Surveillance of antibiotic resistance evolution and detection of class 1 and 2 integrons in human isolates of multiple resistant *Salmonella* Typhimurium obtained in Uruguay from 1976 to 2000.

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Abstract:

Objectives: To study the evolution of antibiotic resistance in isolates of *S.* Typhimurium obtained in Uruguay between the years 1976 and 2000, and to determine the incidence of class-1 and 2 integrons in multiple resistant isolates.

Methods: we studied 258 strains of *Salmonella enterica* serovar Typhimurium from various sources, isolated from 1976 to 2000. By means of disk diffusion assay and PCR, we determined the evolution of the antibiotic resistance and the distribution of class-1 and 2 integrons in all isolates.

Results: During the period 1989-2000 resistance to streptomycin was 57%, tetracycline 13.6%, sulfonamides 11.2% and ampicillin 7.2%. Resistance to gentamicin, kanamicin, cloramphenicol and nalidixic-acid were lower than 5%; on the other hand 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 last period, resistance to streptomycin, tetracycline, sulfonamides and ampicillin were 63.2, 36.8, 32.3 and 27.8%, respectively.

Throughout both periods 29 multiple resistant *S. Typhimurium* strains were isolated, harbouring some class of integrons: 15 strains had only *intI2*, 11 strains presented both *intI1* and *intI2*, and 3 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.

Keywords:

Salmonella, Antibiotics, Resistance, integrons, evolution

Introduction:

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

In Uruguay, *S.* Typhimurium is one of the main causes of human salmonellosis since 1971 [1,2], second only to *S.* Enteritidis since 1995 [3]. This epidemiological situation is similar to other countries in Latin America and the rest of the world [4, 5, 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 small children, elderly, and immunocompromised patients.

Antibiotic resistance in *S.* Typhimurium is a re-emerging problem worldwide. In our country multiple resistant isolates (defined as resistance to four or more classes of antibiotics)[7] have been reported since 1975 [8]; different enteric pathogens like other *Salmonella* serovars and enteropathogenic *E. coli* (EPEC), have also been pointed 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, being class-1 and 2 integrons the most frequently associated to resistance in clinical isolates [11, 12].

Briefly, integrons are classified according to the integrase gene (*intI*) nucleotide sequence. In class-1 integrons, the *intI-1* gene together with P_{ant} (integrase promoter), P_c (cassettes genes promoter) and *attI* (integrase recognition site) constitute the 5´ conserved segment (5´CS). The 3´conserved segment (3'CS) is basically formed by a truncated copy of *qacEΔ1* which confers resistance to quaternary ammonium compounds, and *sul1* gene that 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 to a 59bp 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, which generally code for antibiotic resistance [11].

Class-2 integrons have a similar structure to the integrons mentioned above; 5´ conserved segment is constituted by *intI2* gene; the 3´ conserved segment is formed by five *tns* genes responsible for transposition and, finally, a variable region (VR) located between both conserved segments.

Nevertheless, *intI2* presents a stop codon at the amino acidic 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 to class-1 integrons [11], class-2 integrons-associated antibiotics resistance genes are scarcely more than a dozen. Among others, these stand out: streptothricin acetyltransferase (*sat2*), type I dihydrofolate reductase (*dfrA1*), streptomycin 3'-adenyltransferase (*aadA1*). Recently, other genes have been characterized, chloramphenicol acetyltransferase (*catB2*) [14], erythromycin esterase (*ereA*) [15] and dihidropteroate synthase (*sul1* or *dhps*) [16].

Due to the fact that these genetics elements may be horizontally disseminated and that *Salmonella* antibiotic susceptibility does not have an homogenous distribution, neither geographical nor temporal, surveillance programs must be performed monitoring 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 *S.* 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 multiresistant isolates.

Materials and methods:

Bacterial strains. All *S.* Typhimurium strains of human origin received at the CNS, between the years 1976 and 2000, were selected for the present study. CNS is housed in the Departamento de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina; for the last 60 years the CNS has characterized *Salmonella* isolates from human, animal, food, feed and environmental origins, submitted voluntarily by several private and public laboratories throughout the country. Nevertheless, since the beginning of the V.E.T.A program in 1995 (Vigilancia de las Enfermedades Transmitidas por Alimentos - surveillance of food-borne diseases) samples from food-related outbreaks must be sent to the CNS mandatorily (www.bvsops.org.uy/pdf/**veta**00.pdf)[17].
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 (nutrient broth 8 g, peptone 5 g, NaCl 3 g, $Na_2HPO_4 12H_2O$ 2 g per liter) were isolated on tryptic soy agar (TSA) (Difco, Detroit, MI) and incubated at 37ºC for 18-24 hours.

Every *S.* Typhimurium isolate harboring class-1 and 2 integrons, underwent detection of SGI-1, phage-typing and PFGE.

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

Antibiotic susceptibility testing. Antibiotic susceptibility testing was performed using the standard disk diffusion (Kirby-Bauer) method and 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).

For results analysis, intermediate and absolutely resistant isolates were considered together as "resistant".

Escherichia coli ATCC 25922 and ATCC 35218 were used as reference strains.

Resistance Patterns definitions:

To define different resistance patterns we took into consideration the possible resistance mechanisms, in such sense resistance to ampicillin and cephalotin, including cefuroxime was considered as the same profile. Using the same criteria, resistance to trimethoprim-sulfamethoxazole was not included in table 2 if isolates were resistant to both sulfafurazole and trimethoprim.

155 **PCR amplification**. Detection of *intI1* and *intI2* genes was done employing specific
156 primers, namely I3: GCGTTCGGTCAAGGTTCTGG, I5: ACCGCCAACTTTCAGCA
157 CAT, intI2-F: TTATTGCTGGGATTAGGC, intI2-R: ACGGCTACCCTCTGTTATC,
158 repectively [12]. VR size was determined by PCR using 5'CS and 3'CS primers[20].
159 **PCR detection of SGI1:** detection of SGI-1 was performed employing primers U7-
160 L12/LJR1 for the left junction and 104RJ/104D or C9L2/104D for the right junction as
161 previously described in the literature [20].
162
163 **Pulsed-field gel electrophoresis (PFGE).** Only *Salmonella* isolates with *intI1* and *intI2*
164 genes were further characterized by PFGE in order to detect a possible clonal
165 dissemination of the resistances. PFGE was performed following the PulseNet-Europe
166 protocol (http://www.pulsenet-europe.org/docs.htm). Total DNA was digested with
167 XbaI (Roche Applied Science), and the obtained fragments were separated in 1%
168 agarose (Seakem gold Agarose, Iberlabo Spain) gels using the CHEF-DR-II system
169 (Biorad Laboratories Inc. Hercules, CA, USA). Electrophoresis was carried out with
170 0.5X TBE buffer at 6V/cm and 14ºC. The running time was 21h and the pulse ramp
171 time was 2.2-63.8s. The XbaI digested DNA from *Salmonella* Braenderup H9812 was
172 used as a molecular size marker. Pattern clustering was performed using UPGMA
173 (unweighted pair-group method with an arithmetic mean) and the Dice coefficient with
174 a tolerance index of 0.5%. Fragments smaller than 30kb were disregarded according to
175 the PulseNet guidelines for standardization.
176
177 **Statistical Analysis.** To analyze the evolution of resistance, two periods of time were
178 established (1976-1988 and 1989-2000). Variables were compared using Mantel-

179 Haenszel chi-square test for the comparison of dichotomous variables and trend analysis
180 using Statistical analysis SPSS 10.0 software package (SPSS, Chicago, IL).
181
182 **Results and Discussion:**
183 Two hundred and fifty-eight *S.* Typhimurium isolates were recovered from the 283
184 archived isolates (91.2%). The remaining 25 strains could not be recovered from storage
185 devices. One hundred and thirty-three isolates correspond to the 1976-88 period and 125
186 isolates belong to 1989-2000 period.
187 **Global resistance**. All isolates were susceptible to amikacin, oxyiminocephalosporins,
188 cefamycins, carbapenems and fluoroquinolones. However, only 78 isolates (30.2%)
189 were susceptible to all tested antibiotics. One hundred and one strains (39.1%)
190 presented resistance to a single antibiotic. Of these, 68 strains presented resistance to
191 streptomycin, 16 to tetracycline, 11 to sulfonamides, four to nitrofurantoin and one to
192 ampicillin and nalidixic-acid, respectively.
193 When analyzed individually, the highest levels of resistance were to streptomycin
194 (59%), followed by tetracycline and sulfonamides (26% and 22%, respectively). Results
195 are given in Table 1.
196 Overall, 38/258 strains (14.7%), were multiresistant. Twenty eight different resistance
197 patterns were identified (see Table 2).
198 Within this diversity the most frequently found resistance pattern was
199 AKSSuTmNxCToGTF (five isolates). In the second place, pattern ASSuTm was found
200 four times but these isolates were obtained in different years (1984, 1990, 1995 and
201 2000) making the possibility of a single strain dissemination unlikely. The classic
202 pentaresistant phenotype (ACSSuT), usually conferred by the genomic island I (SGI-1)
203 [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).

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

Detection of class-1 integrons, their VRs and class-2 integrons:

We searched for the occurrence of integrons in multiple resistant *Salmonella* isolates. Twenty-nine out of 38 strains presented integrons: tree 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 to. 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 integron 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) harboured 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 3000bp. According to the presence / absence of these VRs we designated four profiles: 0 (no band), 1 (one band 1000pb), 2 (two bands 900-1100pb) and 3 (three bands 900, 1100 and 3000pb) 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.

Phage-typing and PFGE. Eleven multirresistant *S.* typhimurium isolates (*intI*-1 and *intI*-2 +) were characterized by phage typing and pulsed-field gel electrophoresis. Three belonged to phage-type DT193, one to DT194 and one to DT195, whereas the remaining six were nontypeable. (Fig.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 4 of such isolates were clustered between 1978 and 1979 they had different phage-type as well as different restriction patterns, ruling out the possibility of a single strain circulating among the population.

Other 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 (fig. 1).

Although types DT193, 194 and 195 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 southernmost part of Brazil [22].

Antibiotic resistance evolution in *S.* Typhimurium. Comparison of antibiotic resistance levels between both time periods 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 between 1976 and 1988 (31 isolates versus 7 isolates during the period 1989-2000).

When compared to European antibiotic resistance levels, our findings show that during the years 1989-2000 streptomycin resistance was 57%, similar to values reported in

Spain (61%)[7,23] and slightly lower than in France (71%)[24]. On the other hand, we also found similar low values (i.e.: <5%) of resistance to gentamicin, kanamicin and nalidixic-acid (Table 3). Nevertheless, we did find considerable differences in antibiotic resistance levels between our country and European countries to ampicillin (7,2 vs 65%), sulfonamides (11,2 vs 70%), cloramphenicol (0,8 vs 58%) and tetracycline (13,6 vs 80%) [7,23,24,25].SENTRY surveillance program, which includes other South American countries, has reported similar levels of resistance to ampicillin and tetracyclin, albeit higher 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 such sense, we did not find any strain displaying resistance to those antibiotics throughout the surveillance period.

Resistance to fluoroquinolones generally occurs by small stepwise increases in its minimum inhibitory concentration, in most cases it first involves resistance to non-fluorated quinolones such as nalidixic-acid [26]. Bearing that in mind, the low number of quinolone-resistant strains isolated in this study (2,4%) somehow minimizes the probability of 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.

279 The decrease in antibiotic resistance witnessed during the second time period (i.e.:
280 years 1989-2000) goes hand in hand with a decrease in the occurrence of integrons in
281 those strains isolated during the same time-span. Just one isolate from this period
282 presented these elements (class 1). We have not find a clear explanation to this
283 notorious decrease in antibiotic resistance between both periods since *Salmonella*
284 infections are only antibiotic-treated in serious cases, and therefore they are rarely
285 exposed to this kind of selective pressure. On the other side, the continuous
286 Streptomycin resistance is not related to human usage since this antibiotic has fallen out
287 of use in medicine in our country. Different hypothesis have been proposed, one of
288 them is the availability of drinking water and good water treatment facilities in our
289 country (so far Uruguay is the only country in South America free from cholera); this
290 rules out the possibility of infection through this source.
291 Even when livestock production in Uruguay is extensive, antimicrobial use for growth
292 promotion is not frequent since production system is mostly based on grazing. In recent
293 years, the National Program of Biological Wastes did not find antibiotic traces in cattle
294 (Mendez R, personal report). Other animal sources, through the food chain, are not
295 probably due to little poultry and pork production in the country. In this context,
296 recently, we have found that the genotypes of *Salmonella* Enteritidis involved in human
297 infection derive mainly from poultry and eggs [29], which suggest that this constitutes the
298 main source of transmission to humans. The introduction almost 20 years ago of a live,
299 attenuated *Salmonella* vaccine aimed at protecting poultry against infection by
300 *Salmonella* serovar Gallinarum probably resulted in an important decrease of antibiotic
301 administration to fowl birds. The removal of this selective pressure could have favored
302 the colonization of the avian gut by antibiotic-susceptible strains explaining, partially,
303 the decrease in the recovery of resistant isolates from humans.

Two issues should be kept in mind while analyzing the results obtained: in the first place, although this is a retrospective analysis and some isolates may have lost their antibiotic-resistance while stored or during their recovery, it would be reasonable to think that this is the case of those strains stored for longer periods; nevertheless such strains are the ones with the highest levels of antibiotic-resistance.

Secondly, this report represents the evolution of antibiotic-resistance of every *S.* Typhimurium isolate submitted to CNS; it should be taken into account the non-mandatory character of the studying of food-related outbreaks prior to 1995 as well as the non-mandatory character of the studying of isolated cases. In this sense those strains obtained during this second period can be seen as being more representative of our reality than those isolated during the first period. If we consider only those isolates obtained from 1995 to the year 2000 (75 out of 125 strains obtained throughout the second period), only three of them (004/95, 178/00 y 222/00) were multirresistant (see table 2). These results clearly reflect the drastic reduction of antibiotic resistance levels during the past years of the *S.* Typhimurium strains isolated in Uruguay.

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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No Competing interest declared

References:

1. Peluffo CA, Hormaeche CE, Coubria, MC. Frecuencia de tipos serologicos clasificados en el Centro Nacional de Salmonelas. Rev. Urug. Patol. Clin. Microbiol. 1971;9:143–150.
2. Hormaeche CE, De Marco R, Schelotto F, de Montero C, Rivas CN, Mutti D, Bello N. Frecuencia de serotipos identificados en el Centro de Salmonelas de Montevideo. Rev. Urug. Patol. Clin Microbiol. 1977;15:43–47.
3. Martínez A., Torres ME, Macedo M., Algorta G. Origen y distribución de serotipos de *Salmonella,* 1996-1999. XIV Congreso Latinoamericano de Patología Clínica, V Congreso de Patología Clínica del MERCOSUR, VIII Congreso Uruguayo de Patología Clínica. 2000, Montevideo.
4. Gales AC, Sader HS, Mendes RE, Jones RN. Salmonella spp. isolates causing bloodstream infections in Latin America: report of antimicrobial activity from the SENTRY Antimicrobial Surveillance Program (1997-2000). Diagn. Microbiol. Infect. Dis. 2002;44:313-8.
5. Orman BE, Piñeiro SA, Arduino S, Galas M, Melano R, Caffer MI, Sordelli DO, Centrón D. Evolution of multiresistance in nontyphoid salmonella serovars from 1984 to 1998 in Argentina. Antimicrob. Agents Chemother. 2002;46:3963-70.
6. World Health Organization. Global Salm-Surv Progress Report 2000-2005. 2006
7. Cruchaga S, Echeita A, Aladuena A, Garcia-Pena J, Frias N, Usera MA.. Antimicrobial resistance in salmonellae from humans, food and animals in Spain in 1998. J. Antimicrob. Chemother. 2001; 47:315-21.

356 **8.** Schelotto F, Rivas CM, Alía C, Colensky L. Características de las cepas de *S.*
357 *typhimurium* multirresistentes productoras de infección cruzada en hospitales
358 pediátricos. Rev. Latinoam. Microbiol. 1975;17:9–16.
359 **9.** Vignoli R, Varela G, Mota MI, Cordeiro NF, Power P, Ingold E, Gadea P, Sirok
360 A, Schelotto F, Ayala JA, Gutkind G. Enteropathogenic *Escherichia coli*
361 (EPEC) Strains carrying PER-2 and TEM 116 Extended Spectrum ß-Lactamase
362 (ESBL) Isolated from Children with Diarrhea in Uruguay. J. Clin. Microbiol.
363 2005; 43:2940–2943.
364 **10.** Vignoli R, Cordeiro NF, García V, Mota MI, Betancor L, Power P, Chabalgoity
365 JA, Schelotto F, Gutkind G, Ayala JA. New TEM Derived Extended-Spectrum
366 β-Lactamase and its Genomic Context in Plasmid from *Salmonella enterica*
367 Serovar Derby Isolates from Uruguay. Antimicrob. Agent Chemother. 2006;50:
368 781-784.
369 **11.** Sabaté M. and Prats G. Estructura y función de los integrones. Enferm. Infecc.
370 Microbiol. Clin. 2002; 20: 341-345.
371 **12.** Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M,
372 Liebert C, Summers AO, White DG, Maurer JJ. Incidence of class 1 and 2
373 integrases in clinical and commensal bacteria from livestock, companion
374 animals and exotics. Antimicrob. Agents Chemother. 2001;45:723-726.
375 **13.** Hansson K, Sundström L, Pelletier A, Roy PH. IntI2 Integron Integrase in Tn*7* J.
376 Bacteriol. 2002;184:1712–1721.
377 **14.** Ramirez MS, Quiroga C. Centron D. Novel Rearrangement of a Class 2 Integron
378 in Two Non-Epidemiologically Related Isolates of Acinetobacter baumannii.
379 Antimicrob. Agents Chemother. 2005;49: 5179-5181.

15. Biskri L. and Mazel D. Erythromycin esterase gene ere(A) is located in a functional gene cassette in an unusual class 2 integron. Antimicrob. Agents Chemother. 2003;47: 3326-3331.

16. Ploy MC, Denis F, Courvalin P, Lambert T. Molecular characterization of integrons in Acinetobacter baumannii: description of a hybrid class 2 integron. Antimicrob. Agents Chemother. 2000; 44: 2684-2688.

17. Organización Panamericana de la Salud. Programa de Salud Publica Veterinaria Instituto Panamericano de Protección de Alimentos y Zoonosis 1er. Taller Nacional del Sistema V.E.T.A www.bvsops.org.uy/pdf/**veta**00.pdf.

18. Anderson ES, Ward LR, Saxe MJ and de Sa JD. Bacteriophage typing designation of Salmonella Typhimurium. J Hyg Camb. 1977:78:297-300

19. Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. M100–S16. Clinical and Laboratory Standards Institute, Wayne Pa.

20. Levings RS, Lightfoot D, Partridge SR, Hall RM, Djordjevic SP. The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. J. Bacteriol. 2005;187:4401–4409.

21. Pereira CS, Medeiros LM, Garcia Costa R, Lima Festivo M, Falavina dos Reis EM, Miyuki Seki L; dos Prazeres Rodrigues D. Phage Typing And Multidrug Resistance Profile In *S.* Typhimurium Isolated From Different Sources In Brazil From 1999 To 2004.Braz. J. Microbiol. 2007;38:385-390.

22. Bessa MC, Michael GB, Canu N, Canal CW, Cardoso M, Rabsch W, Rubino S. Phenotypic and genetic characterization of Salmonella enterica subsp. enterica

serovar Typhimurium isolated from pigs in Rio Grande do Sul, Brazil. Res.Vet.Sci. 2007;83:302-10.

23. Soler P, González-Sanz R, Bleda MJ, Hernández G, Echeíta A, Usera MA. Antimicrobial resistance in non-typhoidal Salmonella from human source, Spain, 2001-2003.J. Antimicrob. Chemother. 2006; 58:310-314

24. Weill FX, Guesnier F, Guibert V, Timinouni M, Demartin M, Polomack L, Grimont PA. Multidrug Resistance in *Salmonella enterica* Serotype Typhimurium from Humans in France (1993 to 2003) J. Clin. Microbiol. 2006; 44 :700–708.

25. Streit JM, Jones RN, Toleman MA, Stratchounsk, LS, Fritsche TR. Prevalence and antimicrobial susceptibility patterns among gastroenteritis-causing pathogens recovered in Europe and Latin America and *Salmonella* isolates recovered from bloodstream infections in North America and Latin America: report from the SENTRY Antimicrobial Surveillance Program. 2003. International J. Antimicrob. Agent. 2006;27:367–375.

26. Hooper DC. Mechanisms of action and resistance of older and newer fluorquinolones. Clin. Infect. Dis. 2000;31:S24-S28.

27. Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, Bush K, Hooper DC. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nature Medicine. 2006;12:83-88.

28. Cordeiro N, Robino L, Medina J, Seija V, Bado I, García V, Berro M, Pontet J, López L, Bazet C, Rieppi G, Gutkind G, Ayala JA, Vignoli R. Ciprofloxacin-resistant enterobacteria harboring the aac(6')-Ib-cr variant isolated from feces of inpatients in an Intensive Care Unit in Uruguay. Antimicrob Agents Chemother. 2008;52:806-7

29. Betancor L, Schelotto F, Martinez A, Pereira M, Algorta G, Rodríguez MA, Vignoli R, Chabalgoity JA. Random Amplified Polymorphic DNA and Phenotyping Analysis of *Salmonella enterica* serovar Enteritidis Isolates Collected from Humans and Poultry in Uruguay from 1995 to 2002. J. of Clin. Microbiol. 2004;42: 1155-1162.

30. Ingold, E.; Schelotto, F.; Gadea, P.; Varela, G.; Sirok, A.; Arenas, C.; Vignoli,R.; Calvelo,E.; Tanzi, M.N.; del Monte,A. Antibiotic Resistance of Enteropathogenic Bacteria Isolated From Children with Diarrhea in Montevideo, Uruguay. 9th International Congress on Infectious Diseases. Buenos Aires Argentina 2000.

Table 1. Susceptibility rates throughout the study period. Montevideo, 1976-2000.

	%S	(%R+I)
S	105(40.7)	153 (59,3)
T	192 (74,4)	66 (25,6)
Su	201(77.9)	57 (22.1)
A	212(82.2)	46 (17.8)
PIP	216 (83.7)	42 (16.3)
SAM	219 (84.9)	39(15.1)
Nx	225 (87.2)	33 (12.8)
KF	223 (86.4)	35 (13,6)
Tm	226 (87.2)	32 (12.8)
F	231 (89.5)	27 (10.5)
SXT	229 (88.8)	29 (11.2)
K	230 (89.1)	28 (10.9)
C	240 (93)	18 (7.0)
G	242 (93.8)	16 (6,2)
To	244 (94.6)	14 (5.4)
CXM	254 (98.4)	4 (1.6)
AK	258 (100)	0.0
FEP	258 (100)	0.0
CTX	258 (100)	0.0
FOX	258 (100)	0.0
CAZ	258 (100)	0.0
CIP	258 (100)	0.0
IPM	258 (100)	0.0

S: streptomycin, T: tetracycline, Su: sulfonamides, A: ampicillin, PIP: piperacillin, SAM: ampicillin/sulbactam, Nx: nalidixic-acid, KF: cephalotin, Tm: trimethoprim, F: nitrofurantoine, SXT: trimethoprim-sulfametoxazole, K: kanamycin, C: chloramphenicol, G: gentamycin, To: tobramycin, CXM: cefuroxime, AK: amikacin, FEP: cefepime, CTX: cefotaxime, FOX: cefoxitin, CAZ: ceftazidime, CIP: ciprofloxacin, IPM: imipenem.

Table 2. Main features of multi-resistant strains

Resistance profile	n	Isolates and Integrons[1,2,3]
AKSNx	1	(048/77)$^{intI-2}$
ASSuT	2	(074/77)$^{intI-2}$, (03/92)$^{-}$
ASTmSxt	1	(016/84)$^{intI-2}$
ASNxF	1	(079/77)$^{-}$
SSuToT	1	(018/94)$^{-}$
ASSuTm	4	(015/84,027/90,004/95)$^{-}$ (178/00)$^{intI-1}$
AKSToG	1	(047/81)$^{-}$
AKSSuNx	1	(076/77)$^{intI-2}$
ASSuTmNx	1	(058/77)$^{intI-2}$
AKSSuTm	1	(030/91)$^{-}$
ASTmNxTF	1	(090/78)$^{intI-2}$
AKSSuTmNx	1	(096/78)$^{intI-2}$
ASSuTm**CT**	1	(222/00)$^{-}$
AKSSuNx**CT**	1	(059/77)$^{intI-1}$
AKSTmNxTF	1	(098/78)$^{intI-2}$
AKSTmNxGF	1	(071/77)$^{intI-2}$
ASSuTmNxCF	1	(092/78)$^{intI-2}$
ASSuTmNxGT	1	(020/81)$^{intI-2}$
AKSSuTmNxT	1	(021/81)$^{intI-2}$
AKSSuTmNxTF	1	(130/78)$^{intI-2}$
KSSuNxCToGT	1	(157/79)$^{intI-1}$
AKSSuTmNxCToG	2	($165/79_3$, $102/78_3$)$^{intI-1\ intI-2}$
AKSSuTmNx**CT**F	1	($024/81_2$)$^{intI-1\ intI-2}$
AKSSuTmNx**C**G**T**	1	($068/77_2$)$^{intI-1\ intI-2}$
AKSSuTmNxCToGF	1	($100/78_3$)$^{intI-1\ intI-2}$
AKSSuTmNx**C**ToG**T**	1	($164/79_3$)$^{intI-1\ intI-2}$
AKSSuTmNx**C**G**T**F	2	(091/78,009/81)$^{intI-2}$
AKSSuTmNx**C**ToG**T**F	5	($094/78_3$,$101/78_3$,$116/78_1$,$154/79_3$,$058/83_0$)$^{intI\text{-}1\ intI-2}$

[1] In superscript: presence of class-1 (*intI1*) and/or 2 (*intI2*) are showed. (-) No integrons are detected.

[2] In subscript Profile of VRs size using 5'CS-3'CS primers: 0: no band, 1: one band (1000pb), 2: two band (900-1100 pb), 3 three band (900, 1100, 3000pb)

Bold letter cases indicate penta-resistance profile.

[3] The number after the dash bar indicates the year of isolation.

Table 3. Resistance comparison among both time periods.

ANTIBIOTIC	PERIOD		P value*
	1976 – 1988 n =133 (%)	1989 – 2000 n=125 (%)	
A	37 (27.8%)	9 (7.2%)	<0.001
SAM	34 (25.5%)	5 (4%)	<0.001
PIP	34 (25.5%)	8 (6.4%)	<0.001
KF	29 (21.8%)	6 (4.8%)	<0.001
GM	16 (12%)	0 (0%)	<0.001
K	26 (19.6%)	2 (1.6%)	<0.001
S	84 (63.2%)	71 (56.8)	0.359
To	13 (9,8%)	1 (0.8%)	0.001
Su	43 (32.3%)	14 (11.2%)	<0.001
Tm	25 (18.8%)	7 (5.6%)	0.001
SXT	24 (18%)	5 (4%)	<0.001
C	17 (12.8%)	1 (0.8%)	<0.001
T	49 (36.8%)	17 (13.6%)	<0.001
Nx	30 (22.6%)	3 (2.4%)	<0.001
F	22 (16.5%)	5 (4%)	0.002

* Statistical significance of antibiotic resistance between both time periods

Figure 1: PFGE

Phylogenetic tree of the eleven multirresistant *S.* Typhimurium isolates harboring class-1 and 2 integrons included in the PFGE 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 using a 0.5% Dice coefficient and 1,5% tolerance.

Figure

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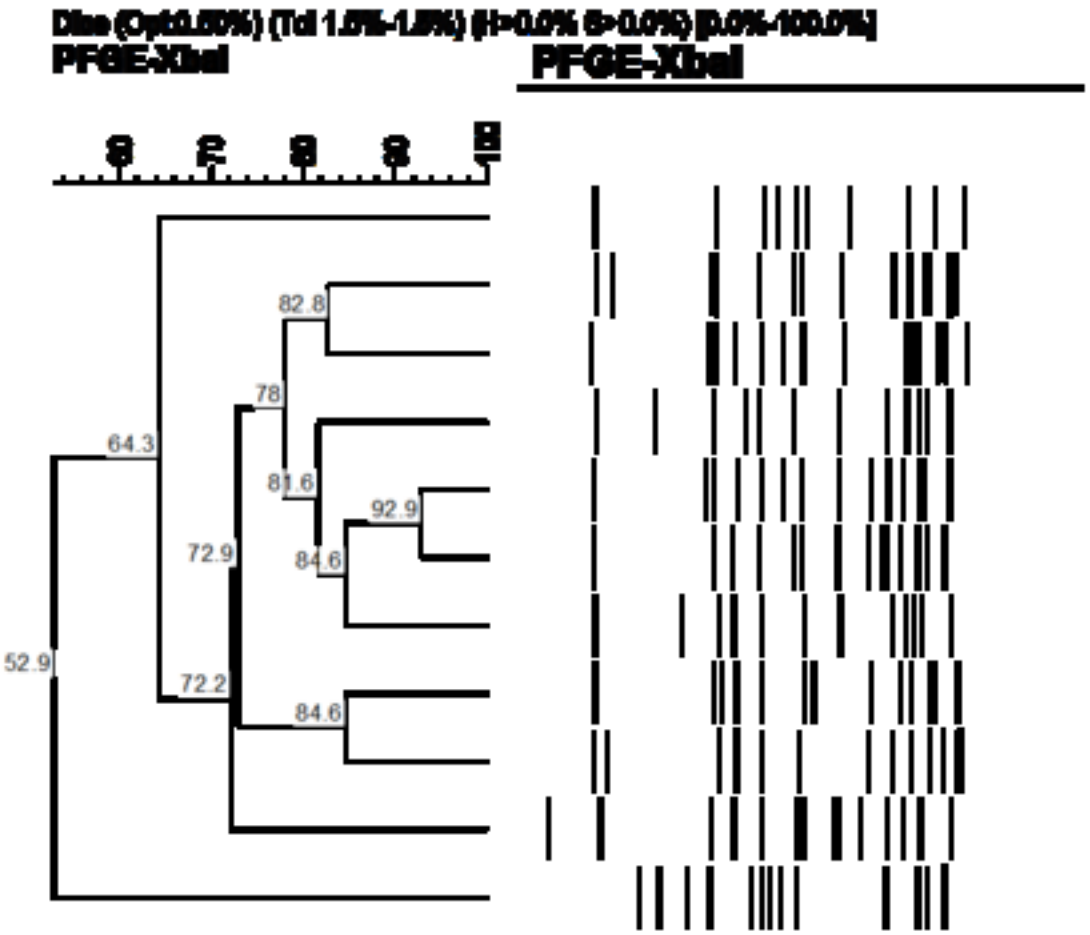

Key	Serotype	Phage-type
0154/79	Typhimurium	NT
0102/78	Typhimurium	193
0164/79	Typhimurium	NT
0024/81	Typhimurium	NT
0100/78	Typhimurium	NT
0058/83	Typhimurium	193
0101/78	Typhimurium	193
0116/78	Typhimurium	194
0165/79	Typhimurium	NT
0094/78	Typhimurium	195
0068/77	Typhimurium	NT