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Characterization of antibiotic resistance genes linked to class 1 and 2 integrons in strains of *Salmonella* spp. isolated from swine

Betty San Martín, Lisette Lapierre, Javiera Cornejo, and Sergio Bucarey

Abstract: The aim of this study was to characterize the antibiotic resistance profiles, the integron-associated resistance determinants, and the potential ability of transferring these determinants by conjugation in *Salmonella enterica* isolated from swine. Fifty-four strains of *Salmonella* spp. were isolated from healthy swine. The percentages of resistance, determined by the plate dilution method were as follows: oxytetracycline (41%), streptomycin (39%), sulphamethoxazol+trimethoprim (19%), enrofloxacin–ciprofloxacin (13%), and amoxicillin (0%). The most important resistance serovars were *Salmonella* Branderburg, *Salmonella* Derby, *Salmonella* Typhimurium, and *Salmonella* Heidelberg. The oxytetracycline-resistant strains amplified the genes *tetA* (36%), *tetB* (64%); and the strains resistant to streptomycin and trimethoprim amplified the genes *aadA1* (100%) and *dfrA1* (100%), respectively. None of the fluoroquinolone-resistant strains amplified the gene *qnr*. Ten strains amplified the class 1 integron harboring the cassette *aadA1*. Six strains amplified the class 2 integron harboring the cassettes *dfrA1*, *sat1*, and *aadA1*. The conjugation assays showed that 2 strains transferred the *tetA* and *aadA1* genes and the class 1 integron to a recipient strain. Taken together, the results obtained in this study show a high percentage of resistance in and the presence of integrons in strains of *S. enterica* isolated from swine. This information should support the implementation of regulations for the prudent use of antimicrobial agents in food-producing animals.

Key words: multiresistance, *Salmonella*, integrons, resistance genes.

Résumé : Le but de cette étude était de caractériser les profiles de résistance aux antibiotiques, les déterminants de résistance associés à des intégrons et la capacité potentielle de transfert de ces déterminants par conjugaison chez *Salmonella enterica* isolée du porc. Cinquante-quatre souches de *Salmonella* spp. ont été isolées de porcs en bonne santé. Les pourcentages de résistance, déterminés par la méthode de dilution en plaques étaient les suivants : oxytétracycline (41 %), streptomycine (39 %), sulphaméthoxazol + triméthoprime (19 %), enrofloxacine–ciprofloxacine (13 %) et amoxicilline (0 %). Les sérovars résistants les plus importants consistaient en *Salmonella* Branderburg, *Salmonella* Derby, *Salmonella* Typhimurium et *Salmonella* Heidelberg. Les souches résistantes à l'oxytétracycline comportaient une amplification des gènes *tetA* (36 %) et *tetB* (64 %) alors que chez les souches résistantes à la streptomycine et au triméthoprime, les gènes *aadA1* (100 %) et *dfrA1* (100 %) étaient respectivement amplifiés. Aucune des souches résistantes au fluoroquinolone ne comportait d'amplification du gène *qnr*. Dix souches comportaient une amplification d'un intégron de classe 1 contenant la cassette *aadA1*. Six souches montraient une amplification d'un intégron de classe 2 contenant les cassettes *dfrA1*, *sat1* et *aadA1*. Les essais de conjugaison ont démontré que 2 souches pouvaient transférer les gènes *tetA* et *aadA1* et l'intégron de classe 1 à une souche réceptrice. En somme, les résultats obtenus dans cette étude révèlent un haut pourcentage de résistance et la présence d'intégrons dans les souches de *S. enterica* isolées du porc. Cette information devrait appuyer la mise en oeuvre d'une réglementation visant l'utilisation avisée d'agents antimicrobiens chez les animaux de consommation.

Mots-clés : résistance multiple, *Salmonella*, intégrons, gènes de résistance.

[Traduit par la Rédaction]

Introduction

The genus *Salmonella* encompasses a large taxonomic group with over 2500 recognized serovars. These microorganisms are difficult to control in food animal environments, since animals may be asymptomatic fecal shedders. Carrier animals play an important role in the spread of infection as sources of food contamination and human infection. Salmonellosis is one of the most important zoonotic diseases caused by food poisoning. The contamination sources of greatest risk are food products coming from poultry, swine, and cattle.

The emergence of antimicrobial resistance in zoonotic bacteria has had a significant worldwide impact. Data suggest that inadequate selection and abuse of antimicrobials

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may lead to resistance in various bacteria and, furthermore, complicate the treatment of bacterial infections (WHO 2001).

The evaluation of drug resistance at a molecular level is an important tool for understanding the participation of genetic elements in the expression of resistance and its possible transference among bacteria (Fluit et al. 2001; Alekhun and Levy 2007).

High levels of multidrug resistance are normally associated with mobile genetic elements that encode specific resistance genes (Randall et al. 2004). Among these genetic elements are the integrons, which are structures that can integrate and express resistance genes. The essential components of an integron include a conserved region with the gene *intI*, which encodes an integrase. Near this gene, there is a specific recombination site and a variable region where different resistance cassettes are integrated. Six classes of integrons have already been identified to date (Nield et al. 2001). The class 1 integron has been associated with the genes *sul1*, *qacE*Δ*1*, and *ORF5*. The class 2 integron has been associated with the transposon Tn*7* and with the genes *dfrA1*, *sat*, and *aadA1* (Carattoli 2001; Fluit and Schmitz 2004). On the other hand, genes encoding for resistance to tetracycline have not been found as a gene cassette within integrons. Genes encoding for tetracycline resistance have been found associated with class 1 integrons in self-transferable plasmids, in gram-negative bacteria (L'Abée-Lund and Sorum 2001; Guerra et al. 2002; Agerso and Sandvang 2005).

Integrons are not able to self-transfer, but they can associate themselves with insertion sequences present in transposons and (or) conjugative plasmids that serve as a vehicle for their inter- or intra-species transmission (Fluit and Schmitz 2004). The transfer of these genetic resistance determinants through mobile elements may lead to the selection of diverse resistance genes in strains of susceptible bacteria that have not been previously exposed to the antimicrobial agent in question (Sunde and Nostrom 2006).

The levels of resistance and multiresistance in Chilean food-producing animals have been evaluated in strains of §Escherichia coli, *Salmonella* spp., and *Enterococcus* spp. from swine, poultry, and cattle (San Martín et al. 2005*a*, 2005*b*, 2005*c*). However, little is known about resistance genes and the transmission of integrons in our country and in South America.

The objective of this study was to characterize, among strains of *Salmonella* spp. isolated from clinically healthy swine, the antibiotic resistance profiles to the common antimicrobials groups used in veterinary medicine. The integron-associated resistance determinants and their potential ability to transfer these determinants by conjugation were assessed. In addition, the presence of *tet* genes, which confer resistance to tetracyclines, were identified in these strains.

Materials and methods

Sampling

A total of 1198 fecal samples were obtained during a period of 10 months with a maximum of 10 samples per origin, to avoid collecting repeated specimens from the same site. Samples were collected in 6 slaughterhouses from swines coming from 126 different farms. Fecal content (5 ± 0.5 g) from the large intestine were deposited in sterile tubes (Fisherbrand) containing Cary–Blair transport medium (Difco). All samples were transported to the laboratory within 24 h.

Isolation and identification of *Salmonella* strains

The fecal samples were cultured in Rappaport Vassiliadis broth (Difco) at 37 °C for 24, 48, and 72 h, and were streaked every 24 h on XLD (xylose lysine deoxycholate) agar. Suspicious colonies were identified by the rapid diagnostic test API20E, (bioMerieux, Dirham, North Caroline, USA) and by the fast agglutination test with polyvalent A-I and Vi sera (Difco). Only one *Salmonella* strain was selected per sample. Serotyping was performed by the Public Health Institute of Chile.

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, following the recommended norms of the Clinical Laboratory Standard Institute (CLSI 2006). *Salmonella* spp. strains were tested for the following antimicrobial drugs: oxytetracycline (Sigma, 96%), streptomycin (Sigma, 98%), trimethoprim–sulfamethoxazole (Sigma, 100%), ciprofloxacin (USP Standard, 100%), enrofloxacin (Lab Chile 100%), and amoxicillin (Oxoid, 98%). The breakpoint given by the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP 2005) was used for streptomycin. The breakpoints of the CLSI were used for the other antimicrobials. Quality control for susceptibility testing was performed using *E. coli* ATCC 25922.

Detection of genetic determinants for resistance by PCR

Detection of resistance genes

The following genes were detected for the strains of *Salmonella* spp. resistant to oxytetracycline, streptomycin, trimethoprim, and fluoroquinolones: *tetA*, *tetB*, and *tetG*; *aadA1*; *dfrA1*; and *qnr*; respectively (Table 1).

The lysed bacteria were obtained according to the protocol described by Ling et al. (2003). Strains were cultivated in Luria–Bertani (LB) broth (Difco) and incubated at 37 °C for 18 h. The amplification of DNA was done according to the protocol described by San Martín et al. (2005*c*). In each reaction, 3 μL of the lysed bacteria was used and was mixed with 1 μL (25 pmol) of each primer (Table 1), 1 μL each of 200 μmol/L dinucleotide triphosphates (Promega), 3 μL of 1.5 mmol/L magnesium chloride (Invitrogen), 5 μL of 10× *Taq* buffer (Invitrogen), 0.2 μL (0.5 UI) of *Taq* polymerase enzyme (Invitrogen), and 36.8 μL of sterile deionized water free of DNase and RNase (Invitrogen). The mixture was carried to a thermocycler (Eppendorf), programmed with 35 cycles each of 1 min at 94 °C, 30 s at the hybridization temperature (shown for each antibiotic in Table 1), and 1 min at 72 °C. The program finished with an extension cycle of 10 min at 72 °C.

Ten microlitres of each amplified DNA was run in 1% agarose gels in an electrophoresis camera (BioRad in Tris–acetate–EDTA (TAE) buffer (Winkler). The DNA bands

Table 1. Primers and annealing temperatures used in the amplification reactions.

Antibiotic	Gene or region(s)	Product size (bp)	Annealing temp. (°C)	Primer sequence (5′ to 3′)	Reference or GenBank acc. No.
Streptomycin	*aadA1*	447	58	Forward: TATCCAGCTAAGCGCGAACT; Reverse: ATTTGCCGACTACCTTGGTC	EF592570
Oxytetracycline	*tetA*	577	52	Forward: GGTTCACTCGAACGACGTCA; Reverse: CTGTCCGACAAGTTGCATGA	Randall et al. 2004
	tetB	751	52	Forward: CTGGATTACTTATTGCTGGC; Reverse: CACCTTGCTGATGACTCTT	Randall et al. 2004
	tetG	604	56	Forward: CCGGTCTTATGGGTGCTCTA; Reverse: GACTGGCTTCGTTCTTCTGG	Randall et al. 2004
Trimethoprim	*dfrA1*	367	45	Forward: GGAGTGCCAAAGGTGAACAGC; Reverse: GAGGCGAAGTCTTGGGTAAAAAC	Toro et al. 2005
Enrofloxacin and ciprofloxacin	*qnr*	670	50	Forward: GGGTATGGATATTATTGATAAAG; Reverse: CTAATCCGGCAGCACTATTTA	Mammeri et al. 2005
	intI1	280	60	Forward: CCTCCCGCACGATGATC; Reverse: TCCACGCATCGTCAGGC	Goldstein et al. 2001
	intI2	232	60	Forward: TTATTGCTGGGATTAGGC; Reverse: ACGGCTACCCTCTGTTATC	Goldstein et al. 2001
	Class 1 integron	Variable*	56	5′CS: GGCATCCAAGCAGCAAG; 3′CS: AAGCAGACTTGACCTGA	Sunde and Nostrom 2006
	Class 2 integron	Variable*	56	5′CS: GACGGCATGCACGATTTGTA; 3′CS: GATGCCATCGCAAGTACGAG	L'Abée-Lund and Sorum 2001
	sul1	435	56	Forward: CTTCGATGAGAGCCGGCGGC; Reverse: GCAAGGCGGAAACCCGCGCC	Gebreyes and Thakur 2005

*The size of the 5′CS–3′CS zone depends on the number of gene inserts in this region of the integron.

were stained with ethidium bromide and visualized with a UV light transilluminator. Control markers with molecular masses of 100 bp and 1 kb (New England) were used.

Salmonella spp. phenotypically resistant to the tested antimicrobial agents and positive for PCR were used as positive control strains. Amplified fragments were sequenced and confirmed using the GenBank database of the program NCBI BLAST. Accession numbers were EF592570, AF071555, J01830, Y19118, and EF592570 for the *aadA1*, *tetA*, *tetB*, *tetG*, and *dfrA1* genes, respectively. A strain of susceptible *Salmonella* spp. was used as a negative control.

Detection of class 1 and class 2 integrons

For all the bacterial strains that presented resistance genes shown in Table 1, the genes *intl1* and *intl2*, the 5′CS–3′CS variable region, and the gene *sul1* were amplified. The PCRs were performed with the methodology previously described, using the primers and hybridization temperatures indicated in Table 1. A marker with a molecular mass of 1 kb was used (New England).

Structural association of the resistance genes and integrons

To determine the gene cassette array harbored in the integrons, the primer 5′CS of the class 1 and class 2 integrons were used. Each primer was associated with a resistance gene (Table 1). The 5′CS primer of the class 1 integron was associated with the *aadA1*(R) primer. The 5′CS primer of the class 2 integron was associated with the *aadA1*(R) primer and the *dfrA1*(R) primer. In addition, the primer *dfrA1*(F) was associated with the *aadA1*(R) primer. All of the PCR amplifications were conducted as previously described, with an annealing temperature of 55 °C. The isolates were divided in groups according to the genes found in the variable region of each integron.

To corroborate that the genes are inside the variable region of the integron, one representative strain of each group was sequenced in the laboratory of Retrogen Inc., San Diego, California, USA. The results of the sequencing were compared with that of GenBank using accession Nos. AM055749 and EF488370.

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 had resistance genes to oxytetracycline that were not structurally associated with the integron. *Escherichia coli* J53 Az^r resistant to sodium azide was used as a recipient strain (Wang et al. 2003). This strain was donated by Dr. Hopper, Boston General Hospital, Boston, Massachusetts, USA.

Conjugation was completed according to the protocol of the laboratory of microbiology of the Department of Microbiology and Immunology of the University of Texas Medical Branch, Texas, USA. The strains were cultivated at 37 °C for 18 h 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. A 1:10 dilution in LB broth was done for each donor strain and a 1:100 for the recipient strain. The dilutions were incubated at 37 °C for 1 h with agitation. A volume of 250 μL of each dilution was mixed in an Eppendorf tube and centrifuged at 8000 for 5 min. The pellet was resuspended and streaked on a LB agar plate without antibiotics. These plates were incubated for mating for 4 h at 37 °C, and the bacterial mix was resuspended in LB broth. One hundred microlitres was streaked in a LB agar plate that contained a combination of sodium azide with the corresponding antimicrobial agent, according to the resistance phenotype. The plates were incubated at 37 °C for 18 h.

The plasmid DNA was extracted from the transconjugant strains by the alkaline lysis protocol of Sambrook et al. (1989). 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. *Escherichia coli* J53 Plac and *E. coli* R1 (Wang et al. 2003) containing a plasmid of 152 and 92 kb, 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 system with Mueller–Hinton agar (Difco) and commercial drug discs (Arlab). The inhibition zones used to classify the strains as resistant or susceptible were those recommended by the CLSI. The presence of the integron and the resistance genes associated with the plasmid was confirmed by PCR using DNA from transconjugant strains as a template.

Results

Susceptibility in vitro

Fifty-four strains of *Salmonella* spp. were isolated from 1198 fecal samples. Of the 54 strains, 65% (35 strains) were resistant to at least one of the tested antimicrobial agents. The minimum inhibitory concentrations (MICs) for each resistant *Salmonella* strain are shown in Table 2. Serotyping was performed in the 35 resistant strains. The most frequent *Salmonella* serovars found were Branderburg, Heidelberg, Derby, and Typhimurium.

Multiresistance profiles

Multiresistance was defined as simultaneous resistance to at least 2 groups of the antimicrobials tested. Twenty-two strains were multiresistant to the tested antibiotics, presenting 5 profiles, which are shown in Table 3. The most common profile was oxytetracycline-streptomycin (TET+STR).

Detection of resistance genes

Of the 22 strains resistant to oxytetracycline, 8 amplified the gene *tetA* and 14 amplified the gene *tetB*, while no strains amplified the gene *tetG*. The 21 strains resistant to streptomycin and the 10 resistant to trimethoprim amplified the gene *addA1* and the gene *dfrA1,*, respectively. None of the 7 strains that presented simultaneous resistance to enrofloxacin and ciprofloxacin amplified the gene *qnr*.

The presence of class 1 and class 2 integrons and their structural association with resistance genes

Of the 35 resistant strains, only 16 presented integrons. Ten amplified the class 1 integron and 6 the class 2 integron. All the strains that presented the class 1 integron am-

Table 2. Percentage of resistance and distribution of minimum inhibitory concentrations (MICs) obtained in strains of *Salmonella* spp. isolated from swine (n = 54).

		No. of isolates at an MIC (μg/mL) of:												
Antimicrobial agent	Percentage of resistant strains	0.125	0.25	0.5	1	2	4	8	16	32	64	128	2/38	4/76	8/152	32/512
Oxytetracycline	41	1	1	2	9	8	5	6	0	7	0	15	NT	NT	NT	NT
Amoxicillin	0	7	0	0	14	12	21	0	0	0	0	0	NT	NT	NT	NT
Streptomycin	39	0	0	0	2	14	5	12	0	0	21	0	NT	NT	NT	NT
Enrofloxacin	13	45	2	0	0	0	7	0	0	0	0	0	NT	NT	NT	NT
Ciprofloxacin	13	47	0	0	0	0	7	0	0	0	0	0	NT	NT	NT	NT
Trimethoprim + sulfamethoxazole	19	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	41	3	9	1

Note: Resistant breakpoint: oxytetracycline, ≥16 μg/mL; amoxicillin, ≥32 μg/mL; streptomycin, ≥64 μg/mL; enrofloxacin, ≥2 μg/mL; ciprofloxacin, ≥2 μg/mL; sulfamethoxazole, ≥4/76 μg/mL. NT, not tested.

Table 3. Multiresistance profiles present in *Salmonella* spp. strains isolated from swine.

Multiresistance profile	No. of multi-resistant strains
TET+Q	3
STR+Q	1
TET+STR	10
STR+SXT	6
TET+Q+STR	2
Total multiresistant strains	22

Note: TET, oxytetracycline; Q, enrofloxacin and ciprofloxacin; STR, streptomycin; SXT, sulphamethoxazol + trimethoprim.

plified the 5′CS–3′CS variable region, with a length of 1.0 kb; the *aadA1* resistance cassette inserted in this region was found. These strains amplified the *sul1* gene, which is found in the 3′CS region. The strains that presented the class 2 integron amplified the variable region with a length of 2.2 kb. The genes *dfrA1*, *sat1*, and *aadA1* were found inside in this region (Table 4).

The presence of the gene *sat1* within the integron was identified by sequencing, and the result obtained was compared with sequences in the nonredundant database (GenBank accession No. AM055749).

Transference of resistance determinants by conjugation

Conjugation assays were performed on 16 strains of *Salmonella* spp. that presented integrons (Table 4). Ten of these strains also presented the gene *tetA* or the gene *tetB*. Two strains (12.5%) were capable of transferring their *tetA* and *aadA1* resistance determinants and class 1 integron to the recipient strain. Both transconjugant strains presented a plasmid of a molecular mass between 92 and 152 kb. The resistance phenotypes and the resistance determinants of these strains were confirmed by the plate diffusion method and PCR, respectively.

Discussion

Monitoring phenotic and genotypic resistance to antibiotics in *Salmonella* spp. isolated from food-producing animals is important for the protection of human and animal health. Strains of *Salmonella* spp. were isolated in 4.5% of the total sampled animals in this study. All the studied animals were clinically healthy and destined for human consumption. Thirty-five strains were resistant to at least one of the tested antimicrobial agents (oxytetracycline, streptomycin, trimethoprim–sulfamethoxazole, ciprofloxacin, enrofloxacin, and amoxicillin). The most frequent resistant serovars found were Branderburg, Derby, and Typhimurium.

All the fluoroquinolone-resistant strains presented simultaneous resistance to ciprofloxacin and enrofloxacin. The emergence of zoonic strains that show cross resistance to fluoroquinolones has generated a controversy over the use of this group of drugs in veterinary medicine worldwide (Norstrom et al. 2006). Considering this risk, the FDA (Food and Drug Administration) of the United States of America prohibited the use of enrofloxacin in poultry in 2005 (FDA 2005).

Frequently, multiresistance in the *Enterobacteriaceae*

Table 4. Characterization of antibiotic resistance genes linked to class 1 and 2 integrons and conjugation ability in different *Salmonella enteric* serovars isolated from swine.

Serovar (no.)	Amplification size (kb)	Gene cassette within the integron	Resistance phenotypes	Conjugation to *Escherichia coli* J53 detected
Class 1 Integron				
Salmonella Branderburg (3)	1	*aadA1*	TET+STR	–
Salmonella Derby (2)	1	*aadA1*	TET+STR	–
Salmonella Senftenberg (1)	1	*aadA1*	TET+STR	+
Salmonella Typhimurium (1)	1	*aadA1*	TET+STR	–
Salmonella Heidelberg (1)	1	*aadA1*	TET+STR	+
Salmonella Agona (1)	1	*aadA1*	TET+STR	–
Salmonella Anatum (1)	1	*aadA1*	TET+STR	–
Class 2 Integron				
Salmonella Branderburg (3)	2.2	*dfrA1-sat1-aadA1*	STR+SXT	–
Salmonella Derby (2)	2.2	*dfrA1-sat1-aadA1*	STR+SXT	–
Salmonella Typhimurium (1)	2.2	*dfrA1-sat1-aadA1*	STR+SXT	–

Note: TET, oxytetracycline; STR, streptomycin; SXT, sulphamethoxazol–trimethoprim.

family has been associated with integrons (Carattoli 2001; Rowe-Magnus and Mazel 2001; Fluit and Schmitz 2004). The presence of these elements increases the virulence of zoonic bacteria, especially when the presence of the class 1 integron is detected. This is due to the fact that more than 60 cassettes have been identified within these elements (Carattoli 2001). In the present study, 22 of 35 resistant strains showed multiresistance. This condition could reduce the alternatives for the treatment of invasive diseases produced by *Salmonella* spp. and increase the risk of therapy failure in human patients mainly due to the presence of fluoroquinolone and β-lactamic-resistant strains. None of the strains tested were resistant to amoxicillin; however, they were resistant to fluoroquinolones. On this regard, a study performed in Denmark reported a significantly greater risk of hospitalization, morbidity and mortality in patients infected by multiresistant *S. enterica* serovar Typhimurium strains (Helms et al. 2002). According to our results, there is a potential risk of transfer of quinolone-resistant strains from healthy swine to the human population.

The class 1 integron is the most frequently found in strains of *Salmonella* spp. With respect to this, van Essen-Zandbergen et al. (2007) indicated that the class 1 integron was observed in 43% of strains isolated from animals and humans, while the class 2 integron was observed in only 1%. On the other hand, Goldstein et al. (2001) showed that in strains of *Salmonella* spp. isolated from poultry, 61.5% presented the class 1 integron and 4.6% presented the class 2 integron. Regarding strains isolated from swine, Gebreyes and Thakur (2005) described that of 28 isolates, 21 were multiresistant and all of them presented the class 1 integron. Likewise, Michael et al. (2006*a*) indicated that 41.6% of the strains presented these molecular elements. In South America, there are few studies about the prevalence of integrons. Peirano et al. (2006), in Brazil, isolated 135 *Salmonella* strains; 55 of them presented the class 1 integron and one of them the class 2 integron. In our study, the resistant strains showed class 1 and class 2 integrons in similar proportions (10 and 6 strains, respectively). The percentage of class 2 integrons found (17%) was larger than the percentage described by the above-mentioned authors. These results could suggest that the use of antibiotics in veterinary medicine in our country differs from that of other countries.

When we were studying the genes inside the class 1 and class 2 integrons, we observed that the gene *aadA1*, which confers resistance to streptomycin and spectinomicin, was present in both integrons. In the class 2 integrons, this gene was associated with the genes *sat* and *dfrA1*, which confer resistance to streptothricin and trimethoprim, respectively (Fluit and Schmitz 2004). Streptothricin is an antimicrobial agent that has never been approved for use in food-producing animals in Chile. On the other hand, streptomycin, spectinomicin, and trimethoprim are drugs that are widely used in our country for the treatment of digestive and respiratory illnesses in swine. According to this, the intensive use of these drugs could have induced the appearance of these resistance gene cassettes within the integrons.

In our study, all the class 1 and class 2 integrons were identical in arrays (class 1 integron *aadA1*; class 2 *dfrA1-sat1-aadA*). These results differ from those of Peirano et al. (2006), who found 10 different rearrays within the variable region of the class 1 integron. These could be due to the fact that these authors took samples from different sources e.g., humans, animal feed, food-producing animals, foodstuff, and other sources, which are sources under different antimicrobial pressure. On the contrary, in this study, all the samples were taken from one source, which was slaughterage pigs destined for human consumption.

There are different hypotheses that could explain the presence of the same resistance gene cassettes within the integrons in the present study. One possibility is the dissemination of a clone with identical resistance determinants for each *Salmonella* serovar. A second theory is the spread of a genetic mobile element and the ulterior selection of certain resistance determinants and integrons related to the antimicrobial pressure (Orman et al. 2002). Taking into account that our isolates came from 126 different farms, which were located in a radius of 550 km, we can assume that this clone could have the same origin because 90% of the sows in Chile came from the same supplier. Michael et al. (2006*b*) in a study performed in Brazil, found 33 clonal

strains of *Salmonella* Agona. These isolates were found in pigs from 15 cities located up to 450 km apart from each other. The authors' explanation was that the tested animals originated from integrated production systems, in which piglets from one producer were distributed to grower farms. This would explain why animals from different herds in different cities carry members of the same clonal group.

Another possible explanation is that infection with *Salmonella* spp. can occur either during the transport of the animals to the slaughterhouse or in the holding pens at the slaughterhouse (Michael et al. 2006*b*). According to Hurd et al. (2002), these 2 sources have been considered as the major causes of *Salmonella* infection. However, the transport to the slaughterhouse can be ruled out as a source of clonal transmission in our study because each farm has its own transport system. On the contrary, the permanence in the holding pen at the slaughterhouse can be a possible explanation, particularly because of the fact that infected animals shed salmonellae under stress conditions.

The major genetic mechanisms of resistance to fluoroquinolones in *Enterobacteriaceae* are (*i*) the mutations in the QRDR region of the gyrase subunit A (*gyrA*) gene of the topoisomerase II, (*ii*) the mutations in *parC* and *parE* genes of the topoisomerase IV, (*iii*) alterations of the bacterial membrane permeability, and (*iv*) the presence of *qnr* gene (Ruiz 2003; Mammeri et al. 2005). Since the aim of our study was to identify the genes described within the integrons or associated with the integrons by plasmids, we only analyzed the presence of the *qnr* gene, which has been currently found in the variable region of the class 1 integrons (Mammeri et al. 2005; Robicsek et al. 2006). In this regard, none of the strains that presented phenotypic resistance to enrofloxacin and ciprofloxacin amplified the gene *qnr*, indicating that other molecular mechanisms of resistance could be present. To answer this question, we performed RFLP–PCR analysis of *gyrA* gene. The results showed that all of the quinolone-resistant strains had one simple mutation in the codon Ser 83 (data not shown).

The *sul1* gene that confers resistance to sulfonamides was also identified. This gene is located in the 3'CS of the class 1 integron, out of the variable region, and is a real indicator of the presence of this integron. According to this, all the strains positive for the class 1 integrase amplified the *sul1* gene.

In the present work, *tet* genes that confer resistance to oxytetracyclines in *Salmonella* were analyzed. All the strains that were resistant to this antibiotic amplified the genes *tetA* or *tetB*, which were located outside the integrons. The *tet* genes have currently been associated with the class 1 integron by conjugative plasmids able to transfer horizontally (Agerso and Sandvang 2005). *tet* genes that confer resistance to oxytetracyclines in the *Enterobacteriaceae* family have also been analyzed by other authors, such as Pasquali et al. (2004).

In the present study, conjugation assays were performed on all the resistant strains that presented integrons. The *tetA* gene and class 1 integrons harboring the *aadA1* cassette were transferred from 2 of the 16 donor *Salmonella* strains to *E. coli* J53 Azr. A high molecular mass plasmid was identified in the transconjugant strains. It is known that integrons are not able to perform self-transference; however, if they are associated with mobile elements, they can potentially transfer themselves horizontally, which could represent a significant risk in the dissemination of multiresistance (Rowe-Magnus and Mazel 2001). Even when we observed that integrons can transfer themselves through plasmids at a low frequency under laboratory conditions, it is important to consider that the efficiency of the transfer in vitro could be very different in vivo. Therefore, it is an important pathway to consider all the evidence in the dissemination of multiresistance in *Salmonella* in the environment. Nevertheless, other mechanisms such as transference by transposons or phages could be contributing to the dissemination of multiresistance.

The results obtained in this study verify the large percentage of resistance in zoonotic strains of *Salmonella* isolated from swine in Chile. These strains represent an important potential reservoir of resistance genes and integrons. This information should be taken into account in the development of public health policy for the use of antimicrobials in food-producing animals, with the aim of protecting animal and human health.

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