5, dfrA17***	+	250	+
4517B	2	ESCp, Chl, Flq, Spc, Tet, Sul, Tri, Flor	+	**1.7**, 0.75	***aadA5, dfrA17***	+		+
22255	2	ESCp, Chl, Flq, Spc, Tet, Sul, Tri, Flor	+	0		+		+
A1-72	3	ESCp, Chl I, Flq, Spc I, Sul, Gen, Flor, Tet	−	0		+	100	+
1745	3	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	0.90		+		−
32	3	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	**1.7**, 0.90	***aadA5, dfr17***	+		−
27315	4	ESCp, Chl, Flq, Spc I, Tet, Flor	+	0		+	140	+
40362	5	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	−	0		+		+
42614B	6	Sul	−	0		−	280, 115	−
1888	7	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	**1.7**, 1.1, 0.75	***aadA5, dfrA17***	+	30, 27	+
18813	7	ESCp, Chl, Flor, Amk I	+	0		+		+
22559	8	ESCp, Chl, Flq, Spc I, Tet, Gen, Sul, Tri, Flor	+	1.4, 0.70		+		+
25055a	8	ESCp, Chl, Flq, Spc I, Tet, Gen, Sul, Tri, Flor	+	0		+		+
25055b	8	ESCp, Chl, Flq, Spc I, Tet, Gen, Sul, Tri, Flor	+	1.3, 0.70		+		+
29610	9	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	1.3, 0.70		+		+
12279	9	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	1.2, 0.60		−		+
21	10	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor I	+	**1.7**, 0.90	***aadA5, dfr17***	+	29	−
42614A	11	ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	−	0		+		+
18397		ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	2.4, 1.2		+		+
23120		ESCp, Chl, Flq, Spc I, Tet, Gen, Sul, Tri, Flor	+	0		+		+
21411		ESCp, Chl, Flq, Spc, Tet, Gen, Sul, Tri, Flor	+	1.4, 0.70		+		+
37		ESCp, Chl, Flq, Spc, Tet, Sul, Tri	+	0		+		−
55-1		ESCp, Chl, Flq, Tet, Sul, Tri, Flor I	+	0		+		−
55-2		ESCp, Chl, Flq, Tet, Sul, Tri, Flor I	+	0		+		−
55-3		ESCp, Chl, Flq, Spc I, Tet, Sul, Tri, Flor	+	0		+		−

[a] A numerical designation was assigned to each distinct DNA fingerprint generated by PFGE with both restriction enzymes *Xba*I and *Bln*I. Isolates with same number have identical PFGE patterns with either restriction enzyme.

[b] ESCp, extended-spectrum cephalosporin resistance, including resistance to the cephalosporins (cefazolin, cefoxitin, cephalothin and ceftioufur), β-lactams (amoxicillin, ampicillin and ticarcillin), and the β-lactam inhibitor clavulanic acid; Flq, fluoroquinolones (enrofloxacin and orbifloxacin). The bacterial isolates were also resistant to chloramphenicol (Chl), gentamicin (Gen) spectinomycin (Spc), sulfonamides (Sul), tetracycline (Tet), and trimethoprim (Tri).

[c] Bacterial isolates were screened for the presence of class 1 integrons by PCR by using the integrase gene *intI* as a marker for this genetic element. A second PCR was done with primers specific for conserved sequences flanking the integron integration site *aatI*. This PCR amplifies a gene cassette(s) present within *aatI* (32). PCR amplicons were sequenced, and the nucleotide or translated amino acid sequences were queried against the GenBank DNA database at the National Center for Biotechnology Information for matches. The genes and the corresponding integron cassette size(s) are highlighted in boldface. Other antibiotic resistance genes for which the isolates were surveyed in this study included bla_{CMY2} (67) and *flo* (27).

[d] Genetic mapping of bla_{CMY2} was done for canine *E. coli* isolates representative of the different genetic types by DNA-DNA hybridization of PFGE generated with restriction endonuclease *Xba*I and hybridized with PCR-generated DNA probe specific for bla_{CMY2}. The molecular sezis of *Xba*I DNA fragment(s) that hybridized with bla_{CMY2} probe are given.

Thirty-two of 34 isolates screened by PCR were positive for the cephamycinase gene (bla_{CMY2}). There was 99.9% identity between the nucleotide sequence of one of the PCR amplicons (GenBank accession no. AF475279) from a canine isolate and the published sequence of the bla_{CMY2} gene (67). The bla_{CMY2} gene is closely related to the *Citrobacter freundii* chromosomal *ampC* gene (66) and to the plasmid-associated *ampC* gene present in *S. enterica* subsp. *enterica* serovar Typhimurium isolates (16) and human and animal *E. coli* isolates (64, 67).

Narrow-spectrum parenteral cephalosporins are used extensively for prophylaxis in cats and dogs with surgical wounds (8). At UGA-SVTH, cefazolin is the cephalosporin most commonly used parenterally. Oral cephalosporins, such as cephalexin, are used for the treatment of skin and urinary tract infections caused by susceptible organisms. Other applications include the treatment of abscesses and wound infections caused by susceptible organisms in dogs and cats. Expanded-spectrum cephalosporins are indicated for mixed infections with anaerobes (aspiration pneumonia, severe bite wound infections, gangrene, peritonitis, pleuritis) and prophylaxis in colonic or perineal surgery. Broad-spectrum cephalosporins are used only for treatment of infections caused by otherwise resistant bacteria in cats and dogs. It has been estimated that 13% of the *E. coli* isolates involved in scours in calves are resistant to broad-spectrum cephalosporins, most likely due to hyperproduction of the chromosomally encoded AmpC β-lactamase (10).

Another unusual resistance phenotype reported among

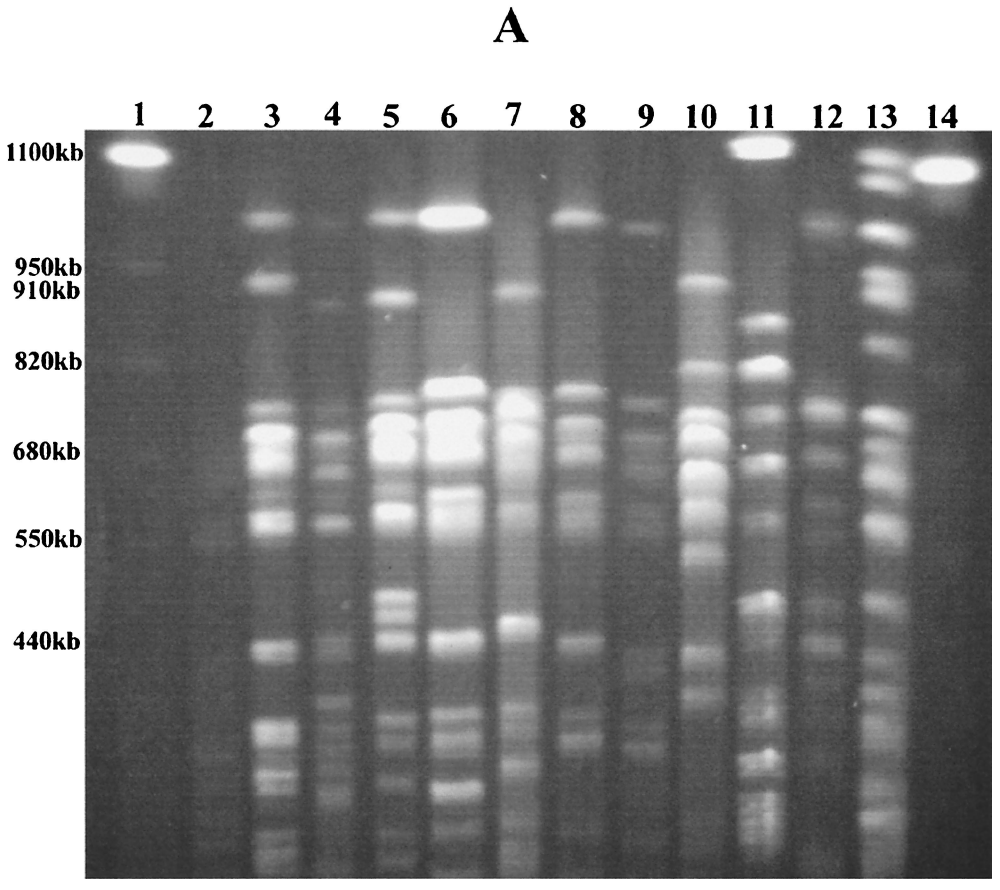

FIG. 1. *E. coli* genetic types identified by PFGE. (A) Patterns obtained by PFGE with *Xba*I. Lanes 1 and 14, *S. cerevisiae* DNA standards (BioWhittaker Molecular Applications, Rockland, Maine); lane 3, PFGE pattern H1 (isolate 29); lane 4, H1.2 (isolate 42614A); lane 5, H1.4 (isolate 38); lane 6, I (isolate 4517A); lane 7, J (isolate A1-72); lane 8, K (isolate 27315); lane 9, L (isolate 40362); lane 10, M (isolate 42614B); lane 11, N (isolate 1888); lane 12, O (isolate 29610); and lane 13, P (isolate 21). (B) Patterns obtained by PFGE with *Bln*I. Lanes 1 and 15, *S. cerevisiae* DNA standards (BioWhittaker Molecular Applications); lane 3, PFGE pattern A1 (isolate 29); lane 4, A2.1 (isolate 38); lane 5, A2.2 (isolate 4479); lane 6, A2.3 (isolate 4517A); lane 7, B1 (isolate 27315); lane 8, B1.1 (isolate 42614A); lane 9, B1.2 (isolate A1-72); lane 10, C (isolate 29610); lane 11, D (isolate 42614B); lane 12, E (isolate 22559); lane 13, F (isolate 1888); and lane 14, G (isolate 21). *E. coli* K-12 LE392 in panels A and B (lanes 2) served as an internal control for the reproducibility of every PFGE run. Alphabetical designations were assigned to PFGE patterns with seven or more band differences (56). The numbers represent four to six band differences within a PFGE pattern, and the numbers following decimal points designate slight genetic differences of one to three bands.

these canine and environmental *E. coli* isolates was resistance to the phenicols chloramphenicol and the veterinary analog florfenicol. This resistance was attributed to widespread dissemination of the *cmlA* homologue *flo* among gram-negative bacteria (7, 13, 14, 24, 27, 28, 61). Twenty of 34 *E. coli* isolates possessed the *flo* florfenicol resistance gene.

The Food and Drug Administration approved the veterinary use of the fluorinated analog of chloramphenicol, florfenicol, for the treatment of bovine respiratory disease in 1996. Florfenicol does not cause irreversible aplastic anemia, and it is not susceptible to inactivation by chloramphenicol transacetylases. Its mechanism of action is very similar to that of chloramphenicol. Although the Food and Drug Administration has approved the use of florfenicol only for the treatment of respiratory disease in cattle caused by highly susceptible bacteria such as *Pasteurella* and *Haemophilus*, other possible future uses of florfenicol have been documented, such as for the treatment of mastitis in cattle (63), to obtain reductions in rates of morbidity and mortality from infections caused by *Actinobacillus pleuropneumoniae* in pigs (J. A. Jackson et al., Proc. 15th Int. Pig VET Congr., abstr. P187, 1998), and for the treatment of furunculosis in fish (50). Of the phenicols, only chloramphenicol is used at UGA-SVTH, but it is used infrequently.

We were able to demonstrate the transfer of extended-spectrum cephalosporin and florfenicol resistance and the antibiotic resistance genes bla_{CMY2} and *flo*, respectively, from canine *E. coli* isolate 4517A to rifampin-resistant *S. enterica* subsp. *enterica* serovar Typhimurium at a frequency of 10^{-6}. Other antibiotics to which resistance was transferred to the recipient included gentamicin, spectinomycin, and sulfadimethoxime. Navarro et al. (42) also reported the transfer of resistance to the same antibiotics to recipient *E. coli* isolates from *Salmonella*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *E. coli* isolates that possessed the bla_{CMY2} cephamycinase gene (42). The identity of the chloramphenicol resistance gene was not determined in that study. Conjugative R plasmids have been reported for canine *E. coli* isolates that confer resistance to four or more antibiotics including ampicillin, tetracycline, chloram-

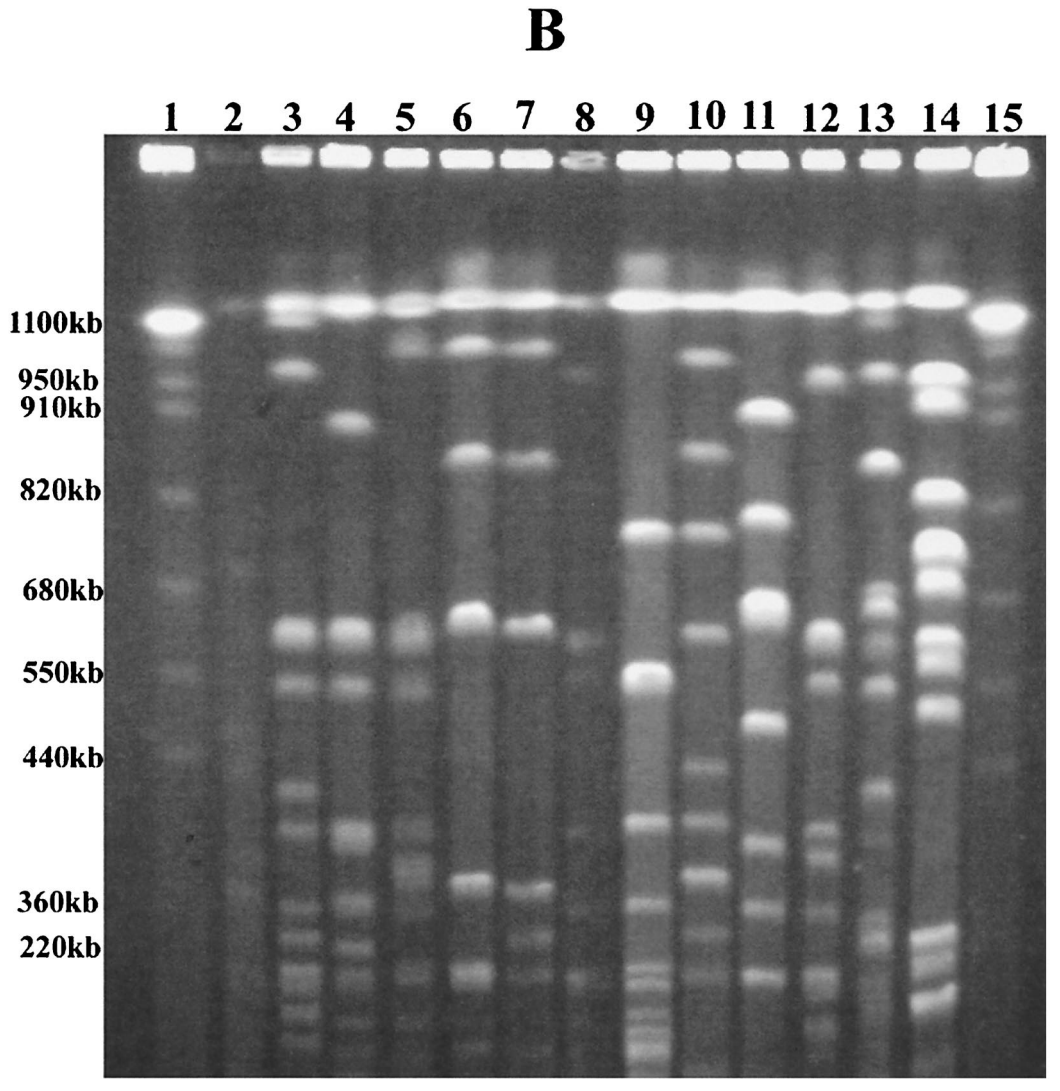

FIG. 1—*Continued.*

phenicol, sulfonamides, and streptomycin (43). Although a specific antibiotic resistance gene was never identified, other investigators (37) reported on *E. coli* isolates from dogs treated with β-lactams which possessed transferable cephamycinases with the spectra of activity, MWs, and pIs characteristic of those of Bla_{CMY2}. There have been other reports on the transmissibility of antibiotic resistance genes (bla_{CMY2} or *flo*) in *Salmonella* (42, 64, 67), *E. coli* (13, 42, 64, 67), *K. pneumoniae* (12, 42), *P. mirabilis* (42), and *Vibrio cholerae* (24). The cephamycinase gene described by Winokur et al. (64) appears to reside on a common plasmid shared between *Salmonella* and *E. coli* isolates from cattle. Since both bla_{CMY2} and *flo* are transferable, is the multidrug resistance present in polyclonal, nosocomial *E. coli* isolates due to the dissemination of a common, conjugative R plasmid in the veterinary teaching hospital?

Southern analysis was used to determine if the bla_{CMY2} cephamycinase gene mapped to a common *Xba*I DNA fragment, indicating dissemination of a common plasmid among these nosocomial isolates. We found that bla_{CMY2} mapped to a diverse array of *Xba*I DNA fragments in these isolates (Table 2). Canine *E. coli* isolates contained a diverse array of high-, intermediate-, and low-molecular-mass plasmids that ranged in size from 2 to 20 kb (Fig. 4). A 6.6-kb plasmid was common to 8 of 22 isolates examined (isolates 22, 34, 53, 18397, 22559, 25055a, 25055b, and 39737). When hybridized with a probe specific for bla_{CMY2} DNA, only the 20-kb plasmid in *E. coli* 4479 contained the bla_{CMY2} cephamycinase gene (data not shown), suggesting that this resistance gene maps to the chromosome or plasmids with higher MWs that cannot be isolated by the procedure used in this study. A similar observation was made with regard to the mapping of the *flo* florfenicol resistance gene in bovine *E. coli* isolates (61), suggesting that these resistance genes are being disseminated not by a common plasmid but, rather, by a transposable element (66). However, extended-spectrum cephalosporin resistance could have emerged in the hospital through the acquisition of a common, conjugative plasmid, but over time this plasmid may have changed as the plasmid acquired a transposon(s) or an integron(s) in this environment. Sequencing of the regions up-

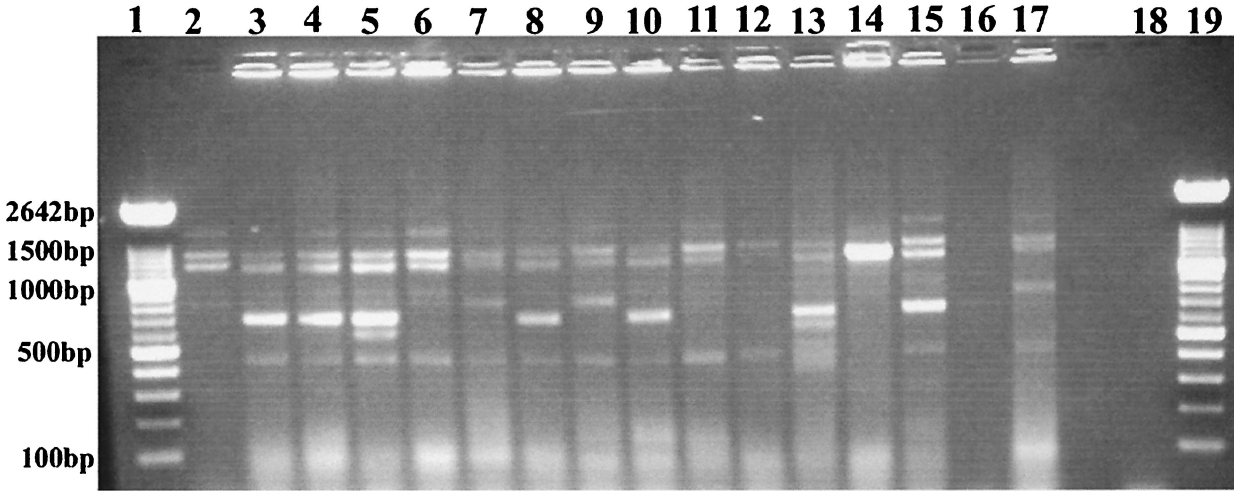

FIG. 2. *E. coli* genetic types identified by ERIC PCR. Lanes 1 and 20, 100-bp ladder (Roche Molecular Biochemicals, Indianapolis, Ind.); lane 3, ERIC pattern II (isolate 29); lane 4, RR (isolate 32); lane 5, LL (isolate 4479); lane 6, NN (isolate 4517A); lane 7, VV (isolate 37); lane 8, KK (isolate 56); lane 9, SS (isolate 42614B); lane 10, QQ (isolate 1745); lane 11, TT (isolate 12279); lane 12, OO (isolate 29610); lane 13, JJ (isolate 21); lane 14, UU (isolate 18397); lane 15, PP (isolate A1-72); lane 16, MM (isolate 38); lane 17, WW (isolate 55-1); lane 18, empty; and lane 19, no-DNA control. *E. coli* K-12 LE392 (lane 2) served as an internal control for the reproducibility of every ERIC PCR run. Since no interpretative criteria have been developed for analysis of the patterns obtained by ERIC PCR, DNA patterns with one or more band differences were given a different alphabetical designation.

stream and downstream of bla_{CMY2} will determine whether we are dealing with a transposable element that left the original plasmid from which the extended-spectrum cephalosporin resistance originated or whether this plasmid has undergone rapid genetic changes since its introduction into the hospital environment.

In addition to extended-spectrum cephalosporin and chloramphenicol resistance, the nosocomial *E. coli* isolates were also resistant to the antibiotics spectinomycin, sulfonamide, and gentamicin. Resistance to multiple antibiotics, especially to spectinomycin and sulfonamides, is often associated with integrons, genetic elements that acquire and trade antibiotic resistance genes (55). Recombination involves the integrase IntI1, integration site *attI*, and a 59-bp element common to genes for antibiotic resistance present within the integron (54, 55). A feature of class 1 integrons, in addition to *intI1*, is the *sul1* sulfonamide resistance gene (55). Thirty of 34 nosocomial isolates in the present study possessed the *intI1* integrase gene.

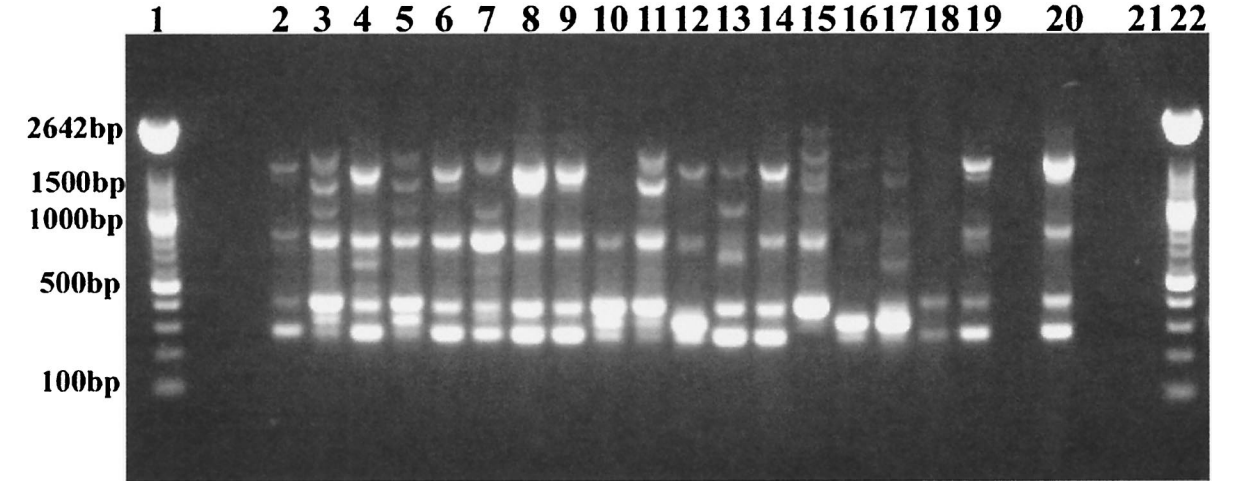

FIG. 3. *E. coli* genetic types identified by RAPD analysis with primer 1290. Lanes 1 and 22, 100-bp ladder (Roche Molecular Biochemicals); lane 2, RAPD 1290 pattern R (isolate 29); lane 3, FF (isolate 21411); lane 4, T (isolate 4479); lane 5, EE (isolate 23120); lane 6, U (isolate 22559); lane 7, Z (isolate 42614B); lane 8, Y (isolate 40362); lane 9, S (isolate A1-72); lane 10, GG (isolate 37); lane 11, HH (isolate 55-1); lane 12, V (isolate 4517A); lane 13, AA (isolate 1888); lane 14, Q (isolate 39737); lane 15, DD (isolate 18397); lane 16, X (isolate 27315); lane 17, BB (isolate 29610); lane 18, CC (isolate 12279); lane 19, W (isolate 1745); and lane 21, no-DNA control. *E. coli* K-12 LE392 (lane 20) served as an internal control for reproducibility of every RAPD analysis run. Since no interpretative criteria have been developed for analysis of RAPD PCR patterns, DNA patterns with one or more band differences were given a different alphabetical designation.

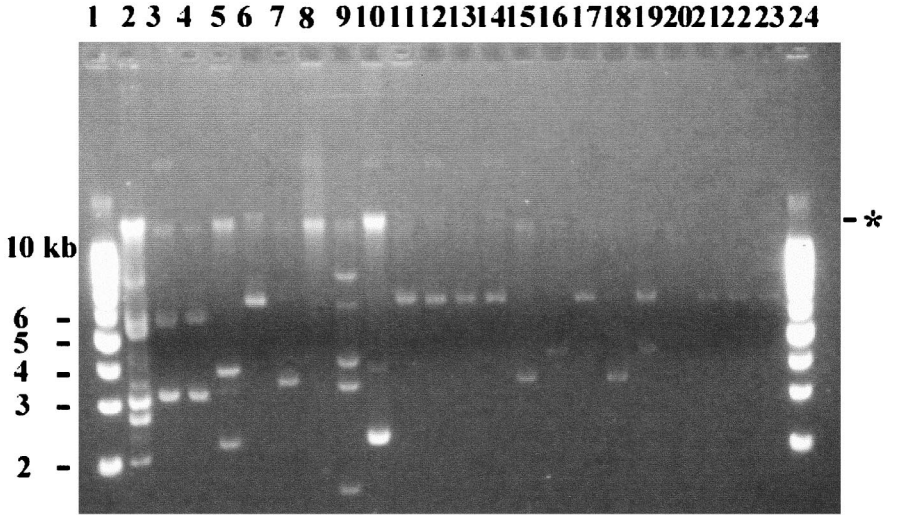

FIG. 4. Plasmid profiles of canine *E. coli* isolates. Lanes 1 and 24, supercoiled DNA ladder; lane 2, V517, plasmid control strain; lane 3, isolate A1-72; lane 4, isolate 1745; lane 5, isolate 1888; lane 6, isolate 4479; lane 7, isolate 4517A; lane 8, isolate 12279; lane 9, isolate 18397; lane 10, isolate 18813; lane 11, 22559; lane 12, isolate 25055a; lane 13, isolate 25055b; lane 14, isolate 25055a; lane 15, isolate 27315; lane 16, isolate 29610; lane 17, isolate 39737; lane 18, isolate 40362, lane 19, isolate 42614A; lane 20, isolate 42614B; lane 21, isolate 22; lane 22, isolate 34; and lane 23, isolate 53. The asterisk indicates the position of chromosomal DNA on the gel.

Among the members of the family *Enterobacteriaceae*, the incidence of class 1 integrons was highest in *E. coli* and *Salmonella* isolates and was associated with multidrug resistance (20). To further characterize the integrons of the nosocomial *E. coli* isolates, PCR was used to amplify the gene cassette(s) associated with class 1 integrons (32). Four *intI1*-positive *E. coli* isolates produced no PCR amplicon, suggesting that the *attI* site of the integron did not contain a gene cassette, while two or more integrons were generally present in the remaining isolates that were positive for class 1 integrase (Fig. 5; Table 2). "Empty" class 1 integrons have been reported for other gram-negative bacteria (34, 52). The gene cassettes present within each integron ranged in size from 0.6 to 2.4 kb, although a 1.7-kb gene cassette was the most prevalent ($n = 8$). This 1.7-kb gene cassette was found in *E. coli* isolates of five different genetic types, suggesting that this integron is disseminated via a common genetic element, possibly a conjugative plasmid. Sequencing of the 1.7-kb PCR amplicon revealed the identities of two antibiotic resistance genes, *dfrA17* and *aadA5*, genes that confer resistance to trimethoprim and spectinomycin, respectively (GenBank accession no. AF475280 and AF475281). The same integron was reported in a human *E. coli* isolate associated with a urinary tract infection (62).

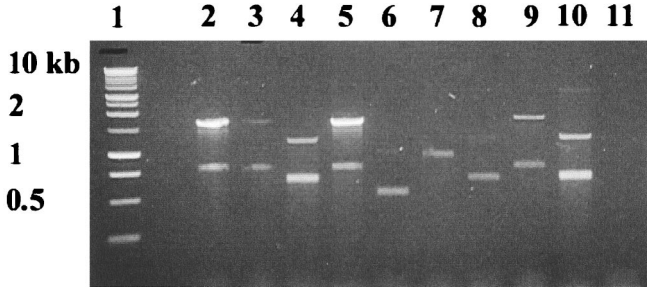

FIG. 5. Gene cassette(s) in class 1 integrons of nosocomial *E. coli.* The gene cassette(s) present within the class 1 integron integration site (*attI*) was amplified by PCR with oligonucleotide primers specific for conserved sequences 5′ and 3′ of the *attI* site (32). PCR amplifications were done with canine *E. coli* isolates positive for the *intI1*class 1 integrase gene, a marker for class 1 integrons. Lanes 2 to 10, canine *E. coli* isolates with representative PCR amplicons associated with class 1 integrons in nosocomial isolates; lane 1, 1-kb ladder (Roche Molecular Biochemicals); lane 2, *E. coli* isolate 1745; lane 3, isolate 1888; lane 4, isolate 4479; lane 5, isolate 4517A; lane 6, isolate 12279; lane 7, isolate 18397; lane 8, isolate 21411; lane 9, isolate 39737; lane 10, isolate 38; and lane 11, no-DNA control.

To eradicate this problem from the veterinary teaching hospital, it was important to implement an effective infection control program (17, 47), to limit contact between affected patients (58), and to institute strict guidelines concerning the judicious use of antibiotics in the hospital. As is evident from our survey of the hospital environment, it is also important to thoroughly clean and disinfect affected areas (6, 58). A regular cleaning schedule was implemented, and we also have in place a program of monitoring for nosocomial infections (17). These measures have reduced the incidence of resistant *E. coli* isolates in the veterinary hospital.

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