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Comparison of integron-linked antibiotic resistance genes in strains of *Salmonella* spp. isolated from swine in Chile in 2005 and 2008

Lisette Lapierre, Betty San Martín, Carolina Araya-Jordán, and Consuelo Borie

Abstract: *Salmonella* spp. isolates obtained from healthy swine in 2008 were analyzed for antibiotic resistance phenotypes and genotypes. The resistance profiles of the 2008 isolates were compared with those of a *Salmonella* collection isolated from the same geographical area in 2005. The 2008 isolates consisted of strains that were 97% oxytetracycline resistant, 33.3% amoxicillin resistant, 31.8% amoxicillin- plus clavulanic acid resistant, 27.5% trimethoprim–sulfamethoxazole resistant, 17.3% streptomycin resistant, and 7.2% enrofloxacin–ciprofloxacin resistant. The presence of integrons and resistance genes and their topological association in resistant strains was assessed by PCR. The prevalence of class 1 integrons was the highest, at 46.2%, while class 2 integrons were present in 17.9% of the isolates. In strains that harboured class 1 integrons, we identified 3 different gene cassette arrangements; a single class 2 integron arrangement of *dfrA1–sat1–aadA1* was found. Comparison of these results with data obtained from the 2005 isolates showed that *Salmonella* strains resistant to amoxicillin and amoxicillin plus clavulanic acid had clearly emerged over the span of 3 years, along with an increase in the prevalence of class 1 integrons and the acquisition of new gene cassette arrangements. These findings highlight the need for continual monitoring of regional isolates to establish more efficient vigilance programs that can address variations in resistance over short periods of time within the same geographical area.

Key words: Salmonella, integrons, resistance genes.

Résumé : Des isolats de *Salmonella* spp. obtenus de porcs sains au cours en 2008 ont été analysés d'un point de vue phénotypique et génotypique relativement à la résistance aux antibiotiques . Les profils de résistance des isolats de 2008 ont été comparés à ceux d'une collection de *Salmonella* isolée dans la même région géographique en 2005. Des souches isolées en 2008, 97 % étaient résistantes à l'oxytétracycline, 33,3 % à l'amoxicilline, 31,8 % à l'amoxicilline-acide clavulanique, 27,5 % à la triméthoprime–sulfaméthoxazole, 17,3 % à la streptomycine et 7,2 % à l'enrofloxacine–ciprofloxacine. La présence d'intégrons et de gènes de résistance, ainsi que leur association topologique dans les souches résistantes, ont été évaluées par PCR. La prévalence des intégrons de classe 1 était la plus élevée, à 46,2 %, alors que les intégrons de classe 2 étaient présents dans 17,9 % des isolats. Chez les souches comportant des intégrons de classe 1, nous avons identifié 3 arrangements de cassettes de gènes; un seul arrangement d'intégron de classe 2 de *dfrA1–sat1–aadA1*a été trouvé. La comparaison de ces résultats avec les données obtenues en 2005 a montré que les souches de *Salmonella* résistantes à l'amoxicilline et à l'amoxicilline plus acide clavulanique ont clairement émergé au cours de ces 3 ans, parallèlement à une augmentation de la prévalence des intégrons de classe 1 et à l'acquisition de nouveaux arrangement de cassettes de gènes. Ces résultats mettent en lumière le besoin de suivre continuellement les isolats régionaux afin d'établir des programmes de vigilance plus efficaces qui pourront se pencher sur les variations de la résistance sur de courtes périodes de temps à l'intérieur d'une même zone géographique.

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

[Traduit par la Rédaction]

The use of antimicrobials in the livestock industry to treat or prevent infectious diseases has contributed in part to the rise in antimicrobial resistance among bacterial strains (Martínez and Baquero 2002). This is particularly true for zoonotic enteropathogens such as *Salmonella* spp. Several studies have reported on the molecular basis of resistance in

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Salmonella spp. isolated from animals around the world (Gebreyes et al. 2004; Randall et al. 2004; Gebreyes and Thakur 2005; Hasman et al. 2005; Vo et al. 2006; Frye et al. 2008). Beyond those descriptions, the aim of current studies is defining the multidrug resistance profiles of *Salmonella* spp. and investigating the mechanisms of transfer of these resistance genes among bacteria. High levels of multidrug resistance are frequently associated with mobile genetic elements, such as integrons, which encode specific resistance genes (Carattoli 2001; Randall et al. 2004). Integrons carrying resistance genes can be transferred by other mobile genetic elements, such as transposons and plasmids, and integrate directly into the bacterial genome. Integrons, together with other mobile genetic elements, facilitate the wide dissemination of resistance genes and the development of multiple drug resistance profiles (Carattoli 2001; Alekshun and Levy 2007).

Previously, we reported a high prevalence of antibiotic resistance genes linked to class 1 and class 2 integrons, as well as evidence of conjugational transfer of these resistance genes, in strains of *Salmonella* spp. isolated from clinically healthy swine in 2005 (San Martín et al. 2008). The aim of the present study was to characterize the resistance phenotype and genotype for several antimicrobial drugs used in veterinary medicine, in strains of *Salmonella* spp. isolated from clinically healthy pigs in 2008. Additionally, the association between genetic resistance determinants and integrons was assessed. The data from the 2005 and 2008 *Salmonella* isolates was compared to characterize possible changes in resistance profiles over time.

Throughout 2008, 290 fecal samples and 290 mesenteric ileocolic lymph node samples were collected at 6 slaughterhouses that received swine from 6 different farms, all of which were within a 550 km radius. Fecal content (5 ± 0.5 g) was collected and processed as described by San Martín et al. (2008). Samples from ileocolic lymph nodes (25 g) were collected and then processed according to Vieira-Pinto et al. (2005). All samples were cultured in Rappaport–Vassiliadis broth (ratio 1:100) (Difco, Detroit, Mich.) at 37 °C for 72 h, during which they were inoculated by streaking every 24 h on xylosine lysine deoxycholate agar (Difco). Putative colonies were identified by rapid diagnostic testing using the BBL Crystal identification system (Becton Dickinson, San Diego, Calif.) and rapid agglutination testing using polyvalent A-I and Vi sera (Difco). One *Salmonella* strain was selected from each sample. Sixty-nine strains were isolated and identified. Minimum inhibitory concentrations (MICs) for the following 8 antimicrobial agents were determined by the agar dilution method according to the protocol of the Clinical Laboratory Standards Institute (2006): oxytetracycline (96%; Sigma–Aldrich, St. Louis, Mo.), enrofloxacin (100%; Laboratorio Chile, Ñuñoa, Chile), ciprofloxacin (100%; USP Reference Standard, Rockville, Md.), trimethoprim–sulfamethoxazole (100%; Sigma–Aldrich), streptomycin (98%; Sigma–Aldrich), amoxicillin (97%; Fluka, St. Louis, Mo.), and amoxicillin plus clavulanic acid (98%; Laboratorio Chile). The breakpoints specified by the Clinical Laboratory Standards Institute were used for all antimicrobials except streptomycin, for which the breakpoint given by the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (Heuer and Hammerun 2005) was used. Quality control was performed using *Escherichia coli* ATCC 25922. Strains that were resistant to oxytetracycline, streptomycin, trimethoprim, amoxicillin, and amoxicillin plus clavulanic acid were analyzed by PCR for the presence of *tetA* and *tetB*, *aadA1*, *dfrA1*, bla_{OXA}, and *carb* (bla_{PSE-1}), respectively (Table 1); quinolone-resistant strains were evaluated by RFLP–PCR to assess the presence of quinolone resistance determining region (QRDR) mutations. The presence of class 1 and class 2 integrons was determined by PCR amplification of the *intI1* and *intI2* genes, the 5′-CS – 3′-CS variable region, and the *sul1* gene (Table 2). Structural associations between integrons and resistance genes were investigated by PCR using different primer combinations, as described by San Martín et al. (2008). Briefly, to determine the gene cassette array harboured by an integron, a 5′-CS primer of the variable region of a class 1 or class 2 integron was used. The primer was associated with a resistance gene present in the strain (see Fig. 1).

Of the 69 strains that were isolated, 97% were resistant to oxytetracycline, 33.3% to amoxicillin, 31.8% to amoxicillin plus clavulanic acid, 27.5% to trimethoprim–sulfamethoxazole, 17.3% to streptomycin, and 7.2% to enrofloxacin and ciprofloxacin. Forty-one strains (59.4%) exhibited multidrug resistance. The multiresistance profile most common was oxytetracycline–amoxicillin–amoxicillin plus clavulanic acid (data not shown). Thus, among the 2008 isolates, there was a high level of single-antibiotic resistance, the most frequent being resistance to β-lactams and drugs of the tetracycline class. These results were in agreement with the results of several related studies (Gebreyes et al. 2004; Gebreyes and Thakur 2005). By comparison, the resistance phenotypes of the 2005 *Salmonella* strains were as follows: 76% were resistant to oxytetracycline, none to amoxicillin, none to amoxicillin plus clavulanic acid, 35% to trimethoprim–sulfamethoxazole, 72% to streptomycin, and 24% to enrofloxacin and ciprofloxacin. Thus, the 3 year period between 2005 and 2008 was marked by the unexpected emergence of *Salmonella* spp. strains resistant to β-lactams.

The changes in *Salmonella* resistance profiles between 2005 and 2008 could reflect selection pressure due to the introduction of ceftiofur, amoxicillin, and (or) amoxicillin plus clavulanic acid into the swine industry over the past several years. As such, our results constitute additional evidence of the high rate at which resistance can emerge after a drug is introduced into practice (Cavaco et al. 2008). Cefotaxime susceptibility was also evaluated to assess the presence of extended spectrum β-lactamases. None of the isolated strains showed third-generation cephalosporin resistance, reflecting the absence of extended spectrum β-lactamases (data not shown).

The emergence of β-lactam resistance in the 2008 collection of *Salmonella* spp. is cause for great concern, because β-lactams, particularly third-generation β-lactams, as well as fluoroquinolones, are the drugs of choice for the treatment of salmonellosis. Despite the fact that salmonellosis is self-limiting in most patients, it can be followed by systemic symptoms in children, elderly, and immunocompromised patients (Galanakis et al. 2007). In this respect it is important to note that none of the strains isolated in the current study exhibited combined resistance to β-lactams and fluoroquinolones. However, genes that encode β-lactamases often coexist with other antimicrobial resistance determinants and can

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

Antibiotic	Gene	Product size (bp)	Annealing temp. (°C)	Primer Sequence (5′→3′)	Reference or GenBank accession No.
Amoxicillin	*bla*$_{OXA}$	713	52	F: ACTGTCGCATCTCCATTATTTGA	Toro et al. 2005
	bla$_{OXA}$			R: ATCGCATTTTTCTTGGCTTTTAT	
	carb	588	55	F: GAATGACCAATTTTAACAATCGC	Vo et al. 2006
	carb			R: CGCTTTTAATACCATCCGTGG	
Streptomycin	*aadA1*	447	58	F: TATCCAGCTAAGCGCGAACT	San Martín et al. 2008
	aadA1			R: ATTTGCCGACTACCTTGGTC	
Oxitetracycline	*tetA*	577	52	F: GGTTCACTCGAACGACGTCA	Randall et al. 2004
	tetA			R: CTGTCCGACAAGTTGCATGA	
	tetB	751	52	F: CTGGATTACTTATTGCTGGC	Randall et al. 2004
	tetB			R: CACCTTGCTGATGACTCTT	
	tetG	604	56	F: CCGGTCTTATGGGTGCTCTA	Randall et al. 2004
	tetG			R: GACTGGCTTCGTTCTTCTGG	
Trimetroprim	*dfrA1*	367	45	F: GGAGTGCCAAAGGTGAACAGC	Toro et al. 2005
	dfrA1			R: GAGGCGAAGTCTTGGGTAAAAAC	

Table 2. Primers and annealing temperatures used for detection of class 1 and class 2 integrons.

Target gene or region (s)	Product size (bp)	Annealing temp. (°C)	Primer Sequence (5′→3′)	Reference
intI 1	280	60	F: CCTCCCGCACGATGATC	Goldstein et al. 2001
intI 1			R: TCCACGCATCGTCAGGC	
intI 2	232	60	F: TTATTGCTGGGATTAGGC	Goldstein et al. 2001
intI 2			R: ACGGCTACCCTCTGTTATC	
5′-CS class 1	Variable*	56	F: GGCATCCAAGCAGCAAG	Sunde and Norström 2006
3′-CS class 1			R: AAGCAGACTTGACCTGA	
5′-CS class 2	Variable*	56	F: GACGGCATGCACGATTTGTA	L'Abée-Lund and Sørum 2001
3′-CS class 2			R: GATGCCATCGCAAGTACGAG	
sul1	435	56	F: CTTCGATGAGAGCCGGCGGC	Gebreyes and Thakur 2005
sul1			R: GCAAGGCGGAAACCCGCGCC	

*The size of the 5′-CS–3′-CS zone depends on the number and type of gene cassettes inserted in this region.

be associated with integrons, thus increasing the potential for enrichment of multidrug-resistant bacteria as well as the spread of resistance determinants among bacterial species (Li et al. 2007).

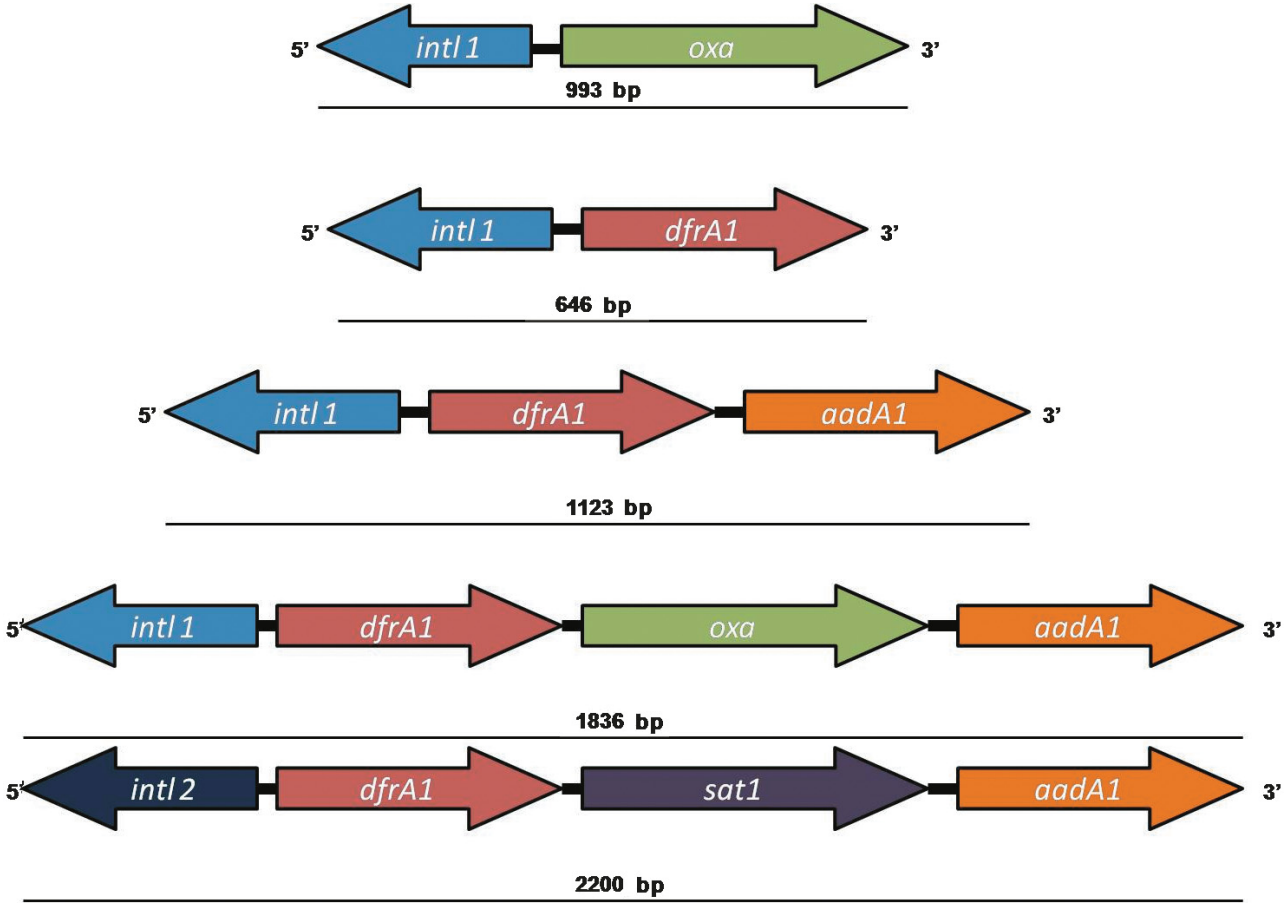
Between 2005 and 2008, there was an apparent reduction in overall resistance rates, with the exception of resistance to tetracycline and β-lactams. The decrease in enrofloxacin–ciprofloxacin resistance is particularly relevant, because the latter is extensively used in human medicine, and the emergence of ciprofloxacin-resistant zoonotic strains could reduce treatment alternatives.

All the resistant strains were analyzed by PCR or RFLP–PCR to elucidate the mechanisms of resistance. The *tetA* gene was amplified from 10 of the 65 strains that were resistant to oxytetracycline, and the *tetB* gene was amplified from 39; 16 of the strains were negative for *tetA* and *tetB*. All 12 streptomycin-resistant strains carried the *aadA1* gene. The *dfrAI* gene was amplified from 17 of the 19 strains that were resistant to trimethoprim. Twenty-three strains were amoxicillin resistant, 22 of which were positive for the *bla*$_{OXA}$ gene. All the amoxicillin plus clavulanic acid resistant strains (n = 22) were positive for the *bla*$_{OXA}$ gene, whereas none of them carried the *carb* (*bla*$_{PSE-1}$) gene. With regard to fluoroquinolone resistance, several resistance determinants have been described in *Enterobacteriaceae*, including (*i*) QRDR mutations of the *gyrA* gene, (*ii*) QRDR mutations of *parC* and *parE* genes, (*iii*) alterations of bacterial membrane permeability, and (*iv*) the presence of *qnr* (Ruiz 2003; Mammeri et al. 2005). In the 2005 samples, a single point mutation in codon 83 of the QRDR region of the *gyrA* gene was identified. In the 2008 samples, every strain resistant to enrofloxacin–ciprofloxacin carried the same single point mutation in codon 83 of the QRDR region of the *gyrA* gene.

Overall, the genetic determinants that were identified in the 2005 isolates (*tetA*, *tetB*, *aadA1*, *dfrAI*) persisted in the 2008 isolates. However, it is important to note the recent emergence of the *bla*$_{OXA}$ gene, possibly due to insertion via an integron in the form of a resistance cassette. To clarify the mechanisms of acquisition of *bla*$_{OXA}$, the presence of class 1 and class 2 integrons in the 2008 isolates and their structural association with resistance genes were analyzed.

The prevalence of class 1 integrons in the 2008 isolates was 44.9%, while class 2 integrons were detected in 17.3% of the strains. Eleven (15.9%) isolates carried both class 1 and class 2 integrons. From strains that harboured class 1 integrons, the *sul1* gene, which is regularly found inside the integron 3′-CS region, was amplified. A marked characteristic of horizontal resistance gene transfer among strains of bacteria is the rate at which mobile genetic elements spread

Fig. 1. A schematic representation of the class 1 and class 2 integrons found among the resistant *Salmonella* spp. strains.



among organisms (Thomas and Nielsen 2005). This could partially explain the relatively large increase in the number of *Salmonella* strains bearing class 1 integrons between 2005 and 2008 (44.9% versus 28.5%, respectively).

Three different cassette arrangements within class 1 integrons were identified among the 2008 isolates, as compared to 1 arrangement (a single copy of *aadA1*) in the 2005 isolates (Fig. 1). A 0.8 kb variable region containing the bla_{OXA} resistance cassette was amplified from 17 of the 42 isolates that were positive for class 1 integrons. From 5 isolates, a 1.5 kb variable region was amplified carrying a *dfrA1–oxa–aadA1* array. From 14 strains, a 1.0 kb variable region with the configuration *dfrA1–aadA1* was amplified. These new rearrangements may have resulted from the use of amoxicillin in swine husbandry and positive selection of strains harbouring a class 1 integron containing a cassette array with the bla_{OXA} gene (Tables 3, 4).

In 5 of the integron-harbouring strains, the resistance cassettes did not fully account for the resistance phenotype of the isolate. A similar phenomenon was described by Yang et al. (2009), in which *E. coli* strains isolated from human fecal samples were analyzed. Fourteen of the *E. coli* isolates contained the *dfrA12–orfF–aadA2* cassette array, but only 8 of them were resistant to streptomycin, and only 9 were trimethoprim–sulfamethoxazole resistant. These results suggest that the presence of a particular resistance determinant is not by and of itself sufficient to account for a resistance phenotype. On the other hand, no integron gene cassette was amplified from 6 of the isolates positive for the class 1 integron. Thus, these strains carried empty integrons. This situation suggests that these bacteria have the potential to rapidly acquire multidrug resistance (Fonseca et al. 2005).

The percentage of strains that were positive for class 2 integrons was stable between 2005 and 2008 (17.9% and 17.3%, respectively). Similar to the 2005 *Salmonella* isolates, a 2.2 kb variable region containing the *dfrA1*, *sat1*, and *aadA1* genes was amplified from the 2008 strains that presented class 2 integrons (Table 4, Fig. 1). The stability of class 2 integrons, as well as the persistence of the *aadA1* gene within class 1 integrons, may be related to the use of streptomycin to control infectious diseases in food-producing animals. In fact, streptomycin is often used in Chile to treat digestive and respiratory bacterial illness in swine. Classic antimicrobials, such as streptomycin and tetracycline, are currently widely used in the swine industries in several South American countries, including Argentina and Brazil (Castagna et al. 2001; Ibar et al. 2009). The stability of class 2 integrons observed in the current study suggests that the use of aminoglycosides, and by extension the positive selection pressure for class 2 integrons, remained constant during the intervening 3 years. The persistence or loss of acquired antimicrobial resistance in bacterial populations previously exposed to drug selective pressure depends on several variables, including rates of reacquisition, effect of resistance traits on bacterial fitness, linked selection, and segregational stability of the resistance determinants (Johnsen et al. 2009).

Table 3. Gene cassettes within class 1 integrons found in phenotypically resistant strains of *Salmonella* spp. isolated from swine.

No. of strains	Resistance genes outside integron	Amplicon size (kb)	Gene cassette	Resistance phenotype
8	—	0.8	*oxa*	AMX + AMC + TET
5	*tetB*	0.8	*oxa*	AMX + AMC + TET
2	*tetA*	0.8	*oxa*	AMX + AMC + TET
1	—	0.8	*oxa*	AMX + TET
1	*tetB*	0.8	*oxa*	AMX–AMC + TET + SXT
1	*tetB*	1.0	*dfrA1–aadA1*	TET + STR + SXT
2	*tetB*	1.0	*dfrA1–aadA1*	TET
3	—	1.0	*dfrA1–aadA1*	TET
1	*tetB*	1.0	*dfrA1–aadA1*	TET + ENR–CIP
2	*tetB*	1.0	*dfrA1–aadA1*	TET
3	—	1.5	*dfrA1–oxa–aadA1*	AMX–AMC + TET + SXT
2	*tetB*	1.5	*dfrA1–oxa–aadA1*	AMX–AMC + TET + SXT

Note: AMX, amoxicillin; AMC, amoxicillin plus clavulanic acid; TET, oxytetracycline; CIP, ciprofloxacin; ENR, enrofloxacin; SXT, trimethoprim–sufamethoxazole; STR, streptomycin.

Table 4. Gene cassettes within class 1 and class 2 integrons found in phenotypically resistant strains of *Salmonella* spp. isolated from swine.

No. of strains	Resistance genes outside integron	Class 1 integron amplicon size (kb)	Gene cassettes in class 1 integrons	Class 2 integron amplicon size (kb)	Gene cassettes in class 2 integrons	Resistance phenotypes
1	*tetB*	1.0	*dfrA1–aadA1*	2.2	*dfrA1–sat1–aadA1*	TET + SXT
3	*tetB*	1.0	*dfrA1–aadA1*	2.2	*dfrA1–sat1–aadA1*	TET + STR + SXT
6	*tetB*	—	Empty	2.2	*dfrA1–sat1–aadA1*	TET + STR + SXT
1	*tetA*	1.0	*dfrA1–aadA1*	2.2	*dfrA1–sat1–aadA1*	TET + STR + SXT

Note: AMX, amoxicillin; AMC, amoxicillin plus clavulanic acid; TET, oxytetracycline; CIP, ciprofloxacin; ENR, enrofloxacin; SXT: trimethoprim–sufamethoxazole; STR, streptomycin.

One representative integron variable region from each group was sequenced (Retrogen Inc., San Diego, Calif.), and the sequences were then compared to the following sequences contained in GenBank: accession Nos. EU089669, DQ923619, EF488370, EF031067, and EF543147. *tetA* and *tetB* gene sequences were not found within any of the sequenced integrons. These 2 genes, which encode tetracycline resistance, have been found in a plasmid that also carries a class 1 integron; thus, they could be transferred horizontally to other bacterial strains through these elements (Sunde and Norström 2006). The apparent physical association of *tet* genes with class 1 integrons on the same plasmid could account for the finding of a high number of *tetA*- and *tetB*-positive strains that were also carriers of class 1 integrons (Tables 1, 2). Further investigation is needed to determine the specific location of the class 1 integrons carried by these strains, that is, whether they are chromosomal or plasmid-borne. There were 16 strains resistant to oxytetracycline, from which neither *tetA* nor *tetB* was amplified; therefore, its tetracycline resistance could be determined by a different resistance gene. Currently, 38 different *tet* and *otr* genes have been described (Roberts 2005).

From an epidemiological standpoint, the emergence of resistant strains within an animal population can lead to the spread of genetic resistance determinants within the original population as well as in different animal groups. The spread of drug-resistant organisms could have variable effects in large swine populations and, depending on the level of infection and whether the infection occurs at the stage of production or slaughter, could reach the level of regional, national, or international concern (Michael et al. 2006). Approximately 90% of the sows in Chile come from one supplier. Antimicrobial selective pressure in these herds could lead to the transfer of newly acquired resistance determinants or clonal expansion of resistant strains in the original population as well as their offspring. Distribution of piglets carrying these resistant strains to different farms likely contributed to the spread of resistant strains to the sampled farms. Our results suggest that the acquisition of resistance genes through vertical and horizontal gene transfer largely contributed to the spread of antimicrobial resistant strains.

In summary, antimicrobial resistance was common among *Salmonella* spp. strains isolated from healthy swine in Chile. Comparison of strains isolated in 2005 and 2008 yielded the following results: (*i*) the emergence of *Salmonella* spp. strains resistant to amoxicillin and amoxicillin plus clavulanic acid; (*ii*) an increase in the prevalence of class 1 integrons; (*iii*) the emergence of class 1 integron positive strains harbouring the *oxa* gene cassette; (*iv*) new gene insertions within the class 1 integrons; and (*v*) the presence of so-called empty integrons among *Salmonella* spp. strains.

An improved understanding of the genetic basis for and epidemiology of emerging antimicrobial resistance is needed to design improved measures for reducing the burden of antimicrobial resistance on health and well-being. Ultimately, this information is needed to develop public health policies that govern the use of antimicrobials in livestock production industries in developing countries.

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References

Alekshun, M.N., and Levy, S.B. 2007. Molecular mechanisms of antibacterial multidrug resistance. Cell, **128**(6): 1037–1050. doi:10.1016/j.cell.2007.03.004. PMID:17382878.

Carattoli, A. 2001. Importance of integrons in the diffusion of resistance. Vet. Res. **32**(3–4): 243–259. doi:10.1051/vetres:2001122. PMID:11432416.

Castagna, S., Bessa, M.C., Carvalho, M., Cardoso, M., and Costa, M. 2001. Antimicrobial resistance patterns of *Salmonella* spp., isolated from slaughtered pigs in the state of Rio Grande do Sul Brasil. Arq. Fac. Vet. **29**: 44–49.

Cavaco, L.M., Abatih, E., Aarestrup, F.M., and Guardabassi, L. 2008. Selection and persistence of CTX-M-producing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrob. Agents Chemother. **52**(10): 3612–3616. doi:10.1128/AAC.00354-08. PMID:18644956.

Clinical Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing. 11th Informational Supplement. NCCLS Document M100-S11. National Committee of Clinical Laboratory Standards, Wayne, Pa.

Fonseca, E.L., Vieira, V.V., Cipriano, R., and Vicente, A.C. 2005. Class 1 integrons in *Pseudomonas aeruginosa* isolates from clinical settings in Amazon region, Brazil. FEMS Immunol. Med. Microbiol. **44**(3): 303–309. doi:10.1016/j.femsim.2005.01.004. PMID:15907453.

Frye, J.G., Fedorka-Cray, P.J., Jackson, C.R., and Rose, M. 2008. Analysis of *Salmonella enterica* with reduced susceptibility to the third-generation cephalosporin ceftriaxone isolated from U.S. cattle during 2000-2004. Microb. Drug Resist. **14**(4): 251–258. doi:10.1089/mdr.2008.0844. PMID:19025468.

Galanakis, E., Bitsori, M., Maraki, S., Giannakopoulou, C., Samonis, G., and Tselentis, Y. 2007. Invasive non-typhoidal salmonellosis in immunocompetent infants and children. Int. J. Infect. Dis. **11**(1): 36–39. doi:10.1016/j.ijid.2005.09.004. PMID:16564718.

Gebreyes, W.A., and Thakur, S. 2005. Multidrug-resistant *Salmonella enterica* serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. Antimicrob. Agents Chemother. **49**(2): 503–511. doi:10.1128/AAC.49.2.503-511.2005. PMID:15673725.

Gebreyes, W.A., Thakur, S., Davies, P.R., Funk, J.A., and Altier, C. 2004. Trends in antimicrobial resistance, phage types and integrons among *Salmonella* serotypes from pigs, 1997–2000. J. Antimicrob. Chemother. **53**(6): 997–1003. doi:10.1093/jac/dkh247. PMID:15150175.

Goldstein, C., Lee, M.D., Sánchez, S., Hudson, C., Phillips, B., Register, B., et al. 2001. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob. Agents Chemother. **45**(3): 723–726. doi:10.1128/AAC.45.3.723-726.2001. PMID:11181350.

Hasman, H., Mevius, D., Veldman, K., Olesen, I., and Aarestrup, F.M. 2005. β-lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J. Antimicrob. Chemother. **56**(1): 115–121. doi:10.1093/jac/dki190. PMID:15941775.

Heuer, O., and Hammerun, A. 2005. DANMAP. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Staten's Serum Institute, Danish Veterinary and Food Administration, Danish Medicines Agency, Danish Institute for Food and Veterinary Research, Copenhagen, Denmark.

Ibar, M.P., Vigo, G., Piñeyro, P., Caffer, M.I., Quiroga, P., Perfumo, C., et al. 2009. Serovars of *Salmonella enterica* subspecies enterica and its antimicrobial resistance in slaughterhouse pigs. Rev. Argent. Microbiol. **41**(3): 156–162. [In Spanish.] PMID:19831314.

Johnsen, P.J., Townsend, J.P., Bøhn, T., Simonsen, G.S., Sundsfjord, A., and Nielsen, K.M. 2009. Factors affecting the reversal of antimicrobial-drug resistance. Lancet Infect. Dis. **9**(6): 357–364. doi:10.1016/S1473-3099(09)70105-7. PMID:19467475.

L'Abée-Lund, T.M., and Sørum, H. 2001. Class 1 integrons mediate antibiotic resistance in the fish pathogen *Aeromonas salmonicida* worldwide. Microb. Drug Resist. **7**(3): 263–272. doi:10.1089/10766290152652819. PMID:11759088.

Li, X.Z., Mehrotra, M., Ghimire, S., and Adewoye, L. 2007. β-Lactam resistance and β-lactamases in bacteria of animal origin. Vet. Microbiol. **121**(3–4): 197–214. doi:10.1016/j.vetmic.2007.01.015. PMID:17306475.

Mammeri, H., Van De Loo, M., Poirel, L., Martinez-Martinez, L., and Nordmann, P. 2005. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob. Agents Chemother. **49**(1): 71–76. doi:10.1128/AAC.49.1.71-76.2005. PMID:15616277.

Martínez, J.L., and Baquero, F. 2002. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin. Microbiol. Rev. **15**(4): 647–679. doi:10.1128/CMR.15.4.647-679.2002. PMID:12364374.

Michael, G.B., Cardoso, M., and Schwarz, S. 2006. Molecular analysis of *Salmonella enterica* subsp. *enterica* serovar Agona isolated from slaughter pigs. Vet. Microbiol. **112**(1): 43–52. doi:10.1016/j.vetmic.2005.10.011. PMID:16289951.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J., and Woodward, M.J. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. J. Antimicrob. Chemother. **53**(2): 208–216. doi:10.1093/jac/dkh070. PMID:14729766.

Roberts, M.C. 2005. Update on acquired tetracycline resistance genes. FEMS Microbiol. Lett. **245**(2): 195–203. doi:10.1016/j.femsle.2005.02.034. PMID:15837373.

Ruiz, J. 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J. Antimicrob. Chemother. **51**(5): 1109–1117. doi:10.1093/jac/dkg222. PMID:12697644.

San Martín, B., Lapierre, L., Cornejo, J., and Bucarey, S. 2008. Characterization of antibiotic resistance genes linked to class 1 and 2 integrons in strains of *Salmonella* spp. isolated from swine. Can. J. Microbiol. **54**(7): 569–576. doi:10.1139/W08-045. PMID:18641703.

Sunde, M., and Norström, M. 2006. The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products. J. Antimicrob. Chemother. **58**(4): 741–747. doi:10.1093/jac/dkl294. PMID:16931539.

Thomas, C.M., and Nielsen, K.M. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat. Rev. Microbiol. **3**(9): 711–721. doi:10.1038/nrmicro1234. PMID:16138099.

Toro, C.S., Farfán, M., Contreras, I., Flores, O., Navarro, N., Mora, G.C., and Prado, V. 2005. Genetic analysis of antibiotic-resistance determinants in multidrug-resistant *Shigella* strains isolated from Chilean children. Epidemiol. Infect. **133**(1): 81–86. doi:10.1017/S0950268804003048. PMID:15724714.

Vieira-Pinto, M., Temudo, P., and Martins, C. 2005. Occurrence of *Salmonella* in the ileum, ileocolic lymph nodes, tonsils, mandibular lymph nodes and carcasses of pigs slaughtered for consumption. J. Vet. Med. **52**(10): 476–481. doi:10.1111/j.1439-0450.2005.00892.x.

Vo, A.T., van Duijkeren, E., Fluit, A.C., Wannet, W.J., Verbruggen, A.J., Maas, H.M., and Gaastra, W. 2006. Antibiotic resistance, integrons and *Salmonella* genomic island 1 among non-typhoidal Salmonella serovars in The Netherlands. Int. J. Antimicrob. Agents, **28**(3): 172–179. doi:10.1016/j.ijantimicag.2006.05.027. PMID:16911867.

Yang, C.M., Lin, M.F., Lin, C.H., Huang, Y.T., Hsu, C.T., and Liou, M.L. 2009. Characterization of antimicrobial resistance patterns and integrons in human fecal *Escherichia coli* in Taiwan. Jpn. J. Infect. Dis. **62**(3): 177–181. PMID:19468175.