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Surveillance of antibiotic resistance evolution and detection of class 1 and 2 integrons in human isolates of multi-resistant *Salmonella* Typhimurium obtained in Uruguay between 1976 and 2000

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Summary
Objectives: To study the evolution of antibiotic resistance in isolates of *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*Salmonella* Typhimurium) obtained in Uruguay between the years 1976 and 2000, and to determine the incidence of class 1 and 2 integrons in the multi-resistant isolates.
Methods: We studied 258 strains of *Salmonella* Typhimurium from various sources, isolated between 1976 and 2000. We determined the evolution of antibiotic resistance and the distribution of class 1 and 2 integrons in all isolates by means of disk diffusion assays and PCR.
Results: During the period 1989—2000 resistance to streptomycin was 56.8%, tetracycline 13.6%, sulfonamides 11.2%, and ampicillin 7.2%. Resistance to gentamicin, kanamycin, chloramphenicol, and nalidixic acid were lower than 5%; no resistance was detected to fluoroquinolones, oxyiminocephalosporins, and amikacin. These results show a dramatic decrease with respect to values found in the period 1976—1988. In this period, resistance to streptomycin was 63.2%, tetracycline 36.8%, sulfonamides 32.3%, and ampicillin 27.8%. Throughout the two periods, 29 multi-resistant

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Salmonella Typhimurium strains were isolated harboring some class of integron: 15 strains had only *intI2*, 11 strains presented both *intI1* and *intI2*, and three isolates only *intI1*.
Conclusions: Our results show a marked decrease in resistance throughout these years, along with a correlation between resistance to different antibiotics and the presence of integrons.

Introduction

Salmonella enterica subspecies *enterica* serovar Typhimurium (*Salmonella* Typhimurium) is an important food-borne pathogen in humans and a broad range of animals. The spectrum of disease in humans comprises gastrointestinal infections and extra-intestinal infections such as bacteremia, central nervous system infections, and bone and urinary tract infections, among others.

In Uruguay, *Salmonella* Typhimurium has been one of the main causes of human salmonellosis since 1971,[1,2] second only to *Salmonella* Enteritidis since 1995.[3] This epidemiological situation is similar to other countries in Latin America and the rest of the world.[4–6]

Taking into consideration that most Salmonella infections consist of mild and self-limited gastrointestinal episodes, antibiotic treatment is administered only in cases of severe infection that may occur in patients such as young children, the elderly, and the immunocompromised.

Antibiotic resistance in *Salmonella* Typhimurium is a re-emerging problem worldwide. In our country, multi-resistant isolates (defined as those having resistance to four or more classes of antibiotics)[7] have been reported since 1975;[8] different enteric pathogens, such as other *Salmonella* serovars and enteropathogenic *Escherichia coli* (EPEC), have also been highlighted as important reservoirs of resistance genes.[9,10] Antibiotic administration generates selective pressure over bacterial species capable of incorporating new genetic material that may confer resistance to such drugs. In this context integrons play an important role in the capture and expression of exogenous genetic material. So far, nine classes of integrons have been described, with those of class 1 and 2 being most frequently associated with resistance in clinical isolates.[11,12]

Briefly, integrons are classified according to the integrase gene (*intI*) nucleotide sequence. In class 1 integrons, the *intI1* gene together with P_{ant} (integrase promoter), P_c (gene cassette promoter), and *attI* (integrase recognition site) constitute the 5′ conserved segment (5′CS). The 3′ conserved segment (3′CS) is basically formed of a truncated copy of *qacEΔ1*, which confers resistance to quaternary ammonium compounds, and the *sul1* gene, which codes for sulfonamide resistance. Between these two conserved segments lies the variable region (VR), containing different arrangements of gene cassettes. These gene cassettes lack a promoter of their own, however they are associated with a 59-bp element (59-be) or *attC*, the recognition site for the integrase. With this structure, class 1 integrons are able to incorporate and express gene cassettes that generally code for antibiotic resistance.[11]

Class 2 integrons have a similar structure to the integrons mentioned above; the 5′CS is constituted of the *intI2* gene, the 3′CS is formed of five *tns* genes responsible for transposition, and, finally, a VR is located between both conserved segments. However, *intI2* presents a stop codon at amino acid position 179, which prevents IntI2 from integrating or excising new gene cassettes.[13] Because of this, while more than 60 antibiotic resistance genes have been found associated with class 1 integrons,[11] class 2 integron-associated antibiotic resistance genes number scarcely more than a dozen. Among others, these stand out: streptothricin acetyltransferase (*sat2*), type I dihydrofolate reductase (*dfrA1*), and streptomycin 3′-adenyltransferase (*aadA1*). Recently, other genes have been characterized: chloramphenicol acetyltransferase (*catB2*),[14] erythromycin esterase (*ereA*),[15] and dihydropteroate synthase (*sul1* or *dhps*).[16]

Due to the fact that these genetic elements may be horizontally disseminated and that Salmonella antibiotic susceptibility does not have a homogeneous distribution (neither geographical nor temporal), surveillance programs must be undertaken to monitor the evolution of antibiotic resistance and the presence of mobile genetic elements.

In this work, we report the results of a study on the evolution of antibiotic resistance in human isolates of *Salmonella* Typhimurium received at the Centro Nacional de Salmonella (CNS) in Uruguay between the years 1976 and 2000; we also determined the presence of class 1 and 2 integrons in the multi-resistant isolates.

Materials and methods

Bacterial strains

All *Salmonella* Typhimurium strains of human origin received at the CNS between the years 1976 and 2000 were selected for the present study. The CNS is housed in the Departamento de Bacteriología y Virología, Instituto de Higiene, Universidad de la República, Montevideo, Uruguay. For the last 60 years the CNS has characterized Salmonella isolates of human, animal, food, feed, and environmental origin, submitted voluntarily by several private and public laboratories throughout the country. Furthermore, since the beginning of the VETA program in 1995 (Vigilancia de las Enfermedades Transmitidas por Alimentos — surveillance of food-borne diseases) it has become mandatory for samples from food-related outbreaks to be sent to the CNS (http://www.bvsops.org.uy/pdf/veta00.pdf).[17] The CNS also participates in the World Health Organization global Salm-Surv program (http://www.who.int/salmsurv/en/).[6]

Bacterial growth

Stock cultures stored in nutrient broth (8 g nutrient broth, 5 g peptone, 3 g NaCl, 2 g/l $Na_2HPO_4{\cdot}12H_2O$) were isolated on tryptic soy agar (TSA) (Difco, Detroit, MI, USA) and incubated at 37 °C for 18–24 h.

Every *Salmonella* Typhimurium isolate harboring class 1 and 2 integrons underwent detection of Salmonella genomic island 1 (SGI-1), phage typing, and pulsed-field gel electrophoresis (PFGE).

Bacterial phage typing

Phage typing of *Salmonella* Typhimurium was performed at the National Reference Laboratory for Salmonella in Spain with phages provided by the International Reference Laboratory (Colindale, London, UK), following international phage typing methods.[18]

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the standard disk diffusion (Kirby—Bauer) method, following the Clinical and Laboratory Standards Institute (CLSI) recommendations.[19] The antibiotics tested included: ampicillin (A), piperacillin (PIP), ampicillin/sulbactam (SAM), cephalothin (KF), cefoxitin (FOX), cefuroxime (CXM), cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), imipenem (IPM), gentamicin (G), amikacin (AK), kanamycin (K), tobramycin (To), streptomycin (S), tetracycline (T), ciprofloxacin (CIP), nalidixic acid (Nx), sulfafurazole (Su), trimethoprim (Tm), trimethoprim/sulfamethoxazole (SXT), chloramphenicol (C), and nitrofurantoin (F). *Escherichia coli* ATCC 25922 and ATCC 35218 were used as reference strains.

For analysis of the results, intermediate and absolutely resistant isolates were considered together as 'resistant'.

Definitions of resistance patterns

To define different resistance patterns we took into consideration the possible resistance mechanisms, hence resistance to ampicillin and cephalothin, including cefuroxime was considered as the same profile. Using the same criteria, resistance to trimethoprim/sulfamethoxazole was not included if isolates were resistant to both sulfafurazole and trimethoprim.

PCR amplification

Detection of *intI1* and *intI2* genes was done employing specific primers, namely I3: GCGTTCGGTCAAGGTTCTGG and I5: ACCGCCAACTTTCAGCACAT, and intI2-F: TTATTGCTGGGATTAGGC and intI2-R: ACGGCTACCCTCTGTTATC, respectively.[12] VR size was determined by PCR using 5′CS and 3′CS primers.[20]

PCR detection of SGI-1

Detection of SGI-1 was performed employing primers U7-L12/LJR1 for the left junction and 104RJ/104D or C9L2/104D for the right junction, as previously described in the literature.[20]

Pulsed-field gel electrophoresis

Only Salmonella isolates with *intI1* and *intI2* genes were further characterized by PFGE in order to detect a possible clonal dissemination of the resistances. PFGE was performed following the PulseNet-Europe protocol (http://www.pulsenet-europe.org/docs.htm). Total DNA was digested with XbaI (Roche Applied Science, Madrid Spain), and the fragments obtained were separated on 1% agarose (Seakem Gold Agarose, Iberlabo, Spain) gels using the CHEF-DR-II system (Biorad Laboratories Inc., Hercules, CA, USA). Electrophoresis was carried out with 0.5× TBE buffer at 6 V/cm and 14 °C. The running time was 21 h and the pulse ramp time was 2.2—63.8 s. The XbaI-digested DNA from Salmonella Braenderup H9812 was used as a molecular size marker. Pattern clustering was performed using UPGMA (unweighted pair-group method with an arithmetic mean) and the Dice coefficient with a tolerance index of 0.5%. Fragments smaller than 30 kb were disregarded according to the PulseNet guidelines for standardization.

Statistical analysis

To analyze the evolution of resistance, two time-periods were established (1976—1988 and 1989—2000). Variables were compared using the Mantel—Haenszel Chi-square test for the comparison of dichotomous variables and trend analysis using SPSS 10.0 software package (SPSS, Chicago, IL, USA).

Results

Two hundred and fifty-eight *Salmonella* Typhimurium isolates were recovered from the 283 archived isolates (91.2%). The remaining 25 strains could not be recovered from storage devices. One hundred and thirty-three isolates were from the 1976—1988 period and 125 isolates were from to 1989—2000 period.

Global resistance

All isolates were susceptible to amikacin, oxyiminocephalosporins, cephamycins, carbapenems, and fluoroquinolones. However, only 78 isolates (30.2%) were susceptible to all tested antibiotics. One hundred and one strains (39.1%) presented resistance to a single antibiotic. Of these, 68 strains presented resistance to streptomycin, 16 to tetracycline, 11 to sulfonamides, four to nitrofurantoin, one to ampicillin, and one to nalidixic acid.

When analyzed individually, the highest levels of resistance were to streptomycin (59.3%), followed by tetracycline and sulfafurazole (25.6% and 22.1%, respectively). Results are given in Table 1.

Overall, 38/258 strains (14.7%) were multi-resistant. Twenty-eight different resistance patterns were identified (see Table 2). Within this diversity the most frequently found resistance pattern was AKSSuTmNxCToGTF (five isolates). The second most frequently found pattern was ASSuTm, which was found four times; however these isolates were obtained in different years (1984, 1990, 1995, and 2000) making the possibility of dissemination of a single strain unlikely. The classic penta-resistant phenotype (ASSuCT), usually conferred by the genomic island I (SGI-1),[20] was present in 12 isolates that also showed resistance to seven or more antibiotics. In this context, we observed a high frequency of co-resistance to Tm, K, and Nx (11/12), G (9/12), F (8/12), and To (6/12) (see Table 2).

Table 1 Susceptibility rates of *Salmonella* Typhimurium isolates ($N = 258$); Montevideo, 1976–2000.

Antibiotic	Susceptible *n* (%)	Resistant *n* (%)
Streptomycin	105 (40.7)	153 (59.3)
Tetracycline	192 (74.4)	66 (25.6)
Sulfafurazole	201 (77.9)	57 (22.1)
Ampicillin	212 (82.2)	46 (17.8)
Piperacillin	216 (83.7)	42 (16.3)
Ampicillin/sulbactam	219 (84.9)	39 (15.1)
Nalidixic acid	225 (87.2)	33 (12.8)
Cephalothin	223 (86.4)	35 (13.6)
Trimethoprim	226 (87.6)	32 (12.4)
Nitrofurantoin	231 (89.5)	27 (10.5)
Trimethoprim/ sulfamethoxazole	229 (88.8)	29 (11.2)
Kanamycin	230 (89.1)	28 (10.9)
Chloramphenicol	240 (93.0)	18 (7.0)
Gentamicin	242 (93.8)	16 (6.2)
Tobramycin	244 (94.6)	14 (5.4)
Cefuroxime	254 (98.4)	4 (1.6)
Amikacin	258 (100)	0.0
Cefepime	258 (100)	0.0
Cefotaxime	258 (100)	0.0
Cefoxitin	258 (100)	0.0
Ceftazidime	258 (100)	0.0
Ciprofloxacin	258 (100)	0.0
Imipenem	258 (100)	0.0

Most of these isolates (10/12) were recovered at the end of the 1970s or early 1980s. All of these isolates were obtained before the description of SGI-1-bearing *Salmonella* Typhimurium.

Detection of class 1 integrons, class 2 integrons, and their variable regions

We searched for the occurrence of integrons in the multi-resistant *Salmonella* Typhimurium isolates. Twenty-nine out of 38 strains presented integrons: three isolates had only class 1 integrons, 15 isolates showed only class 2 integrons, and 11 isolates presented both.

The presence of these genetic elements was related to the number of different antibiotics to which the strains displayed resistance. In this sense, strains that were resistant to four to six different drugs presented no integrons or only one class of integrons (mainly class 2). When strains showed resistance to seven different drugs, at least one type of integrons could be detected (mainly class 2). The presence of both classes of this genetic element was common in those strains that showed resistance to at least nine different antibiotics. All isolates belonging to the most frequent phenotype (AKSSuTmNxCToGTF) harbored both classes of integrons, while most isolates (3/4) belonging to the second most frequent (ASSuTm) had none.

Isolates harboring class 1 and 2 integrons were found to be carrying VRs of four different sizes, namely 900, 1000, 1100, and 3000 bp. According to the presence/absence of these VRs, we designated four profiles: 0 (no band), 1 (one band; 1000 bp), 2 (two bands; 900–1100 bp), and 3 (three bands; 900, 1100, and 3000 bp) (see Table 2). Although some of the VRs showed sizes similar to those present in SGI-1,[20] PCR assays using primers U7-L12/LJR1 and 104RJ/104D or C9L2/104D were negative, ruling out the occurrence of SGI-1 (regardless of the variant) in these isolates.

Table 2 Main features of the multi-resistant strains.

Resistance profile[a]	*n*	Isolates and integrons[b,c,d]
AKSNx	1	$(048/77)^{intI2}$
ASSuT	2	$(074/77)^{intI2}$, $(03/92)^{-}$
ASTmSxt	1	$(016/84)^{intI2}$
ASNxF	1	$(079/77)^{-}$
SSuToT	1	$(018/94)^{-}$
ASSuTm	4	$(015/84,027/90,004/95)^{-}$ $(178/00)^{intI1}$
AKSToG	1	$(047/81)^{-}$
AKSSuNx	1	$(076/77)^{intI2}$
ASSuTmNx	1	$(058/77)^{intI2}$
AKSSuTm	1	$(030/91)^{-}$
ASTmNxTF	1	$(090/78)^{intI2}$
AKSSuTmNx	1	$(096/78)^{intI2}$
ASSuTmN**CT**	1	$(222/00)^{-}$
AKSSuNxT**CT**	1	$(059/77)^{intI1}$
AKSTmNxTF	1	$(098/78)^{intI2}$
AKSTmNxGF	1	$(071/77)^{intI2}$
ASSuTmNxCF	1	$(092/78)^{intI2}$
ASSuTmNxGT	1	$(020/81)^{intI2}$
AKSSuTmNxT	1	$(021/81)^{intI2}$
AKSSuTmNxTF	1	$(130/78)^{intI2}$
KSSuNxCToGT	1	$(157/79)^{intI1}$
AKSSuTmNxCToG	2	$(165/79_3, 102/78_3)^{intI1\ intI2}$
AKSSuTmNx**CT**F	1	$(024/81_2)^{intI1\ intI2}$
AKSSuTmNx**CGT**	1	$(068/77_2)^{intI1\ intI2}$
AKSSuTmNxCToGF	1	$(100/78_3)^{intI1\ intI2}$
AKSSuTmNx**CToGT**	1	$(164/79_3)^{intI1\ intI2}$
AKSSuTmNx**CGT**F	2	$(091/78,009/81)^{intI2}$
AKSSuTmNx**CToGT**F	5	$(094/78_3,101/78_3,116/78_1,$ $154/79_3,058/83_0)^{intI1\ intI2}$

S, streptomycin; T, tetracycline; Su, sulfafurazole; A, ampicillin; Nx, nalidixic acid; Tm, trimethoprim; F, nitrofurantoin; Sxt, trimethoprim/sulfamethoxazole; K, kanamycin; C, chloramphenicol; G, gentamicin; To, tobramycin.

[a] Bold letters indicate the penta-resistance profile.

[b] The presence of class-1 (*intI1*) and/or 2 (*intI2*) integrons is shown in superscript; (−) no integrons detected.

[c] The profile of the variable region size using 5′CS–3′CS primers is shown in subscript; 0: no band, 1: one band (1000 bp), 2: two bands (900–1100 bp), 3: three bands (900, 1100, 3000 bp).

[d] The number after the slash (/) indicates the year of isolation.

Phage typing and PFGE

Eleven multi-resistant *Salmonella* Typhimurium isolates (*intI1* and *intI2* positive) were characterized by phage typing and PFGE. Three belonged to phage-type DT193, one to DT194, and one to DT195; the remaining six were non-typeable (Figure 1).

Five of these strains displayed the same antibiotic resistance profile, AKSSuTmNxCToGTF (strains 154/79, 58/83, 101/78, 116/78, 94/78). Even though four of these isolates

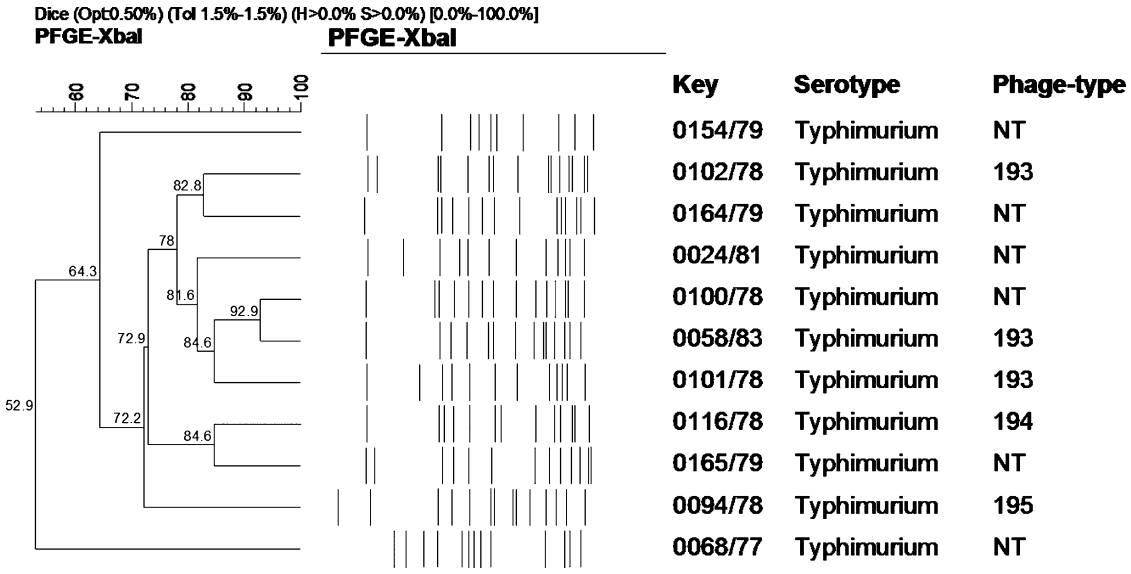

Figure 1 Phylogenetic tree of the 11 multi-resistant *Salmonella* Typhimurium isolates harboring class 1 and 2 integrons included in the pulsed-field gel electrophoresis assay. Along with the restriction pattern obtained for each strain is the information regarding its serotype and phage type. The tree was obtained by the UPGMA method (unweighted pair-group method with an arithmetic mean) using a 0.5% Dice coefficient and 1.5% tolerance.

were clustered between 1978 and 1979 they had different phage types as well as different restriction patterns, ruling out the possibility of a single strain circulating in the population.

Another two isolates displayed similar antibiotic resistance profiles, namely strains 102/78 and 165/79 (AKSSuTmNxCToG), yet their restriction patterns were different as well as their phage-type (Figure 1).

Although types DT193, DT194, and DT195 are not among the most frequently found in our region, DT193 has been detected in human isolates from Brazil.[21] Interestingly, type DT195 is the main phage-type recovered from pork products in the southern-most part of Brazil.[22]

Antibiotic resistance evolution in *Salmonella* Typhimurium

Comparison of antibiotic resistance levels between the two time periods (1976–1988 and 1989–2000) shows a statistically significant decrease in resistance to almost every antibiotic between the years 1989–2000, except for streptomycin, to which resistance remained constant, as reflected in the high *p*-value (Table 3). Accordingly, a larger number of multi-resistant isolates were recovered for the earlier period (31 isolates versus seven isolates during the period 1989–2000).

Discussion

When compared to European antibiotic resistance levels, our findings show that during the years 1989–2000 streptomycin resistance was 56.8%, similar to values reported in Spain (61%)[7,23] and slightly lower than those reported in France (71%).[24] We also found similar low values (i.e., <5%) of resistance to gentamicin, kanamycin, and nalidixic acid (Table 3). However, we did find considerable differences between our country and European countries in antibiotic resistance levels to ampicillin (7.2% vs. 65%), sulfafurazole (11.2% vs. 70%), chloramphenicol (0.8% vs. 58%), and tetracycline (13.6% vs. 80%).[7,23–25] The SENTRY surveillance program, which includes other South American countries, has reported similar levels of resistance to ampicillin and tetracycline, but higher levels for other antibiotics such as nalidixic acid and trimethoprim/sulfamethoxazole.[4,25]

International guidelines recommend the use of oxyiminocephalosporins and fluoroquinolones to treat serious or invasive Salmonella infections. In this regard, we did not find any

Table 3 Resistance comparison for the two time periods.

Antibiotic	Period		
	1976–1988 (*n* = 133) *n* (%)	1989–2000 (*n* = 125) *n* (%)	*p*-Value[a]
Ampicillin	37 (27.8%)	9 (7.2%)	<0.001
Ampicillin/sulbactam	34 (25.6%)	5 (4%)	<0.001
Piperacillin	34 (25.6%)	8 (6.4%)	<0.001
Cephalothin	29 (21.8%)	6 (4.8%)	<0.001
Gentamicin	16 (12.0%)	0 (0%)	<0.001
Kanamycin	26 (19.5%)	2 (1.6%)	<0.001
Streptomycin	84 (63.2%)	71 (56.8%)	0.359
Tobramycin	13 (9.8%)	1 (0.8%)	0.001
Sulfafurazole	43 (32.3%)	14 (11.2%)	<0.001
Trimethoprim	25 (18.8%)	7 (5.6%)	0.001
Trimethoprim/ sulfamethoxazole	24 (18.0%)	5 (4%)	<0.001
Chloramphenicol	17 (12.8%)	1 (0.8%)	<0.001
Tetracycline	49 (36.8%)	17 (13.6%)	<0.001
Nalidixic acid	30 (22.6%)	3 (2.4%)	<0.001
Nitrofurantoin	22 (16.5%)	5 (4%)	0.002

[a] Statistical significance of the difference in antibiotic resistance between the two time periods.

strain displaying resistance to these antibiotics throughout the surveillance period.

Resistance to fluoroquinolones generally occurs by small stepwise increases in its minimum inhibitory concentration, and in most cases it first involves resistance to non-fluorinated quinolones such as nalidixic acid.[26] Bearing this in mind, the low number of quinolone-resistant strains isolated in this study (2.4%) somehow minimizes the probability of the occurrence of resistance to newer quinolones. Moreover, we did not find resistance to amikacin, which could lead us into thinking about the potential participation of a recently described variant of an aminoglycoside-acetylating enzyme, namely Aac(6′)-Ib-cr.[27] This enzyme has already been detected in our country.[28] However, surveillance programs aimed at detecting resistance to such antibiotics are necessary due to the continuous detection of new transferable quinolone resistance mechanisms.

The decrease in antibiotic resistance witnessed during the second time period (i.e., the years 1989—2000) goes hand in hand with a decrease in the occurrence of integrons in these strains isolated over the same time-span. Just one isolate from this period presented these elements (class 1). We have not found a clear explanation for this notable decrease in antibiotic resistance between the two periods, since Salmonella infections are only treated with antibiotics in serious cases, and therefore they are rarely exposed to this kind of selective pressure. On the other hand, the continuous streptomycin resistance is not related to human usage, since this antibiotic has fallen out of use in medicine in our country. Different hypotheses have been proposed, one of which is related to the availability of drinking water and good water treatment facilities in our country (so far Uruguay is the only country in South America free from cholera); this rules out the possibility of infection from this source.

Even though livestock production in Uruguay is extensive, antimicrobials are not frequently used for growth promotion since the production system is mostly based on grazing. In recent years, the National Program of Biological Wastes did not find antibiotic traces in cattle (Mendez R, personal report). Infection from other animal sources, through the food chain, is low due to the low level of poultry and pork production in the country . In this context, we have recently found that the genotypes of *Salmonella* Enteritidis involved in human infection derive mainly from poultry and eggs,[29] which suggest that this constitutes the main source of transmission to humans. The introduction almost 20 years ago of a live, attenuated Salmonella vaccine aimed at protecting poultry against infection by *Salmonella* serovar Gallinarum, probably resulted in an important decrease in antibiotic administration to fowl birds. The removal of this selective pressure could have favored the colonization of the avian gut by antibiotic-susceptible strains, therefore partially explaining the decrease in the recovery of resistant isolates from humans.

Two issues should be kept in mind while analyzing the results obtained. Firstly, although this is a retrospective analysis and some isolates may have lost their antibiotic-resistance during storage or during recovery, it would be reasonable to think that this would be the case for those strains stored for longer periods; yet these strains are the ones with the highest levels of antibiotic-resistance. Secondly, this report represents the evolution of antibiotic-resistance of every *Salmonella* Typhimurium isolate submitted to the CNS; however, it should be taken into account that the study of food-related outbreaks prior to 1995 was not mandatory, and that the study of isolated cases is also not mandatory. In this sense those strains obtained during the second time period can be seen as being more representative of our reality than those isolated during the first time period. If we consider only those isolates obtained from 1995 to 2000 (75 out of 125 strains obtained throughout the second time period), only three of them (004/95, 178/00, and 222/00) were multi-resistant (see Table 2). These results clearly reflect the marked reduction in antibiotic resistance levels of the *Salmonella* Typhimurium strains isolated in Uruguay during recent years.

These results are similar to those observed with other enterobacteria in our country, such as EPEC, where resistance levels have fallen during the past 15 years.[30]

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