Genetic Characterization of Antibiotic Resistance Genes Linked to Class 1 and Class 2 Integrons in Commensal Strains of *Escherichia coli* Isolated from Poultry and Swine

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The aim of this research was to identify the presence of integrons among *Escherichia coli* strains isolated from poultry and swine and to characterize the topological association of these integrons with resistance genes and assess their potential ability to transfer these elements by conjugation. One hundred and seventy-two strains of *E. coli* were isolated. Their resistance to tetracycline, streptomycin, sulfamethoxazole-trimethoprim, ciprofloxacin, and enrofloxacin was studied by plate dilution. In resistant strains the presence of integrons and resistance genes was assessed by PCR. In the variable region, genes *aadA1*, *dfrA1*, and *qnr* were analyzed. Also, presence of *tetA*, *tetB*, and *sul1* was assessed. Transference of these genes and integrons *in vitro* was evaluated by conjugation assays, using *E. coli* J53 Az^r as recipient strain. Seventy-eight percent and 83% of the poultry and swine strains, respectively, were resistant to at least one of the studied antimicrobials. Of the isolated strains 91 presented integrons. Resistance genes detected within the integrons were *aadA1*, *dfrA1*, and *sat1*. Gene *qnr* was not detected. Genes *tet* and *sul1* were identified in 105 and 53 strains, respectively. Seven strains transferred their resistance determinants by conjugation. The results verify the high percentage of antibiotic resistance in the *E. coli* strains isolated, and these represent a reservoir of resistance genes and integrons.

Introduction

The emergence of bacterial antibiotic multiresistance has had a significant worldwide impact.[49] In this regard, programs for monitoring resistance have been implemented in many countries for the purpose of protecting the health of humans as well as animals.[1,20] These programs monitor zoonotic organisms, pathogens, and indicator bacteria such as *Escherichia coli* and *Enterococcus* spp. These bacteria are part of the normal enteric microbiota and can be reservoirs of antibiotic resistance genes transferable to other zoonotic bacteria and pathogens that can cause infections in animals and humans.[42] The evaluation of antibiotic resistance at a molecular level constitutes a tool for comprehending the participation of genetic elements in the expression of resistance and its possible transference between bacterial species.[4]

Integrons are found among the genetic elements that have contributed to the development of antimicrobial multiresistance in Gram-negative bacteria.[6,10] These elements capture different resistance cassettes and express them. The general structure of the integron consists of a conserved region that codes an integrase (*Int*) and a variable region (5′CS-3′CS) where various resistance cassettes can be integrated themselves.[30,43] Different classes of integrons have been described to date, among which are (i) class 1 integrons associated with the genes *sul1*, *qacED1*, and *ORF5*; (ii) class 2 integrons associated with the transposon *Tn7* and the genes *dfr1*, *aad1*, and *sat*; and (iii) class 3 integrons associated with the gene $bla_{imp.}$[6,16] Genes resistant to tetracycline have not been found as cassettes in class 1 integrons, but have been found associated with class 1 integrons on self-transferable plasmids in Gram-negative bacteria.[2,17,22]

Several studies have shown that the most common integrons in enterobacteria such as *E. coli* are those of class 1 and class 2.[15,17] Epidemiological studies of Enterobacteriaceae in France and The Netherlands showed that over half of the isolates tested carried integrons. These results could be extended to the rest of Western Europe.[10] Studies in China show that the incidence of integrons in *E. coli* differs among animal species and is greater in poultry than in swine.[50]

The integrons cannot perform self-transference, but can become associated with transposon, insertion sequences, and/or conjugative plasmids that serve as vehicles for their

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transmission.[11] The transference of these resistance determinants through mobile elements can lead to the selection of diverse resistance genes even if the bacterial strains have not been previously exposed to the antimicrobial agent in question.[44]

The levels of resistance and multiresistance have been evaluated for strains of *Salmonella* spp. isolated from poultry,[38] *E. coli* and *Enterococcus* spp. isolated from pigs and poultry,[36] and *E. coli* isolated from cattle[36] in Chilean food animals. However, little is known so far about resistance genes and the transmission of integrons.

The aim of this study was to characterize, among strains of *E. coli* isolated from healthy poultry and swine, the presence of integrons and their structural association with gene cassettes encoding for resistance to streptomycin, trimethoprim, and fluoroquinolones harbored in the variable regions of these genetic elements. In addition, the presence of *tet* and *sul1* genes was identified in these strains. The *sul1* gene is associated with sulfonamide resistance and is a good indicator of class 1 integrons. Finally, we evaluated the possible transference of these molecular structures by conjugation.

Materials and Methods

Acquisition of samples

Samples were collected during a period of ten months. Studies were performed on 85 strains of *E. coli* isolated from laying hens and broiler chickens from 30 different farms. Samples were obtained at a cloacal level with sterile swabs. A maximum of three isolates from each origin were required for acceptance.

As well, 87 strains of *E. coli* were isolated from swine from 15 different farms. The swine samples were collected in slaughterhouses, taking from the large intestine 5 ± 0.5 g of fecal content and depositing it in sterile bottles. A maximum of six isolates from each origin were taken. All of the samples were transported to the laboratory by means of Cary-Blair transport (Difco; Becton, Dickinson and Company, Franklin Lakes, NJ).

Isolation and identification of E. coli strains

The samples were streaked directly in McConkey agar (Difco) and incubated at 37°C for 18 to 24 hours. The suspect colonies were identified by rapid diagnostic test API20E (bioMerieux, Durham, NC). One colony was selected as a sample for analysis. The isolated samples were frozen at −70°C in brain/heart broth containing 15% glycerol.

Antimicrobial susceptibility test

Antimicrobial susceptibility was established by the plate dilution method (to determine the distribution of minimum inhibitory concentration [MIC]), following the recommended norms of the Clinical and Laboratory Standards Institute (CLSI).[8] *E. coli* strains were tested for the following antimicrobial drugs: tetracycline (Sigma 96%; Sigma-Aldrich Corp., St. Louis, MO), streptomycin (Sigma 98%), sulfamethoxazol-trimethoprim (Sigma 100%), ciprofloxacin (USP Standard 100%; United States Pharmacopeia, Rockville, MD), and enrofloxacin (Lab Chile 100%; Laboratorios Chile, Santiago, Chile). For streptomycin the breakpoint given in the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP)[20] was used. For the other antimicrobials the breakpoints of the CLSI were used. *E. coli* ATCC 25922 was used as quality control.

Detection of resistance determinants by PCR in strains of E. coli

In the *E. coli* strains resistant to tetracyclines the genes *tetA* and *tetB* were detected. In the case of the strains resistant to streptomycin, gene *aadA1* was assessed. For sulfamethoxazol-trimethoprim resistant strains, genes *sul1* and *dfrA1* were detected, and for the fluoroquinolone-resistant strains gene *qnr* was assessed (Table 1).

The bacterial strains were streaked in Luria-Bertani (LB) broth (Difco) and incubated at 37°C for 18 hours, to obtain lysed bacteria according to the protocol described by Ling *et al.*[24] DNA amplification was performed according to the protocol described by San Martín *et al.*[38] The mixture was transferred to a thermocycler (Eppendorf North America, Westbury, NY), programmed with 35 cycles of 1 minute at 94°C, 30 seconds at the annealing temperature specific for each antimicrobial agent indicated in Table 1 and 2 minutes at 72°C, with a final extension of 10 minutes at 72°C. Ten microliters of each sample of amplified DNA were run in 1% agarose gels in an electrophoresis chamber (Bio-Rad Laboratories, Hercules, CA) in TAE buffer (Winkler Ltda,

Table 1. Primers and Annealing Temperatures Used in the PCR Assays for Each Resistance Gene

Antimicrobial	*Gene*	*Product size (bp)*	*Annealing temp (°C)*	*Primer sequence (5′ to 3′)*	*Reference or GenBank accession no.*
Streptomycin	*aadA1(F)* *aadA1(R)*	447	58	TATCCAGCTAAGCGCGAACT ATTTGCCGACTACCTTGGTC	EF 560799
Tetracycline	*tetA(F)* *tetA(R)*	577	56	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	28
	tetB(F) *tetB(R)*	634	56	CCTCAGCTTCTCAACGCGTG GCACCTTGCTGATGACTCTT	28
Trimethoprim	*dfrA1(F)* *dfrA1(R)*	367	45	GGAGTGCCAAAGGTGAACAGC GAGGCGAAGTCTTGGGTAAAAAC	45
Fluoroquinolone	*qnr(F)* *qnr(R)*	670	50	GGGTATGGATATTATTGATAAAG CTAATCCGGCAGCACTATTTA	25

Table 2. Primers and Annealing Temperatures Used for the Detection of Class 1 and Class 2 Integrons

Target gene or region(s)	*Product size (bp)*	*Annealing temp (°C)*	*Primer sequence (5′ to 3′)*	*Reference*
intI1(F)	280	60	CCTCCCGCACGATGATC	15
intI1(R)			TCCACGCATCGTCAGGC	
intI2(F)	232	60	TTATTGCTGGGATTAGGC	15
intI2(R)			ACGGCTACCCTCTGTTATC	
5′CS class 1	variable[a]	56	GGCATCCAAGCAGCAAG	44
3′CS class 1			AAGCAGACTTGACCTGA	
5′CS class 2	variable[a]	56	GACGGCATGCACGATTTGTA	22
3′CS class 2			GATGCCATCGCAAGTACGAG	
sul1(F)	435	56	CTTCGATGAGAGCCGGCGGC	14
sul1(R)			GCAAGGCGGAAACCCGCGCC	

[a]The sizes of the 5′CS-3′CS zone depend on the number of inserted genes in this region of the integron.

Santiago, Chile). Markers with molecular weights of 100 bp and 1 kbp (New England Biolabs, Ipswich, MA) were used. The bands of DNA were stained with ethidium bromide for visualization in a UV light transilluminator.

As a positive control, strains of *E. coli* phenotypically resistant to the studied antimicrobial agents and positive for PCR were used. Amplified fragments were sequenced and confirmed using Basic Local Alignment Search Tool (BLAST), against GenBank database, accession numbers EF560799, DQ464880, J01830, EF450247, and EF543147, for the genes *aadA1*, *tetA*, *tetB*, *sul1*, and *dfrA1*, respectively. As a negative control, a strain of susceptible *E. coli* was used.

Detection of class 1 and class 2 integrons by PCR. In all of the bacterial strains that presented the resistance genes shown in Table 1, the genes *intI1*, *sul1*, and *intI2* and the 5′CS-3′CS variable region for both class 1 and class 2 integrons were amplified. The PCRs were performed with the methodology previously described, using the primers and annealing temperatures indicated in Table 2. A marker with a molecular weight of 1 kbp (New England) was used.

Structural association between resistance genes and integrons by PCR. To determine which array of gene cassettes form part of the integrons, different primer associations were used. The 5′CS primer of the variable region of the class 1 integron was associated with *aadA1(R)*, *dfrA1(R)*, *tetA(R)* or *tetB(R)*. The 5′CS primer of the variable region of the class 2 integron was associated with *aadA1(R)*, *dfrA1(R)*, *tetA(R)* or *tetB(R)*. The primer *dfrA1(F)* was associated with *aadA1(R)*. All of the PCR amplifications were conducted as previously described, at an annealing temperature of 55°C. In accordance with the genes found in the variable region and their multiresistance profiles, the isolates were divided into seven groups.

Transference of resistance genes and integrons by conjugation

Conjugation experiments were carried out with donor strains that presented resistance genes within integrons. Some of these strains also presented resistance genes to tetracycline that were not structurally associated with the integron. *E. coli* J53 Az^r resistant to sodium azide was used as a recipient strain.[47]

Conjugation was completed according to the protocol prescribed by the Laboratory of Microbiology of the Department of Microbiology and Immunology of the University of Texas Medical Branch. The strains were cultivated at 37°C for 18 hours in LB broth. The donor strains were supplemented with the antimicrobial agents to which they were resistant. The recipient strains were supplemented with 100 μg/ml of sodium azide.[47] A dilution of 1:10 was performed in LB broth for each donor strain and 1:100 for the recipient strain, incubating at 37°C during 1 hour of agitation. Two hundred and fifty microliters of each strain were mixed in an eppendorf tube and centrifuged at 8000 rpm for 5 minutes. The pellets were suspended and streaked on an LB agar plate without antibiotics. They were incubated for 4 hours at 37°C, and the bacterial colonies were resuspended in LB broth. Hundred microliters were streaked in an LB agar plate that contained a combination of sodium azide with the corresponding antimicrobial agent according to resistance phenotype. The plates were incubated at 37°C for 18 hours.

The plasmid DNA was extracted from the transconjugant strains by alkaline lysis.[34] Visualization of the plasmids was accomplished by electrophoresis in 0.8% agarose gels in TAE buffer. The plasmid DNA was stained with ethidium bromide and visualized in a UV light transilluminator. *E. coli* J53 plac and *E. coli* R1,[47] which present plasmids of 152 kbp and 92 kbp, respectively, were used as controls. Transconjugants were tested for resistance to antimicrobial agents (corresponding to the resistance profile of the donor) in a standard disc diffusion test using commercial drug discs (Arlab, Santiago, Chile), with Mueller–Hinton agar (Difco). The inhibition zones used to classify the strains as resistant or susceptible were those recommended by the CLSI.[8] The presence of the integron and the resistance genes associated with the plasmid were confirmed by PCR using DNA from transconjugant strains as a template.

Results

Susceptibility tests in vitro

Of the 85 poultry strains and 87 swine strains that were isolated, 78% and 83% respectively were resistant to at least one antimicrobial agent. The distribution of the MICs for each antimicrobial agent is shown in Table 3.

Table 3. Percentages of Resistance and Distribution of Minimum Inhibitory Concentrations (MICs) Obtained in Strains of *Escherichia coli* Isolated from Poultry ($N = 85$) and Swine ($N = 87$)

			Distribution of MICs μg/ml (N)										
Antimicrobial	*Animal species*	*% Resistant strains*	*0.125*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*
Tetracycline	Poultry	77%	—	—	—	4	11	4	—	5	14	7	40
	Swine	77%	—	—	—	—	7	12	1	14	19	12	22
Streptomycin	Poultry	45%	—	3	1	2	17	5	7	12	29	6	3
	Swine	74%	3	1	—	—	—	—	15	3	18	33	14
Enrofloxacin	Poultry	23%	64	1	—	—	—	20	—	—	—	—	—
	Swine	10%	68	10	—	—	—	9	—	—	—	—	—
Ciprofloxacin	Poultry	23%	65	—	—	—	—	20	—	—	—	—	—
	Swine	10%	78	—	—	—	—	9	—	—	—	—	—

			Distribution of MICs μg/ml (N)			
			2/38	4/76	8/152	32/512
Trimethoprim Sulfamethoxazole	Poultry Swine	14% 38%	63 49	10 5	12 33	— —

N = Number of strains.

Multiresistance profiles

Multiresistance was defined as simultaneous resistance to at least two different groups of antimicrobials tested. According to this, five profiles of multiresistance were determined in the *E. coli* strains, being greater in those isolated from swine (Table 4).

Detection of resistance genes and integrons

A large percentage of the *E. coli* strains that were resistant to tetracycline presented the assessed genes. In the poultry strains a similar proportion of the genes *tetA* and *tetB* was detected. In swine isolates, *tetB* gene was more frequent. A large percentage of the strains resistant to streptomycin and trimethoprim amplified the *aadA1* and *dfrA1* genes, respectively. With regard to the strains resistant to fluoroquinolones, none presented the gene *qnr* (Table 5).

Of the 25 poultry strains, 37.3% presented integrons. Of the 66 swine strains, 90% presented this genetic structure. The class 1 integron was the most common in both animal populations. All strains containing class 1 integrons were positive for the presence of the *sul1* gene (Table 5).

Table 4. Profiles of Multiresistance in Strains of *Escherichia coli* Isolated from Poultry and Swine

Profiles of multiresistance	*No. of poultry strains*	*No.of swine strains*
TET + STR	14	29
TET + STR + SXT	5	9
TET + STR + CIP/ENR	13	6
TET + STR + CIP/ENR + SXT	1	3
STR + SXT	5	18
Total multiresistant strains	38	65

TET, Tetracycline; STR, Streptomycin; SXT, Trimethoprim/Sulfamethoxazole; CIP, Ciprofloxacin; ENR, Enrofloxacin.

Integrons and their relationship with resistance genes

Of the 91 strains that presented integrons, 79 amplified the variable region, with different molecular sizes. For the class 1 integrons variable regions of 1.0 kbp and 1.6 kbp were obtained, while for the class 2 integrons the variable regions sizes were 1.5 kbp, 2.2 kbp, and 2.5 kbp (Table 6). Twelve strains positive for integrases did not amplify the variable region.

The *aadA1* resistance cassette was found inserted in all of the class 1 and class 2 integrons. This resistance cassette was found associated with the *dfrA1* gene cassette in 21 strains, and with *estX-sat2* in four strains. The three resistance cassette genes (*dfrA1-sat-aadA1*) were found inserted in 18 strains (Table 6). The genes *tetA*, *tetB*, and *qnr* were not found within the integrons.

Table 5. Distribution of Genes Encoding for Resistance to Antimicrobial Agents and Class 1 and Class 2 Integrons in the *Escherichia coli* Strains Isolated from Poultry and Swine

	No. of resistant strains			*Strains positive to genetic marker (%)*	
Antimicrobial/ Integron	*Poultry*	*Swine*	*Genetic marker*	*Poultry*	*Swine*
Tetracycline	66	67	*tetA*	33	1.5
			tetB	38	85
			tetA + tetB	1.5	0
Streptomycin	38	65	*aadA1*	100	95
Trimethoprim	12	33	*dfrA1*	100	100
Fluoroquinolones	20	9	*qnr*	0	0
Class 1 Integron			*intI1*	23%	57%
			sul1	100%	100%
Class 2 Integron			*intI2*	15%	35%

Table 6. Genes Cassettes Within the Class 1 and Class 2 Integrons in Phenotypically Resistant Strains of *Escherichia. coli*

Groups	No. of poultry strains	No.of swine strains	Amplicon size and genes cassette	Resistance phenotypes
Strains of E. coli *positive for class 1 integrons*				
I	7	29	1.0 kbp, *aadA1*	TET + STR
II	5	8	1.6 kbp, *dfrA1-aadA1*	TET + STR + SXT
III	0	4	1.6 kbp, *dfrA1-aadA1*	STR + SXT
Strains of *E. coli* positive for class 2 integrons				
IV	0	4	1.5 kbp, *dfrA1-aadA1*	STR + SXT
V	5	12	2.2 kbp, *dfrA1-sat1-aadA1*	STR + SXT
VI	1	0	2.2 kbp, *dfrA1-sat1-aadA1*	TET + STR + ENR/CIP + SXT
VII	1	3	2.5 kbp, *estX-sat2-aadA1*	TET + STR + ENR/CIP

TET, Tetracycline; STR, Streptomycin; SXT, Trimethoprim/Sulamethoxazole; CIP, Ciprofloxacin; ENR, Enrofloxacin.

In order to confirm our results, one representative strain from each group was sequenced in the Retrogen Inc. Laboratory of San Diego, CA. The results of the sequencing were compared with the GenBank database, using the following accession numbers: EF543148 (group I), EF488370 (groups II and III), EF031067 (group IV), EF543147 (groups V and VI), and AB161463 (group VII). The presence of the genes *sat1* and *estX-sat2* within the integron was identified when the sequencing was performed. This was confirmed with the GenBank using *E. coli* EF543147 and *Salmonella* Enteritidis AB161461, respectively (Table 6).

Transference of resistance determinants by conjugation

Conjugation assays were performed on 79 strains that presented integrons containing resistance genes. Fifty-four of these strains also presented *tet* resistance genes. Of the total strains assessed, seven (8.8%) were capable of transferring their genetic resistance determinants by conjugation. Furthermore, in three of the swine transconjugant strains, *tetB* gene and a plasmid of approximately 92 kbp were found. Also, two of the swine transconjugant strains presented a plasmid of molecular weight between 92 and 152 kbp and a class 1 integron with the cassette *aadA1*, in addition to the *tetB* gene. The two transconjugant poultry strains presented a plasmid of molecular weight between 92 and 152 kbp, and the class 1 integron with the *aadA1* gene as well as the *tetA* gene were found probably inside the plasmid. The resistance phenotypes and the resistance determinants of each transconjugant strain were confirmed by Kirby Bauer method and PCR, respectively.

Discussion

In the present study more than 78% of *E. coli* strains, isolated from poultry and swine, presented resistance to at least one studied antimicrobial agent (tetracyclines, streptomycin, sulfamethoxazole + trimethoprim, and fluoroquinolones). The resistance to these drugs has also been described in programs performed by different countries that monitor bacterial resistance in veterinary medicine. Among these programs, we can mention DANMAP[20] from Denmark, NORM-VET[44] from Norwegia, and the JVARM[5] from Japan. In fact, in some countries more than half of all commercial antibiotics are used in food-producing animals.[13,40]

The most frequently observed multiresistance profiles were TET-STR, STR-SXT, and TET-STR-CIP-ENR. Our results confirm the broad distribution of multiresistance in strains obtained from food-producing animals. This situation has been widely reported at the international level[18,23,50] as well as at the national level.[37,38]

Integrons present among animal isolates are disseminated worldwide and are considered as important contributors to the development of antibiotic resistance.[15,18] Among these, the class 1 integron is the most prevalent type. In this regard, van Essen-Zadbergen *et al.*,[46] Golstein *et al.*,[15] and Phongpaichit *et al.*[26] determined the percentage of class 1 integrons in this species of bacteria as 76%, 66%, and 63.5%, respectively.

In this study we found that a large percentage (66%) of *E. coli* strains harbored integrons, the class 1 integron being the most prevalent. The strains isolated from poultry showed lower percentages of integrons than those isolated from swine. Our results contrast with the studies by Yang *et al.*,[50] who described greater percentages of integrons in isolates of chickens compared to the strains obtained from swine. These discrepancies could be due to the fact that Yang *et al.* analyzed *E. coli* strains isolated from diseased animals, while in the present study the isolates were obtained from healthy animals. Thus, the prevalence of integrons could be associated with the different usage of antimicrobials between food-producing animal species, or it could be associated with the different kind of bacterial illness present in determinate geographic regions.

Of all the strains that presented integrons, 12 isolates (13%) did not carry gene cassettes, the so-called empty integrons. This situation also has been described by other authors,[12,39] pointing out that these bacteria can have the potential in the future to convert themselves rapidly into multiresistant strains. In this regard, Rosser and Young[31] proposed that "empty" integrons represent ancestral elements that have not yet acquired gene cassettes inserted between the conserved segments of the integrons.

While studying the genes that form part of the integron, we observed that the cassette array most frequently found associated with class 1 integrons was *aadA1.* On the other

hand, *dfrA1-sat1-aadA1* was most often associated with class 2 integrons.

In the analysis of each gene individually, the *aadA1* gene, which confers resistance to streptomycin and spectinomycin, was found in all of the strains that presented class 1 or class 2 integrons. The predominance of this cassette suggests that this could be the first to be acquired by an integron and/or could be more stable than the other genetic cassettes as indicated by Kang *et al.*[21] On the other hand, the high predominance of this gene could be related to the extensive use of these aminoglucosides in the control of infectious diseases in these food-producing animals. In fact, streptomycin and spectinomycin are antimicrobians used often in Chile in the treatment of digestive and respiratory bacterial illness in poultry and swine.

The second most frequent gene within integrons was the *dfrA1*, which confers resistance to trimethoprim. This gene has been described in class 1 and class 2 integrons.[21,35,51] In the analysis of the genetic profiles of the integrons, this gene, *dfrA1*, was always found associated with the genes *aadA1* and *sat*. Both of these genes confer resistance to aminoglucosides, and the *sat* gene in particular confers resistance to streptothricin. Furthermore, four strains presented the gene cassette array *estX-sat2-aadA1.* This infrequent array has been described by other authors in *Salmonella* Enteritidis strains isolated from humans and from river water in Japan, and in *E. coli* strains isolated from swine in Korea and Norway.[3,21,43] In the United States, Singh *et al.*[41] detected the presence of the gene *sat* in strains of *E. coli* isolated from food-producing animals, even though the use of streptothricin is not approved in that country. On the other hand, resistance to streptothricin has been reported in Gram-negative bacteria following use of nourseothricin.[48] Streptothricin and nourseothricin are antimicrobial agents that have never been approved for use in Chile in these food-producing species. So the finding of the gene *sat* in our study could be associated with the use of other antibiotics such as trimethoprim or streptomycin.

Among the genetic mechanisms of resistance to fluoroquinolones is the *qnr* gene, which can be found within the variable region of class 1 integron. This gene has been described in strains of *Klebsiella* pneumoniae, *E. coli*, and *Salmonella* Enteritidis, isolated from human patients and has been currently associated with conjugative plasmids, which confer them horizontal transferability.[25,29,47] In this regard, none of the isolated strains that presented phenotypic resistance to both enrofloxacin and ciprofloxacin amplified the gene *qnr*, so another molecular mechanism of resistance could be present in these strains. Within these mechanisms, alterations of the bacterial membrane permeability as well as mutations in the QRDR region of the gene gyrase subunit A have been described.[27,33] Mutations in this region have been previously described by San Martín *et al.*[38] in *Salmonella* spp. strains isolated from laying hens and broiler chickens. However, as the aim of the present study was to determine the resistance associated with integrons, the QRDR region was not characterized.

In the present study we also identified the *sul1* gene, which is out of the variable region of the integron. Located in the 3′CS region of the class 1 integron, this gene confers resistance to sulfonamides and is a real indicator of the presence of this integron.[6] Thus, all the strains positive to class 1 integron amplified the *sul1* gene.

The genes encoding resistance to tetracyclines, *tetA* and *tetB*, were also analyzed. These genes are widely spread among Enterobacteriaceae.[7,9,23] It has been demonstrated that the *tet* genes can be present in a plasmid that carry a class 1 integron, so they can transfer horizontally to other bacterial strains themselves through these elements.[44] The strains isolated in this work showed a high percentage of resistance to tetracyclines (77%), and a large number of them amplified the *tetB* and *tetA* genes. None of these genes were found in the variable region of the integrons; however, seven strains that amplified the *tetB* or *tetA* transferred these genes by conjugation to the recipient strains, *E. coli* J53 Az^r. Four of these seven strains also transferred the class 1 integron and the gene *aadA1*. In all of the transconjugant strains a self-transferable plasmid was identified.

The integrons present principally in fermenting Gram-negative bacilli of the Enterobacteriaceae family cannot perform self-transference. However, when associated with conjugative plasmids, the potential horizontal transfer ability of integrons is considered a risk with regard to the dissemination of multiresistance.[32] Although we observed a low efficiency of transfer by conjugation of integrons at the laboratory conditions, it is important to consider that the efficiency of transfer *in vivo* could be very different.

The results obtained in this study verify the large percentage of resistance in indicator strains of *E. coli* isolated from poultry and swine in Chile. These strains represent an important potential reservoir of resistance genes and integrons. This information should ultimately provide important guidance for the development of public health policy for use of antimicrobials in food animal production in developing countries.

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Disclosure Statement

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