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Short communication

Prevalence, antimicrobial susceptibility, and molecular characterization by PCR and pulsed field gel electrophoresis (PFGE) of *Salmonella* spp. isolated from foods of animal origin in San Luis, Argentina

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A R T I C L E I N F O

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ABSTRACT

This study was aimed to determine the prevalence of *Salmonella* spp. in foods of animal origin sold at retail stores over the period 2005–2011 in San Luis, Argentina. Characterization of isolates was performed by biochemical and serological tests, antimicrobial susceptibility assays, detection of *invA* invasion gene by PCR and comparison of genomic profiles by *Xbal* DNA restriction and PFGE. Twenty seven *Salmonella* strains were detected in 27 (6.32%) of 427 samples of foods analysed. Sixteen S. Enteritidis and one S. Montevideo strains from chicken meat (17 positive samples/115 total samples), six S. Anatum strains from pork sausages (6/90), two S. Typhimurium strains from liquid egg (2/60) and two S. Montevideo strains from chicken giblets (2/62) were isolated. No *Salmonella* strains were recovered from chicken carcasses (0/100). *Salmonella* strains were susceptible to antimicrobials commonly used for clinical treatment. All isolates carried the *invA* gene. DNA restriction and PFGE analysis revealed similar genomic profiles within each *Salmonella* serovar regardless of the food type, sampling year, or retail store where samples were purchased, suggesting the possibility of circulation and transmission of clones of limited diversity in our region.

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1. Introduction

Salmonella spp. includes a large number of serovars that inhabit the intestinal tract of various domestic and wild animal species. Some of them have a predilection for a particular host and cause disease in humans and animals, while others are in a large number of reservoirs allowing them to spread to the environment (Ibar et al., 2009).

In humans, salmonellosis is usually related to the consumption of contaminated foods. Typical foodstuffs from which *Salmonella* has been isolated include pork (Prendergast et al., 2009), chicken meat (Chen, Hwang, Wang, Shih, & Tsen, 2011; White et al., 2007), eggs (Fearnley, Raupach, Lagala, & Cameron, 2011; Murchie et al., 2007), bovine meat (Gallegos-Robles et al., 2009), dairy products (De Buyser, Dufour, Maire, & Lafarge, 2001) and vegetables (Sant'Ana, Landgraf, Destro, & Franco, 2011).

In recent years, *Salmonella* infections have been the most frequently illnesses reported (17.6 per 100,000 persons) in United States. The most common serovars were *S*. Enteritidis, *S*. Newport

and *S.* Typhimurium (CDC, 2011). In Latin America, PulseNet Latin America and Caribbean Network working between 2005 and 2009 was able to collect 849 *Salmonella* spp. strains associated to outbreak and sporadic cases. These strains included 70 *S.* Typhi, 554 *S.* Typhimurium and 225 *S.* Enteritidis from Argentina, Brazil, Chile, Colombia, Costa Rica and Paraguay (Campos et al., 2012). Otherwise, Sant'Ana et al. (2011), demonstrated the presence of *S.* Enteritidis and *S.* Typhimurium from minimally processed vegetables in Brazil, while in Chile, *Salmonella* was isolated from 6 out of 560 samples of chicken carcasses and giblets (Ulloa, González, Hernández, Villanueva, & Fernández, 2010). In Uruguay, eggs and chicken meat were identified as the source of infection during an outbreak due to *Salmonella* Enteritidis (Betancor et al., 2010).

In Argentina, a significant increase in the number of *S*. Enteritidis isolates from foods associated with foodborne outbreaks has been observed since 1987 (Caffer & Eiguer, 1994). During the period 2000–2005, the National Reference Laboratory studied 443 *Salmonella* isolates derived from foods associated to outbreaks and reported *S*. Enteritidis (31.8%), *S*. Typhimurium (19.6%), *S*. Newport (6.9%), *S*. Agona (6.1%) and other serovars in a lower frequency. On the other hand, from 2006 *S*. Typhimurium was the most frequent serovar isolated from humans, animals and foods (Caffer et al., 2010). Although *Salmonella* foodborne outbreaks are uncommon





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in our region, the consumption of slightly cooked poultry, pork and meat products and the preparation of pastry, mousse, mayonnaise and other foods using unpasteurized raw eggs constitute the major risk of *Salmonella* illness for consumers (Satorres, Pederiva, & Centorbi, 1998). In this regard, the vertical and horizontal contamination of eggs by *Salmonella* serovars has been demonstrated (Martelli & Davies, 2012).

Concern about poultry, meats and other foodstuffs contaminated with foodborne pathogens has gained considerable attention because of the increased incidence of antimicrobial-resistant bacteria associated with human illness (Khan, Melvin, & Dagdag, 2007). Some authors have reported that the antimicrobial resistance of *Salmonella* strains may be associated with the expression of certain virulence genes including *invA* (Dione et al., 2011). This gene is carried on a region of the bacterial chromosome known as the *Salmonella* pathogenicity island 1 (SPI1) and encodes a factor related to the process of invasion of the intestinal epithelium. The demonstration of *invA* gene in *Salmonella* isolates can be epidemiologically relevant (Chacon, Barrantes, Garcia, & Achi, 2010).

A molecular technique such as pulsed-field gel electrophoresis (PFGE) is used for subtyping of *Salmonella* spp. isolates. Several studies indicate that PFGE may offer an improved level of discrimination over other genotypic methods for the epidemiological characterization of *Salmonella* spp. The use of PFGE in combination with *Xbal* DNA restriction has been widely recognized as a reference method for fingerprinting *Salmonella* serovars (Ammari et al., 2009a; Kalender, Sen, Hasman, Hendriksen, & Aarestrup, 2009).

The objectives of this study were i) to determine the prevalence of *Salmonella* spp. in foods of animal origin for human consumption in our region ii) to investigate the antimicrobial susceptibility of the isolated strains, iii) to assess the presence of the *invA* virulence gene by PCR, and iv) to determine possible genetic relationships among these *Salmonella* isolates by PFGE.

2. Materials and methods

2.1. Sample collection

A total of 427 samples were purchased at random over the period 2005–2011 from twelve retail stores and supermarkets located in San Luis City, Argentina. The studied samples were: i) chicken meat (n = 115), including chicken thigh, leg and breast, ii) chicken carcass (n = 100), iii) chicken giblets (n = 62), iv) liquid eggs (n = 60) and v) pork sausages (n = 90). After purchase, each sample was transported in its original container under refrigeration and used for *Salmonella* isolation and identification within 24 h of collection.

2.2. Microbiological analysis

Salmonella cultures from all samples were performed according to the FDA Bacteriological Analytical Manual (Andrews & Hammack, 2007) with some modifications. Typical colonies from each medium were picked and tested by Gram staining and standard biochemical methods (Le Minor, 1984). Serological tests for Salmonella strains were performed using poly-O antiserum (Sanofi SA, Paris, France). Results were confirmed by Reference Center for Enterobacteria, National Institute of Infectious Diseases INEI-ANLIS, Buenos Aires, Argentina.

2.3. Antimicrobial susceptibility

The antimicrobial susceptibility of *Salmonella* isolates was determined by the disk diffusion method on Mueller Hinton agar

(MH; Britania, Buenos Aires, Argentina) performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The following antibiotic disks (Britania) were used: ampicillin, 10 μ g (AMP); colistin 10 μ g (COL); chloramphenicol, 30 μ g (CHL); gentamicin, 10 μ g (GEN); tetracycline, 30 μ g (TET); trimethoprim—sulfamethoxazole, 25 μ g (TMS); ciprofloxacin, 5 μ g (CIP); neomycin, 30 μ g (NEO); kanamycin, 30 μ g (KAN); furazolidone, 5 μ g (FUR); nalidixic acid, 30 μ g (NAL); cefotaxime, 30 μ g (CTX); ceftriaxone, 30 μ g (CRO) and phosphomycin, 50 μ g (PHO). Zones of growth inhibition were evaluated according to CLSI standards (2009). *Escherichia coli* ATCC 25922 was used as a control strain.

2.4. Polymerase chain reaction for detection of invA gene

DNA extraction was made using a boiling protocol as described by Leotta et al. (2005). PCR was performed in a thermal cycler Techne TC-512 (Techne Inc., Duxford, UK). A 25 µl volume of a reaction mix containing 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 1 pmol/µl of each specific primer (forward 5'-CAA AGG TTC AGA ACG TGT CG-3'and reverse 5'-GAT ATT CCG CCC CAT ATT ATC-3'), 0.04 U/µl Taq polymerase, 2 µl template, and ultrapure water was prepared. The primers were designed in this study using the PubMed database (GenBank accession number M90846.1) and OligoCalc software (Kibbe WA. 'OligoCalc: an online oligonucleotide properties calculator', 2007). PCR was performed as follows: 94 °C for 3 min, then 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, and a final extension of 72 °C for 5 min. The products were analysed by 2% agarose gel electrophoresis in comparison with a 100-bp molecular weight DNA marker (Productos Biológicos, Quilmes, Argentina). The bands were visualized by staining with GelRed® Acid Gel Stain (Biotum, Hayward, CA, USA), 1.5 µl stock solution/40 µl gel, and photographed with a 6.0 mega pixels digital camera (Sony Cybershot; Sony Corporation, New York, USA).

2.5. Pulsed-field gel electrophoresis (PFGE)

DNA preparation was as described previously (Ribot et al., 2006). Chromosomal DNA contained in agarose plugs was digested with 10 U of *Xba*l (Fermentas, Burlington Ontario, Canada), and PFGE was performed with a CHEF-DRIII system (Bio-Rad, Hercules, CA, USA), using an electric field of 6 V/cm at 14 °C, angle of 120° and switching times of 1.8–20 s over 20 h. Migration of the DNA fragments was achieved in a 1.0% pulsed-field agarose gel (Bio-Rad) submerged in 0.5X TBE buffer (45 mmol/l Tris-Borate and 1 mmol/l EDTA) and *Salmonella* Braenderup H9812 was used a molecular reference marker. The gels were stained with Gel Red[®] Acid Gel Stain (Biotum) and photographed.

2.6. Statistical analysis

Statistical analysis on detection frequency of *Salmonella* strains related to the food type studied was performed using Chi-square test (Analytical Software, Tallahassee FL, USA). Calculations were based on confidence level equal or higher than 95% ($p \le 0.05$ was considered statistically significant).

The discrimination index (DI) values of PFGE were calculated by Simpson's diversity index, as presented by Hunter and Gaston (1998). Clustering of the patterns obtained by the PFGE was performed using Statistica 6.0 software (StatSoft Inc., Tulsa, OK, USA) and the unweighted pair group method with arithmetic average (UPGMA). G.I. Favier et al. / Food Control 29 (2013) 49-54

3. Results

4. Discussion

3.1. Prevalence of Salmonella serovars in foods

A total of 27 (6.32%) out of 427 samples of foods were *Salmonella* spp. positive; the frequency of isolation was significantly higher from chicken meat than from the other samples ($p \le 0.05$). The strains classified into four serovars were distributed as follows: 16 *S*. Enteritidis and one *S*. Montevideo strains from chicken meat (17 positive samples/115 total samples), six *S*. Anatum strains from pork sausages (6/90), two *S*. Montevideo strains from chicken giblets (2/62) and two *S*. Typhimurium strains from liquid egg (2/60). No *Salmonella* strains were recovered from chicken carcasses (0/100). The most frequently isolated serovar was *S*. Enteritidis (59.26%) followed by *S*. Anatum (22.22%), *S*. Montevideo (11.11%) and *S*. Typhimurium (7.40%) (Table 1).

3.2. Antimicrobial susceptibility

All *Salmonella* strains were susceptible to ciprofloxacin, chloramphenicol, colistin, gentamicin, kanamycin, trimethoprim sulfamethoxazole, cefotaxime and ceftriaxone. All *S.* Enteritidis and *S.* Montevideo strains were resistant to furazolidone, while *S.* Anatum and *S.* Montevideo isolated from chicken giblets were resistant to tetracycline. Resistance to nalidixic acid, ampicillin, phosphomycin, or tetracycline was observed in some *S.* Enteritidis strains. Two *S.* Typhi strains were resistant to neomycin (Table 2).

3.3. PCR

All strains amplified a 421 pb region corresponding to *Salmonella invA* gene (data not shown).

No association between the antimicrobial resistance profiles and the presence of *invA* gene was observed.

3.4. PFGE

PFGE of *Xba*I-digested genomic DNA from 27 *Salmonella* isolates showed four different macrorestriction profiles, each corresponding to a different serovar. No differences were observed between PFGE profiles of *S*. Montevideo when isolates from different foods (chicken meat and chicken giblets) were compared. DNA restriction patterns of 11, 14, 16 and 14 fragments ranging from 20.5 to 1135 kb were obtained for *S*. Enteritidis, *S*. Montevideo, *S*. Anatum and *S*. Typhimurium, respectively (Fig. 1).

Table 1

Prevalence	of Salmone	lla in	chicken	meat,	chicken	carcass,	chicken	giblets,	liquid
eggs and p	ork sausage	s and	the sero	vars id	lentified.				

Source	No of samples analysed	No of positive samples ^a	Serovar ^b
Chicken meat	115	17 ^A (14.8)	S. Enteritidis (59.26)
			S. Montevideo (3.70)
Pork sausages	90	$6^{B}(6.6)$	S. Anatum (22.22)
Chicken giblets	62	$2^{C}(3.2)$	S. Montevideo (7.40)
Liquid eggs	60	2 ^c (3.3)	S. Typhimurium (7.40)
Chicken carcasses	100	0 ^D	
Total of samples	427	27 (6.32%)	

Values followed by different capital letters indicate statistically significant difference (P < 0.05).

^a Values in the parenthesis indicate the percentage of positive samples per type of sample.

^b Values in the parenthesis indicate the percentage of each *Salmonella* serovar relating to the total of positive samples.

The Salmonella search was positive in a 6.32% of 427 foods of animal origin for human consumption analysed in our region. The 77.7% of positive Salmonella samples (21 samples/27 total samples) corresponded to chicken products and the remaining 22.3% (6 samples/27 total samples) corresponded to pork sausages $(p \le 0.05)$. In a study performed in Mexico, Zaidi et al. (2006) found high Salmonella spp. prevalence from pork (58.1%), beef (54%) and poultry (39.7%). A high Salmonella prevalence was also observed by Ibar et al. (2009) who reported 24.1% of Salmonella recovery from 386 porcine samples in slaughterhouses from Argentina. Salmonella spp. is often found in the ovary or intestinal tract of food-producing animals such chicken and pigs, respectively. During the slaughter, this pathogen can contaminate carcasses and meats, resulting in a source of foodborne illness. Several surveys have been undertaken in poultry slaughterhouses to identify processing areas where microbial cross-contamination can occur (Hinton, Cason, & Ingram, 2004; Northcutt, Buhr, & Dickens, 2002). Similarly, pigs can be reservoirs of serovars potentially pathogenic to humans and source of infection through the consumption of their products (Griffith, Schwartz, & Meyerholdz, 2006). In this study, twenty seven strains were isolated and the prevalence order of Salmonella serovars was: S. Enteritidis (59.26%), S. Anatum (22.22%), S. Montevideo (11.11%) and S. Typhimurium (7.40%). Rivoal et al. (2009) obtained 63.9% and 17.4% of S. Enteritidis and S. Typhimurium respectively, from 144 whole liquid egg samples in France, and Ammari et al .(2009b) reported the isolation of S. Enteritidis as prevalent serovar from food samples in Morocco. Negative Salmonella detection on chicken carcasses could be attributed to a low contamination level undetectable by culture techniques, or to the presence of injured bacteria which could not be re-grown during enrichment.

In this study, resistances to ampicillin and nalidixic acid were observed in 25 and 31% of S. Enteritidis strains, respectively. Resistance to penicillins by Salmonella is attributable to the acquired ability of the strains to produce β -lactamase. Instead, quinolone resistance has been initially attributed to point mutations in the gyrA gene encoding the A subunit of gyrase, whose complex with DNA is the primary target. Changes at Ser-83 or at Asp-87 of the quinolone resistance-determining region (QRDR) in the A subunit are the most frequent changes observed in nalidixic acid-resistant strains (Hur, Jawale, & Lee, 2012). S. Typhimurium isolated from liquid eggs were neomycin-resistant. Resistance to aminoglycoside antibiotics in Salmonella is associated with modifying enzymes which function by phosphorylating, acetylating or adenylating certain aminoglycosides. Another resistance mechanism is associated with the modification of the drug-binding target within the cell (Hur et al., 2012). Several Salmonella strains showed resistance to tetracycline. This is highly associated with the acquisition and expression of efflux pumps that reduce toxic levels of the drug in the bacterial cells. In Salmonella, these efflux pumps are encoded by the tet genes (Hur et al., 2012). Resistance to furazolidone and phosphomycin were also observed. However, most of strains isolated in the present study were susceptible to antimicrobials commonly used for clinical treatment.

The *invA* gene has been widely used in studies for the detection of *Salmonella* spp. in food samples (Chacon et al., 2010). In a study on prevalence of virulence genes in non-typhoidal *Salmonella* isolated from humans, animals and food products in Gambia and Senegal, Dione et al. (2011) observed high prevalence (99.5%) of strains carrying *invA*. They found significant association between the presence of this gene and other virulence genes and the antimicrobial resistance to amoxicillin, ticarcillin, trimethoprim–sulfamethoxazole, tetracy-cline, trimethoprim, spectinomycin, streptomycin, sulfonamides and

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Table 2			
Antimicrobial	susceptibility	of Salmonel	la strains isolated from chicken meat, pork sausage, chicken giblets and liquid eggs.
Source	N°	Serovar	Percent suscentibility ^a

Source	N°	Serovar	Percent susceptibility ^a													
			NAL	AMP	CIP	CHL	COL	PHO	FUR	GEN	KAN	NEO	TET	TMS	CTX	CRO
Chicken meat	16	S. Enteritidis	69	75	100	100	100	94	0	100	100	100	75	100	100	100
Chicken meat	1	S. Montevideo	100	100	100	100	100	100	0	100	100	100	100	100	100	100
Pork sausages	6	S. Anatum	100	100	100	100	100	100	100	100	100	100	0	100	100	100
Chicken giblets	2	S. Montevideo	100	100	100	100	100	100	0	100	100	100	0	100	100	100
Liquid eggs	2	S. Typhimurium	100	100	100	100	100	100	100	100	100	0	100	100	100	100
All sources	27		81	85	100	100	100	96	45	100	100	100	50	100	100	100

^a NAL: nalidixic acid (30 μg), AMP: ampicillin (10 μg), CIP: ciprofloxacin (5 μg), CHL: chloramphenicol (30 μg), COL: colistin (10 μg), PHO: phosphomycin (50 μg), FUR: furazolidone (100 μg), GEN: gentamicin (10 μg), KAN: kanamycin (30 μg), NEO: neomycin (30 μg), TET: tetracycline (30 μg), TMS: trimethoprim—sulfamethoxazole (25 μg), CTX: cefotaxime (30 μg) and CRO: ceftriaxone (30 μg).

nitrofurantoin. Hur et al. (2011) also demonstrated the presence of *invA* in 42 S. Typhimurium strains isolated from piglets with diarrhoea in Korea.

Epidemiological investigation of this pathogen using molecularbased methods is especially valuable. PFGE has been widely used to determine strain relatedness, confirm outbreaks and identify the sources of strains (Hur et al., 2011). In this study, PFGE was used for subtyping *Salmonella* spp. strains. The PFGE analysis indicated that some of the *Salmonella* isolates are indistinguishable and/or highly related; the dendrogram showed that PFGE differentiated into groupings that correlated with serovars. Moreover, PFGE analysis revealed matching between *S.* Montevideo strains isolated from



Fig. 1. Fingerprints and dendrogram obtained by PFGE of 27 Salmonella strains isolated in the present study.

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chicken meat and giblets samples, suggesting that chicken can be reservoirs and source of *Salmonella* dissemination in the region. The similarity observed in DNA profiles within each *Salmonella* serovar regardless of the food type, sampling year or retail store where samples were purchased, suggested the possibility of circulation and transmission of clones of limited diversity in our region. In contrast, Rivoal et al. (2009) could discriminate different DNA profiles among *S.* Enteritidis, *S.* Typhimurium or *S.* Infantis isolates by using *Spel* and *Xbal* restriction followed by PFGE. These authors noted that *Xbal* enzyme permitted better discrimination than *Spel* enzyme within the Enteritidis serovar.

5. Conclusions

The present study showed low prevalence of *Salmonella* serovars in foods of animal origin for human consumption in our region. The highest *Salmonella* recovery was observed in chicken meat being *S*. Enteritidis the predominant serovar. The invasion potential of strains was assessed by detecting the *invA* gene by PCR. Significant antimicrobial susceptibility was observed in all cases and PFGE revealed homogeneous DNA restriction patterns within each serovar.

Isolation of *Salmonella* species from foods of animal origin in our region emphasizes the need to continue the consumer's education on proper food handling and cooking practices to decrease the risk of transmission of *Salmonella* from contaminated meat, poultry and eggs products.

The *Salmonella* surveillance on these foods and the characterization of the isolates can contribute to the understanding of the epidemiology of this pathogen and to alert to public health organisms about risks for the human health.

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