Veterinary Microbiology 144 (2010) 363–370

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

The role of class 1 and 2 integrons in mediating antimicrobial resistance among canine and feline clinical *E. coli* isolates from the US

Bashar W. Shaheen [a], Omar A. Oyarzabal [b], Dawn M. Boothe [a,*]

[a] Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, 109 Greene Hall, Auburn, AL 36849, USA
[b] Department of Biological Science, Alabama State University, Montgomery, AL, USA

ARTICLE INFO

Article history:
Received 15 April 2009
Received in revised form 8 January 2010
Accepted 25 January 2010

Keywords:
Integron
Gene cassette
Escherichia coli
Antimicrobial resistance

ABSTRACT

Integrons are mobile genetic elements that incorporate an open reading frame or gene cassettes. They have an important role in the acquisition and dissemination of antimicrobial resistance genes. Yet the occurrence of integrons carrying antimicrobial resistance genes in bacterial pathogens of pet animals is seldom addressed. The purpose of this study was to describe the incidence of class 1 and 2 integrons in clinical isolates of *Escherichia coli* ($n = 82$) from cats and dogs provided by diagnostic laboratories in five States of the USA. An association between resistance genes in the integrons and the isolates' phenotypes was found. Integrons were detected using PCR and then further characterized by restriction fragment-length polymorphism analysis and amplicon sequencing. Class 1 integrons were detected in 27% of the isolates, while only 2% ($n = 2$) of the isolates were positive for the presence of class 2 integrons. Seventy-two percent ($n = 59$) of the isolates did not carry integrons. Eleven gene cassettes were found either alone or in combination with other gene cassettes, which encoded resistance to aminoglycosides (aadA1, aadA2, aadA5, aacA4, and aadB), trimethoprim (dfrA1, dhfrA17, and dfrA12), chloramphenicol (*catB3* and cmlA6), and streptothricin (sat1), respectively. All integron-positive isolates were characterized by resistance to least two drug classes and 35% produced extended-spectrum β-lactamases. The association of integrons carried on plasmids and antimicrobial resistance was confirmed by curing experiments for three isolates. Resistance was resolved once large plasmids (size range 97–169 kb) carrying the class 1 integron were lost. Therefore, integrons appear to have an essential role in facilitating the dissemination of the resistance genes and contributing to the creation of multi-drug resistant phenotypes.

1. Introduction

Escherichia coli is a common cause of canine and feline urinary tract infection and pyometra (Chen et al., 2003; Hagman and Kühn, 2002). Historically a range of antimicrobial agents has been used to treat these infections in veterinary medicine, including penicillins, cephalosporins, macrolides, lincosamides, fusidic acid, tetracyclines, chloramphenicol, potentiated sulfonamides, aminoglycosides and fluoroquinolones. The use of antimicrobial drugs has been associated with an increasing trend of antimicrobial resistance among canine and feline clinical *E. coli* isolates in the last decade (Normand et al., 2000b). In addition to an increasing frequency, the type of resistance expressed by *E. coli* also is changing, with more isolates expressing multi-drug resistance (MDR) in the US and Europe (Cohn et al., 2003; Guardabassi et al., 2004; Normand et al., 2000a; Shaheen et al., 2008).

Integrons play an essential role in facilitating the transfer of the resistance genes, contributing to the

* Corresponding author. Tel.: +1 334 844 4751; fax: +1 334 844 7188.
E-mail address: boothdm@auburn.edu (D.M. Boothe).

0378-1135/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2010.01.018

creation of MDR phenotypes (Hall and Collis, 1998; Rowe-Magnus and Mazel, 2002). Studies documenting class 1 and 2 integrons from clinical isolates of *E. coli* from cats and dogs have been reported from Switzerland, Germany and Australia (Cocchi et al., 2007; Kadlec and Schwarz, 2008; Lanz et al., 2003; Sidjabat et al., 2006). Only 22% and 15% of isolates were found to carry class 1 integrons in a Swiss (Cocchi et al., 2007) and Australian study (Sidjabat et al., 2006), respectively. One US study found 30 of 34 (88%) nosocomial isolates harbored the intI1 integrase gene, and those isolates were resistant to more than one drug (Sanchez et al., 2002).

Previous studies (Cocchi et al., 2007) found that more isolates from livestock carried class 1 integrons than isolates from cats and dogs and it was concluded that this could be attributed to the extensive use of antibiotics in livestock. The purpose of this study is to further define the role of integrons and their associated gene cassettes in mediating antimicrobial resistance in clinical isolates of *E. coli* from cats and dogs. In this study, we build on previous investigators' findings (van Essen-Zandbergen et al., 2007) by describing the distribution of class 1 and 2 integrons among clinical *E. coli* isolates associated with disease in dogs and cats, and associating type 1 or type 2 integrons with their antimicrobial resistant gene cassettes

2. Materials and methods

2.1. Bacterial isolates and culture conditions

Canine and feline clinical *E. coli* isolates ($n = 82$) were acquired from five clinical veterinary diagnostic laboratories (IDEXX) between May and December 2008. Isolates were originally received by the diagnostic laboratories from veterinarians located throughout the continental United States. Isolates had been cultured by the laboratories upon receipt from veterinary practitioners who had collected samples from dogs or cats with presumed infections. Each isolate was identified as *E. coli* by the submitting diagnostic laboratory prior to submission to the Clinical Pharmacology Laboratory (CPL) at Auburn University. Upon receipt by the CPL, isolates were plated on CHROMagar™ Orientation (Becton, Dickinson, Franklin Lakes, NJ) to allow for rapid differentiation and identification of *E. coli* and *Enterococcus* without confirmatory testing confirmation.

2.2. Susceptibility testing

Isolates were streaked onto trypticase agar (Becton Dickinson, Franklin Lakes, NJ) and then incubated at 35 °C for 18–24 h. For standard susceptibility testing, cells were harvested and suspended in 4.5 ml of 0.9% normal saline and adjusted to 0.5 McFarland standard turbidity ($\sim 10^8$ CFU) using SENSITITER® Nephelometer (TREK Diagnostic Systems, Cleveland, OH) before testing. Antimicrobial susceptibility testing was performed using custom made broth micro-dilution susceptibility plates (TREK Diagnostic Systems, Cleveland, OH) according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretive standards (CLSI, 2008). The antimicrobials used in the study were ampicillin, amoxicillin/clavulanic acid, cephalothin, cefoxitin, cefpodoxime, cefotaxime, ceftazidime, meropenem, enrofloxacin, gentamicin, doxycycline, chloromphenicol, and trimethoprim/sulfamethoxazole. Micro-dilution plates were incubated at 35 °C for 20–24 h. The MIC values were recorded using the SENSITITER® VIZION system (TREK Diagnostic Systems, Cleveland, OH). For quality control purposes, *E. coli* ATCC® 25922 (American Tissue Cell Culture, Manassas, VA) and *Enterococcus fecalis* ATCC® 29212 (Manassas, VA) were used. MDR was defined as resistance to two or more drug classes. Eighty-two *E. coli* isolates were randomly selected to represent different phenotypes: single drug resistance (SDR) phenotype to β-lactam antibiotics ($n = 9$); SDR phenotype to chloramphenicol ($n = 3$); susceptible or non-resistance (NR) phenotypes ($n = 17$); and MDR phenotypes ($n = 53$). According to the CLSI guidelines, isolates for which the MIC for either ceftazidime or cefotaxime increased more than 8-fold when tested with either drug or clavulanic acid was identified as potential ESBL producers and shortlisted for confirmation of ESBL production. Among the selected isolates, extended-spectrum β-lactamase-producers were confirmed in 12 isolates using the CLSI interpretive standards (CLSI, 2008).

2.3. Bacterial DNA preparation, PCR assays and DNA sequencing

Bacterial DNA for PCR reaction was prepared by boiling bacterial cultures in 200 μl of PreMan® Ultra Sample Preparation Reagent (Applied Biosystem, Foster City, CA) for 10 min, followed by centrifugation. The variable regions (i.e., the region situated between the conserved segments (CS): 5′CS and 3′CS) and integrase genes (i.e., *Int1* and *IntII*) of class 1 and 2 integrons were amplified with a PCR reaction using primers listed in Table 1. All primers were synthesized by Operon, Inc. (Huntsville, AL). Amplification reactions were carried out in 25 μl reaction volumes of Omnimix (Cepheid) including 3U TaKaRa hot start Taq polymerase, 200 μM dNTP, 4 mM $MgCl_2$, 25 mM HEPES buffer, pH 8.0 ± 0.1, 1 μl of DNA preparation, and 1 μl of the forward and reverse primers. Distilled water was added to bring the final volume to 25 μl. The PCR conditions for class 1 and 2 integrons are listed in Table 1. The reaction products were analyzed by electrophoresis in agarose gels stained with ethidium bromide, and visualized under UV light. The image was recorded using a gel imaging system (Syngene, Frederick, MA, USA). For each set of PCR reactions, *Salmonella typhimurium* DT104 was included as a positive control for class 1 integrons.

2.4. Characterization of class 1 and 2 integrons by sequencing and restriction fragment-length polymorphism (RFLP)

RFLP was used to differentiate different integron fragments similar in size. Each RFLP pattern was sequenced, with one isolate randomly selected among those with a similar RFLP pattern. The PCR amplicons were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA) and were sequenced using ABI automatic DNA sequencer (Model 377; Perkin-Elmer) at the Genomic Sequencing Laboratory (Auburn University, Auburn, AL,

Table 1
Primers sequences and PCR conditions used in this study.

Primer	Oligonucleotide sequence (5′–3′)	PCR conditions	Reference
IntI1-F	GGT CAA GGA TCT GGA TTT CG	1 cycle of 4 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 62 °C, 1 min at 72 °C; 1 cycle of 8 min at 72 °C	Machado et al. (2005)
IntI1-R	ACA TGC GTG TAA ATC ATC GTC		Machado et al. (2005)
5′CS	GGC ATC CAA GCA GCA AG	1 cycle of 4 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 66 °C, 2 min at 72 °C; 1 cycle of 10 min at 72 °C	Levesque et al. (1995)
3′CS	AAG CAG ACT TGA CCT GA		Levesque et al. (1995)
IntI2-F	CAC GGA TAT GCG ACA AAA AGG T	Same as for *int1*	Machado et al. (2005)
IntI2-R	GTA GCA AAC GAG TGA CGA AAT G		Machado et al. (2005)
attI2-F	GAC GGC ATG CAC GAT TTG TA	Same as for 5′CS	Machado et al. (2005)
orfX-R	GAT GCC ATC GCA AGT ACG AG		Machado et al. (2005)

USA) using the above-described forward and reverse primers. DNA sequences were analyzed and compared to published sequences by searching the GenBank database of the National Center for Biotechnology Information via the BLAST network service (http://www.ncbi.nlm.nih.gov/blast/).

Typing of class 1 and class 2 integrons was performed by RFLP. For RFLP analysis, the purified PCR amplicons of the variable regions of class 1 and 2 integrons were digested using *Alu*I and *HaeIII* restriction endonucleases, respectively, as described (Machado et al., 2005, 2008). Digestions were performed according to the manufacturer's instructions.

Plasmid extraction was carried out on integron-positive isolates with the Qiagen miniprep using the mini-scale alkaline lysis method. Pulsed-field gel electrophoresis (PFGE) was performed to determine DNA fingerprinting profiles of plasmids for the cured and non-cured isolates and to estimate the size of the plasmids present. Plasmid samples (17 μl) were loaded onto a 1% agarose–Tris buffer gel (SeaKem Gold Agarose; BioWhittaker Molecular Applications, Rockland, ME), and PFGE was performed with a CHEF Mapper XA apparatus (CHEF Mapper XA; Bio-Rad Laboratories, Hercules, CA). DNA was electrophoresed for 9 h at a constant voltage of 200 V (6 V/cm), with a pulse time of 6.75–21.7 s, an electric field angle of 120°, and a temperature of 14 °C, before being stained with ethidium bromide.

2.5. Plasmid curing experiments

Natural curing experiments were performed for all integron-positive isolates ($n = 23$). Curing was carried out by daily subculturing for 10 weeks onto trypticase soy agar (TSA) slants incubated at 10 °C and the colonies were tested for antimicrobial susceptibility phenotypically as previously described. Because curing was unsuccessful despite 10 weeks of subculture, the sodium dodecyl sulfate (SDS) method of curing was implemented on 11 isolates representing both class 1 and 2 integrons (Tomoeda et al., 1968). An overnight culture of *E. coli* was diluted in Brucella broth containing 10% SDS and was shaken at 37 °C or 40 °C for 72 h. Cells were checked for their susceptibility to all drug classes. The MICs for the cured cells were compared to the MICs before curing to investigate the contribution of the integrons carried on plasmids to the MDR phenotypes.

2.6. Statistical analysis

Susceptibility data were compared between integron-positive and -negative *E. coli*. A statistical comparison of the frequencies and the association of integron presence in *E. coli* was conducted by using odds ratios and 95% confidence intervals. Significance (P-value), in terms of the number of resistant and susceptible integron-positive and -negative isolates, was calculated by the Pearson's χ^2-square test ($P < 0.05$). Analyses were performed using the statistical software MINITAB® 15 package (Minitab Inc., PA, USA).

3. Results

3.1. Presence of integrons and the association between integrons and antimicrobial resistance

Of the 82 *E. coli* isolates tested, 27% ($n = 22$) carried class 1 integrons. Class 2 integrons where found only in 2.4% ($n = 2$) isolates. One of these isolates also carried a class 1 integron. Of the resistant phenotypes ($n = 65$), 35% ($n = 23$) were positive for either class 1 or class 2 integrons. Integrase genes (i.e., *Int1* and *IntII*) were detected in 28% ($n = 23$) of the total isolates and found in all integron-positive isolates. The association between antimicrobial resistant profile and occurrence of integrons is shown in Fig. 1.

All (100%) of the integron-positive isolates were characterized by resistance to three or more antimicrobial agents, compared to only 37% (22/59) of integron-negative isolates. All four isolates resistant to all 13 antimicrobial agents carried a class 1 integron (Fig. 1).

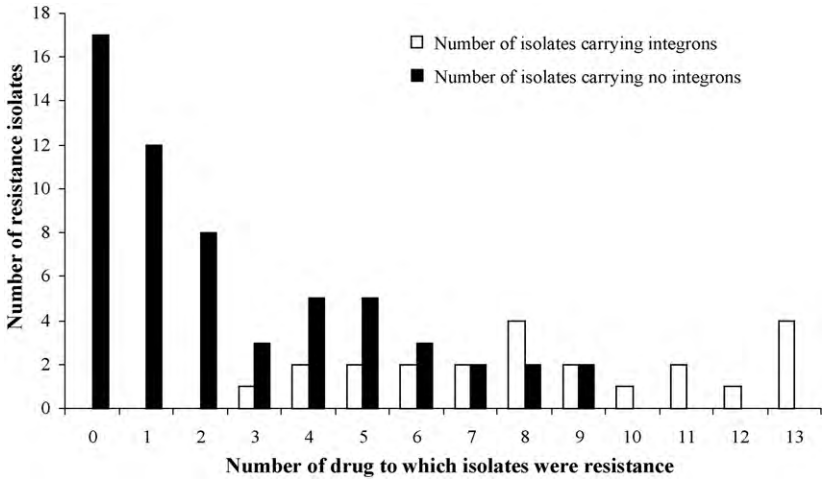

Fig. 1. Association between antimicrobial resistant profile and occurrence of class 1 and 2 integrons in *Escherichia coli* stains isolated from dogs and cats.

A significant association was found between the presence of integrons and resistance to cefotaxime, ceftazidime, meropenem, and trimethoprim–sulfamethoxazole.

3.2. The relationship between integron-positive isolates and MIC

A comparison between integron-positive and -negative isolates, with respect to MIC range, MIC_{50}/MIC_{90} value and percentage resistant to each drugs tested, is shown in Table 2. The percentage of isolates resistant to the penicillin-based β-lactams, ampicillin and amoxicillin–clavulanic acid, was higher in the integron-positive group (82.6% and 56.5%, respectively) compared to the integron-negative group (32.2% and 13.6%, respectively). Resistance to carbapenem and meropenem, was less in both integron-positive and -negative isolates (21.7% and 1.7% resistant, respectively) compared to the other penicillins. For the cephalosporin-based β-lactams, the proportion of resistance varied among the generations. For integron-positive and -negative isolates, the proportion of resistance expressed toward the first generation cephalosporin, cephalothin, was 86.9% and 62.7%, respectively, and toward the second-generation cephalosporin, cefoxitin, 56.5% and 15.3% resistant, respectively. The range of the proportions of resistance for integron-positive and -negative isolates for the third-generation cephalosporins (cefpodoxime, cefotaxime and ceftazidime) was 30.4–47.8% and 1.7–13.6% resistant, respectively. Furthermore, almost 35% ($n = 8$) of integron-positive isolates were confirmed phenotypically to be extended-spectrum β-lactamase-producers. At least 8-fold differences in the MIC_{90s} were observed for β-lactam antibiotics between integron-positive and -negative isolates except for ampicillin. The MIC_{50} and MIC_{90} of enrofloxacin were in the intermediate and high range of resistance (i.e., 2 μg/ml and >128 μg/ml), respectively for integron-positive isolates and in the susceptible and low range of resistance (i.e., 0.06 μg/ml and 4 μg/ml) for integron-negative isolates. Only 13.6% of integron-negative isolates were resistant to enrofloxacin, while 47.8% of integron-positive isolates were resistant.

For integron-negative isolates, the MICs were below the susceptible break points ($MIC_{90} = 4$ μg/ml) for both

Table 2
Antimicrobial susceptibility of *E. coli* isolates.

Antimicrobial agents	Integron-positive isolates ($n = 23$)				Integron-negative isolates ($n = 59$)			
	MIC (μg/ml)			Number (%) of resistant isolates	MIC (μg/ml)			Number (%) of resistant isolates
	Range	MIC50	MIC90		Range	MIC50	MIC90	
Ampicillin	1 to >256	>256	>256	19 (82.6)	2 to >256	4	>256	19 (32.2)
Amoxicillin/clavulanic acid	4 to >1024	32	256	13 (56.5)	0.5 to 256	4	16	8 (13.6)
Cephalothin	4 to >1024	128	>1024	20 (86.9)	4 to >1024	16	128	37 (62.7)
Cefoxitin	2 to >1024	32	>1024	13 (56.5)	2 to 256	4	16	9 (15.3)
Cefpodoxime	0.25 to >128	4	>128	11 (47.8)	0.25 to >128	1	4	8 (13.6)
Cefotaxime	1 to 1024	8	256	10 (43.5)	1 to 512	1	4	3 (5.1)
Ceftazidime	0.5 to 256	2	128	7 (30.4)	0.5 to 16	0.5	1	1 (1.7)
Meropenem	0.25 to >512	0.25	512	5 (21.7)	0.25 to >512	0.25	0.25	1 (1.7)
Enrofloxacin	0.06 to >128	2	>128	11 (47.8)	0.06 to >128	0.06	4	8 (13.6)
Doxycycline	0.5 to >512	32	256	17 (73.9)	0.5 to >512	2	64	17 (28.8)
Chloramphenicol	2 to >1024	32	1024	14 (60.9)	2 to >1024	8	64	15 (25.2)
Gentamicin	0.12 to 128	8	128	11 (47.8)	0.5 to 16	1	4	6 (10.2)
Trimethoprim/sulfamethoxazole	0.06 to >128	>128	>128	15 (65.2)	0.06 to >128	0.06	4	7 (11.9)

gentamicin and trimethoprim/sulfamethoxazole, whereas the percentage of isolates resistant to gentamicin and trimethoprim/sulfamethoxazole among integron-positive isolates was significantly greater with 47.8% and 65.2%, respectively, compared with 10.2% and 11.9%, respectively for integron-negative group.

3.3. Characterization of gene cassettes associated with class 1 and 2 itegrons

A total of 11 gene cassettes (Table 3) were identified by PCR amplification of the conserved regions of class 1 integron. The size of PCR product varied from 1 kb to 3.2 kb. The integrons were found to contain 1–3 gene cassettes and to differ in the location of the cassettes. RFLP characterization of class 1 integrons revealed seven distinct profiles of gene cassette arrays. These were *dfrA1-aadA1*, *dfrA17-aadA5*, *dfrA12-aadA2*, *aadB-aadA1d*, *aacA4-catB3-dfrA1* and *aadB-aadA1-cmlA6*. Among these profiles, the gene cassette aadA1 was found most frequently (68%, $n = 15/22$) in class 1 integrons either alone or in combination with other gene cassettes. The antimicrobial resistance gene cassettes found in class 1 and 2 integrons are listed in Table 3.

The two isolates with class 2 integrons contained the cassette array *dfrA1-sat1-aadA1*; the class 1 gene cassette array found in one of the isolates was *aacA4-catB3-dfrA1*.

3.4. Curing experiments for isolates carrying plasmids harboring class 1 integrons

Three isolates were successfully cured of their class 1 integron-bearing plasmids (gene cassettes): L8055239 (*aadA1*), I3195059 (*aacA4-catB3-dfrA1*), and B5729897 (*aadB-aadA1-cmlA6*). Class 1 integrons were detected in the plasmid DNA extracted from these isolates before but not after curing using PCR protocol (Fig. 2). PFGE revealed that several cured isolates lost 2.3-kb, 23-kb, 97-kb, 145.5-kb, 169-kb, and 194-kb bands after curing (Fig. 2). All *E. coli* cured isolates become susceptible to the antimicrobials for which the integron's gene cassette array encoded resistance (Table 4). Interestingly, some isolates become susceptible to some β-lactam antibiotics, including ampicillin, cefotaxime, ceftazidime, and ticarcillin/clavulanic acid, although none of the gene cassettes identified within the integrons from those isolates conferred resistance to β-lactam antibiotics.

4. Discussion

The increase in the incidence of antimicrobial resistance among canine and feline *E. coli* isolates has been reported in many countries. In our study, we documented the prevalence of class 1 and 2 integrons among clinical isolates of *E. coli* collected from dogs and cats with clinical infections in the US. Additionally, different types of class 1 integrons were characterized which carried different gene cassettes. Furthermore, class 2 integrons were reported for the first time among canine and feline clinical *E. coli* isolates in the US.

In the present study, the incidence of class 1 integrons among canine and feline clinical samples (27%) was similar to that reported from Switzerland where 22% of the isolates from companion animals carried class 1 integrons (Cocchi et al., 2007). However, many studies have documented a higher proportion of class 1 integrons among *E. coli* clinical isolates from farm animals; 52% of isolates from farm animals (Cocchi et al., 2007), 59% from calf diarrhoea isolates (Du et al., 2005), 63% of isolates from chickens (Bass et al., 1999), 64% of swine diarrhoea isolates (Kang et al., 2005) and 82% isolates from chickens (Keyes et al., 2000). The differences between cats and dogs and farm animals may reflect difference in selection pressure from antibiotic use (Rosser and Yound, 1999), with *E. coli* strains from food production animals more frequently exposed to antimicrobial pressure compared with companion animals (Cocchi et al., 2007).

In this study we have also reported a very low percentage of isolates that carried a class 2 integron (only two isolates). Although other studies have reported a higher proportion of class 2 integrons compared to our study, the prevalence of class 2 integrons appears to be lower than class 1 integrons (Goldstein et al., 2001; Sunde, 2005). Furthermore, in contrast to class 1 integrons, for

Table 3
Antibiotic resistance patterns of *E. coli* isolates and their relationship with occurrence of the integrons.

Length of variable region (bp)	Gene cassettes and order	Resistance phenotype[a]	Number of isolates	Species	Tissue source	US State (n)[b]
Class 1 integrons						
1000	*aadA1*	XACDEGR	1	Feline	Urine	CA (1)
1500	*dfrA1-aadA1*	XACS	1	Canine	Urine	NC (1)
1500	*dfrA17-aadA5*	ACDGRERS	2	Canine	Urine	MA (1), NC (1), CA (1)
1800	*dfrA12-aadA2*	HDS	1	Canine	Urine	CA (1)
1500	*aadB-aadA1d*	ACDG	1	Canine	Urine	CA (1)
2500	*aacA4-catB3-dfrA1*	XATOPZCHDERSGM	4	Canine	Urine, Tracheal wash	OH (1), NC (2), IL (2)
3200	*aadB-aadA1-cmlA6*	AHDGSXTOPZCER	2	Canine	Urine	CA (2)
2000	*aadA1-unknown*	XATOPCHDEMGRSZ	10	Canine, Feline	Urine, Nasal cavity, Vagina	IL (2), CA (5), OH (1), NC (1)
Class 2 integrons						
2200	*dhfr1-sat1-aadA1*	XATOPZCHDEGRS	2	Feline, Canine	Urine	NC (2)

[a] Abbreviation of antimicrobial drugs; A, ampicillin; X, amoxicillin/clavulanic; C, cephalothin; O, cefoxitin; P, cefpodoxime; T, cefotaxime; Z, ceftazidime; M, meropenem; E, enrofloxacin; D, doxycycline; H, chloramphenicol; G, gentamicin; S, trimethoprim/sulfamethoxazole.
[b] Abbreviation of the origin of the isolates; CA, California; NC, North Carolina; IL, Illinois; MA, Massachusetts; OH, Ohio.

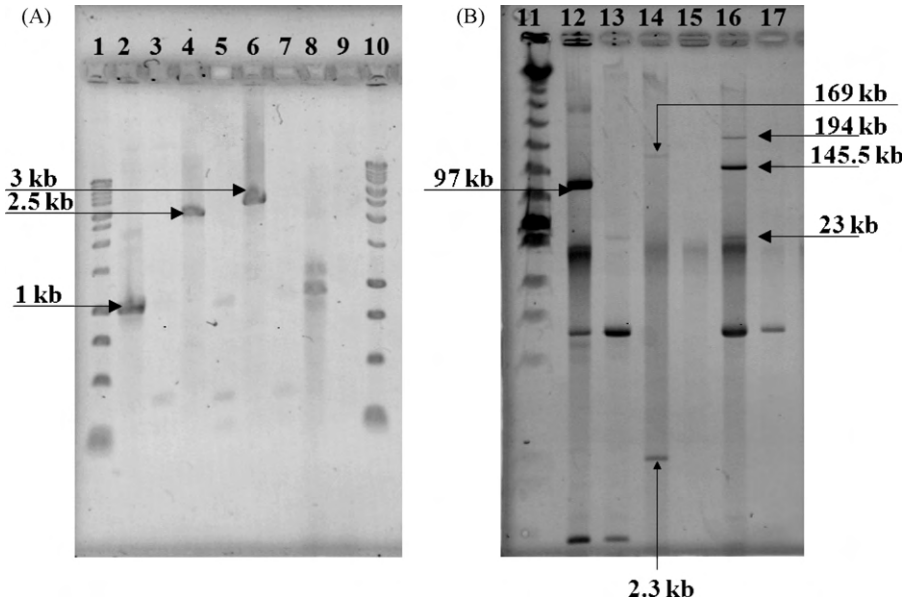

Fig. 2. PFGE profiles of the extracted plasmid DNA of the three isolates before and after curing. PCR amplification (A) and PFGE profiles (B) for the isolates before and after curing. Lanes 2, 4, and 6: PCR positive result for the variable regions of class 1 integrons for isolates L8055239, I3195059, and B5729897 with corresponding amplicons size 1-kb, 2.5-kb, and 3.2-kb, respectively before curing. Lanes 3, 5, and 7: negative results of class1 integrons for isolates L8055239, I3195059, and B5729897, respectively. Lanes 8 and 9 represent isolates *Salmonella serovar typhimurium* DT104 and *E. coli* ATCC® 25922, respectively used as positive and negative control for class 1 integrons. Lanes 12, 14, and 16: isolates L8055239, I3195059, and B5729897, respectively before the curing. Lane 13, isolate L8055239 lost a 97-kb band; lane 15, isolate I3195059 lost a 169-kb and 2.3-kb bands; lane 17, isolate B5729897 lost a 194-kb, 145.5-kb and 23-kb bands after curing.

which 11 different gene cassettes have been identified, only three different gene cassettes have identified (i.e., *dhfr1-sat1-aadA1*) in class 2 integrons.

Class 1 integrons were significantly associated with resistance to all the antimicrobials tested in this study. However, integron-positive strains were significantly more common among isolates with resistance to β-lactam antibiotics (including the 1st, 2nd and 3rd generation of cephalosporins and meropenem). The higher rate of resistance to several classes of β-lactam drugs in integron-positive isolates is probably attributable to an association of β-lactamase genes within integron-carrying plasmids. Many β-lactamase genes within the integron have been reported, including Bla-, Oxa-, CMY-, and CTX-M-types. These genes impart resistance to most penicillins, including penicillin-β-lactamase inhibitors, extended-spectrum β-lactams, and carbapenems (Fluit and Schmitz, 2004; Li et al., 2007). Furthermore, integrons are associated with a greatly increased resistance to fluoroquinolones. This is not surprising as resistance to fluoroquinolones can be derived through plasmid mediated quinolone resistance (PMQR) (Strahilevitz et al., 2009). PMQR also are associated with integrons that often simultaneously carry other resistance determinants. Examples include *bla*, *aad* or

Table 4
Antimicrobial resistance profiles and MICs for the isolates before and after curing.

Antimicrobial drugs	Bacterial isolate(s), MIC[a]					
	L8055239 (B)	L8055239 (A)	I3195059 (B)	I3195059 (A)	B5729897 (B)	B5729897 (A)
Amoxicillin/clavulanic acid	16	8	64	16	8	1
Ampicillin	**>256**	**4**	**>256**	**4**	**>256**	**≤0.5**
Cefotaxime	≤1	≤1	**16**	**≤1**	≤1	≤1
Cefoxitin	8	8	128	16	2	2
Ceftazidime	≤0.5	≤0.5	**64**	**2**	≤0.5	≤0.5
Cephalothin	256	32	>1024	32	**16**	**2**
Chloramphenicol	4	8	**1024**	**8**	**32**	**8**
Doxycycline	32	32	64	32	16	8
Enrofloxacin	64	64	>128	64	1	1
Gentamicin	**128**	**1**	**8**	**1**	**128**	**2**
Meropenem	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Tricarcillin/clavulanic acid	**256**	**4**	**256**	**8**	16	≤2
Trimethoprim/sulfamethoxazole	0.25	0.25	**>128**	**≤0.06**	**>128**	**0.12**

Numbers in boldface represent resistant phenotypes that becomes susceptible after curing. Only the curing of isolate B5729897 resulted in susceptible phenotype.

[a] MICs were determined using broth micro-dilution methods according to CLSI standards (CLSI, 2008).

aac, and *cat* genes which confer resistance to β-lactams, aminoglycoside, and chloramphenicol drugs (Li, 2005; Li et al., 2007; Robicsek et al., 2006). The molecular characterization of the relationship between PMQR and extended-spectrum β-lactamases and resistance in isolates carrying integrons is warranted.

The presence of an integron appears to have had a significant effect on the susceptibility to the aminoglycosides and the potentiated sulfa drugs tested. Our study characterized gene cassettes that confer resistance to aminoglycosides, including *aadA1*, *aadA2 aadA5*, *aacA4* and *aadB*, and genes that confer resistance to trimethoprim, including *dfrA1*, *dhfrA17* and *dfrA12*. The most common detectable gene cassette is the resistant gene, *aadA1*, which encodes for an aminoglycoside adenyltransferase that confers resistance to streptomycin–spectinomycin. A similar finding has been reported in other studies of *E. coli* isolates from veterinary sources (Goldstein et al., 2001; Kadlec and Schwarz, 2008). However, our study is the first to report the presence of *aacA4-catB3-dfrA1* in *E. coli* from companion animals in the US.

A BLAST search for the published sequence in GenBank revealed high similarities for the most prevalent gene cassettes found in *E. coli* isolates from different geographical locations and sources. This data suggests that class 1 integrons may contribute significantly to the horizontal transfer of antimicrobial resistance genes among bacterial species from different sources or geographical locations (Chang et al., 2000; Yu et al., 2003). However, the gene cassettes observed in class 1 and class 2 integrons did not correlate completely with the resistance phenotypes observed in these isolates.

The process of plasmid curing was more effective when 10% SDS was used compared with natural curing at low temperature. The mechanism of SDS action is not completely understood (El-Mansi et al., 2000). Consistent with a previous study (Tomoeda et al., 1968), the efficiency of curing in this study was low. It is possible that plasmids may survive by integration into the genome, even in the absence of selective pressure (El-Mansi et al., 2000). Furthermore, we found that some isolates lost other resistant determinants after curing (for example, resistance to some β-lactam drugs) which were not carried by class 1 integrons in this study (Table 4). It might be possible that other plasmids carrying resistance genes were also cured from the isolates.

The present study demonstrates the presence of class 1 and 2 integrons in clinical isolates of *E. coli* from dogs and cats. The proportion of isolates carrying class 1 and 2 integrons is not as high as found in food production animals and humans, which may be attributed to different antimicrobial pressures for isolates from companion animals. The gene cassettes characterized in the class 1 and class 2 integrons might reflect the selective pressures resulting from different antimicrobial therapy, drugs used, and the regions from which the isolates originated.

Acknowledgements

This work was supported in part by grant D07-MS 006 from Morris Animal Foundation. We thank IDEXX Diagnostic Laboratories for collecting and providing us with the isolates from throughout the US.

References

Bass, L., Liebert, C.A., Lee, M.D., Summers, A.O., White, D.G., Thayer, S.G., Maurer, J.J., 1999. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in Avian *Escherichia coli*. Antimicrob. Agents Chemother. 43, 2925–2929.

Chang, C.Y., Chang, L.L., Chang, Y.H., Lee, T.M., Chang, S.F., 2000. Characterization of drug resistance gene cassettes associated with class 1 integrons in clinical isolates of *Escherichia coli* from Taiwan, ROC. J. Med. Microbiol. 49, 1097–1102.

Chen, Y.M., Wright, P.J., Lee, C.S., Browning, G.F., 2003. Uropathogenic virulence factors in isolates of *Escherichia coli* from clinical cases of canine pyometra and feces of healthy bitches. Vet. Microbiol. 94, 57–69.

Clinical and Laboratory Standards Institute, 2008. Performance Standards for Antimicrobial Susceptibility Testing. 18th Informational Supplement. CLSI Document M100-S18 Wayne, PA.

Cocchi, S., Grasselli, E., Gutacker, M., Benagli, C., Convert, M., Piffaretti, J.C., 2007. Distribution and characterization of integrons in *Escherichia coli* strains of animal and human origin. FEMS. Immunol. Med. Microbiol. 50, 126–132.

Cohn, L.A., Gary, A.T., Fales, W.H., Madsen, R.W., 2003. Trends in fluoroquinolone resistance of bacteria isolated from canine urinary tracts. J. Vet. Diagn. Invest. 15, 338–343.

Du, X., Shen, Z., Wu, B., Xia, S., Shen, J., 2005. Characterization of class 1 integrons-mediated antibiotic resistance among calf pathogenic *Escherichia coli*. FEMS Microbiol. Lett. 245, 295–298.

El-Mansi, M., Anderson, K.J., Inche, C.A., Knowles, L.K., Platt, D.J., 2000. Isolation and curing of the *Klebsiella pneumoniae* large indigenous plasmid using sodium dodecyl sulphate. Res. Microbiol. 151, 201–208.

Fluit, A.C., Schmitz, F.J., 2004. Resistance integrons and super-integrons. Clin. Microbiol. Infect. 10, 272–288.

Goldstein, C., Lee, M.D., Sanchez, S., Hudson, C., Phillips, B., Register, B., Grady, M., Liebert, C., Summers, A.O., White, D.G., Maurer, J.J., 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob. Agents Chemother. 45, 723–726.

Guardabassi, L., Schewarz, S., Lloyd, D.H., 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. J. Antimicrob. Chemother. 54, 321–332.

Hagman, R., Kühn, I., 2002. *Escherichia coli* strains isolated from the uterus and urinary bladder of bitches suffering from pyometra: comparison by restriction enzyme digestion and pulsed-field gel electrophoresis. Vet. Microbiol. 84, 143–153.

Hall, R.M., Collis, C.M., 1998. Antibiotic resistance in gramnegative bacteria: the role of gene cassettes and integrons. Drug Resist. Updates 1, 109–119.

Kadlec, K., Schwarz, S., 2008. Analysis and distribution of class 1 and class 2 integrons and associated gene cassettes among *Escherichia coli* isolates from swine, horses, cats and dogs collected in the BfT-GermVet monitoring study. J. Antimicrob. Chemother. 62, 469–473.

Kang, S.G., Lee, D.Y., Shin, S.J., Ahn, J.M., Yoo, H.S., 2005. Changes in patterns of antimicrobial susceptibility and class 1 integron carriage among *Escherichia coli* isolates. J. Vet. Sci. 6, 201–205.

Keyes, K., Hudson, C., Maurer, J.J., Thayer, S., White, D.G., Lee, M.D., 2000. Detection of florfenicol resistance genes in *Escherichia coli* from sick chickens. Antimicrob. Agents Chemother. 44, 421–424.

Lanz, R., Kuhnert, P., Boerlin, P., 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. 91, 73–84.

Levesque, C., Piche, L., Larose, C., Roy, P.H., 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. 39, 185–195.

Li, X.Z., 2005. Quinolone resistance in bacteria: emphasis on plasmid-mediated mechanisms. Int. J. Antimicrob. Agents 25, 453–463.

Li, X.Z., Mehrotra, M., Ghimire, S., Adewoye, L., 2007. beta-Lactam resistance and beta-lactamases in bacteria of animal origin. Vet. Microbiol. 121, 197–214.

Machado, E., Cantón, R., Baquero, F., Galán, J.C., Rollán, A., Peixe, L., Coque, T.M., 2005. Integron content of extended-spectrum-β-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. Antimicrob. Agents Chemother. 49, 1823–1829.

Machado, E., Coque, T.M., Cantón, R., Sousa, J.C., Peixe, L., 2008. Antibiotic resistance integrons and extended-spectrum {beta}-lactamases

among Enterobacteriaceae isolates recovered from chickens and swine in Portugal. J. Antimicrob. Chemother. 62, 296–302.

Normand, E.H., Gibson, N.R., Reid, S.W., Carmichael, S., Taylor, D.J., 2000a. Antimicrobial-resistance trends in bacterial isolates from companion-animal community practice in the UK. Prev. Vet. Med. 46, 267–278.

Normand, E.H., Gibson, R.N., Taylor, D.J., Carmichael, S., Reid, S.W., 2000b. Trends of antimicrobial resistance in bacterial isolates from a small animal referral hospital. Vet. Rec. 146, 151–155.

Robicsek, A., Strahilevitz, J., Jacoby, G.A., Macielag, M., Abbanat, D., Park, C.H., Bush, K., Hooper, D.C., 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat. Med. 12, 83–88.

Rosser, S.J., Young, H.K., 1999. Identification and characterization of class l integrons in bacteria from an aquatic environment. J. Antimicrob. Chemother. 44, 11–18.

Rowe-Magnus, D.A., Mazel, D., 2002. The role of integrons in antibiotic resistance gene capture. Int. J. Med. Microbiol. 292, 115–125.

Sanchez, S., McCrackin Stevenson, M.A., Hudson, C.R., Maier, M., Buffington, T., Dam, Q., Maurer, J.J., 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. J. Clin. Microbiol. 40, 3586–3595.

Shaheen, B.W., Boothe, D.M., Oyarzabal, O.A., Smaha, T., 2008. Characterization of clinical *Escherichia coli* isolates expressing multidrug resistance recovered from canine and feline with spontaneous disease. In: Congress Proceedings of ACVIM Forum, San Antonio, TX, June 4–7, p. 786.

Sidjabat, H.E., Townsend, K.M., Lorentzen, M., Gobius, K.S., Fegan, N., Chin, J.J., Bettelheim, K.A., Hanson, N.D., Bensink, J.C., Trott, D.J., 2006. Emergence and spread of two distinct clonal groups of multidrug-resistant *Escherichia coli* in a veterinary teaching hospital in Australia. J. Med. Microbiol. 55, 1125–1134.

Strahilevitz, J., Jacoby, G.A., Hooper, D.C., Robicsek, A., 2009. Plasmid-mediated quinolone resistance: a multifaceted threat. Clin. Microbiol. Rev. 22, 664–689.

Sunde, M., 2005. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. J. Antimicrob. Chemother. 56, 1019–1024.

Tomoeda, M., Inuzuka, M., Kubo, N., Nakamura, S., 1968. Effective elimination of drug resistance and sex factors in *Escherichia coli* by sodium dodecyl sulfate. J. Bacteriol. 95, 1078–1089.

van Essen-Zandbergen, A., Smith, H., Veldman, K., Mevius, D., 2007. Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the Netherlands. J. Antimicrob. Chemother. 59, 746–750.

Yu, H.S., Lee, J.C., Kang, H.Y., Ro, D.W., Chung, J.Y., Jeong, Y.S., Tae, S.H., Choi, C.H., Lee, E.Y., Seol, S.Y., Lee, Y.C., Cho, D.T., 2003. Changes in gene cassettes of class 1 integrons among *Escherichia coli* isolates from urine specimens collected in Korea during the last two decades. J. Clin. Microbiol. 41, 5429–5433.