View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Infection, Genetics and Evolution 19 (2013) 145–151

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Molecular epidemiology and virulence markers of *Salmonella* Infantis isolated over 25 years in São Paulo State, Brazil

Fernanda Almeida [a], André Pitondo-Silva [a], Maria Aparecida Oliveira [b], Juliana Pfrimer Falcão [a,*]

[a] *Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
[b] *Instituto Adolfo Lutz de Ribeirão Preto, SP, Brazil*

ARTICLE INFO

Article history:
Received 11 March 2013
Received in revised form 3 June 2013
Accepted 5 July 2013
Available online 13 July 2013

Keywords:
Salmonella Infantis
ERIC-PCR
PFGE
MLST
Virulence markers
Brazil

ABSTRACT

Infection of humans and animals caused by *Salmonella* is a major public health problem worldwide. Among the more than 2500 serovars, *S.* Infantis has been one of the 15 most isolated serovars in the world. Despite its clinical importance, little is known about the molecular characteristics of *S.* Infantis strains from Brazil. The aims of this study were to type *S.* Infantis isolates of this country and to assess their pathogenic potential. The molecular epidemiology of 35 *S.* Infantis strains, isolated from human sources (25) and food items (10) between 1984 and 2009 in São Paulo State, Brazil, were investigated using ERIC-PCR, PFGE and MLST. Furthermore, the presence of some virulence markers from *Salmonella* pathogenicity islands (SPIs) SPI-1 and SPI-2 and from the virulence plasmid was assessed by PCR. Using ERIC-PCR, 34 *S.* Infantis strains exhibited a high genetic similarity (⩾93.7%) and using PFGE, 32 strains exhibited a similarity ⩾80.6%. Additionally, MLST showed a high clonal similarity among strains that all presented the same ST32. Thirty-two isolates under investigation contained the virulence markers *invA*, *sopB*, *sopD*, *sipA*, *sipD*, *ssaR*, *sifA*, *flgK*, *fljB* and *flgL*. In conclusion, the *S.* Infantis strains studied were genetically similar, suggesting that a prevalent subtype has been causing disease and food contamination during a 25 year period in São Paulo State, an important metropolitan region in Brazil. Furthermore, the contamination between strains from food items and sick humans indicates that better control measures for *S.* Infantis may be needed in this country.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Salmonella is an important foodborne pathogen that causes illness worldwide. It is estimated that 93.8 million cases occur globally each year, which results in approximately 155,000 deaths (Majowicz et al., 2010). The genus consists of two species, *Salmonella enterica* and *Salmonella bongori*, and more than 2500 serovars. Some serovars, such as Enteritidis, Typhimurium, Newport, Infantis, Virchow, Hadar and Agona have been the most frequently isolated serovars from humans throughout the world from 2001 to 2007 (Guibourdenche et al., 2010; Hendriksen et al., 2011).

Specifically, *S.* Infantis has been one of the 15 most isolated serovars in African, Asian, European, North American, Oceanic and Latin American countries (Hendriksen et al., 2011). *S.* Infantis has been isolated from veterinary and human hospitals, foods such as vegetables and meat and production animals, such as broiler chickens (Dunowska et al., 2007; Merino et al., 2003; Nógrády et al., 2008; Shahada et al., 2006).

Salmonella pathogenicity involves chromosomal genes present in the *Salmonella* pathogenicity islands (SPIs), of which SPI-1 and SPI-2 are the most extensively studied. The SPI-1-associated proteins include the following: effectors proteins, Sop (SopA–E); proteins associated with invasion, SipA and InvA; translocon assembly protein SipD and flagella associated proteins, FlgK, FljB and FlgL. The SPI-2-associated proteins, SsaR and SifA, are associated with survival and replication within host cells (Giacomodonato et al., 2007; Hur et al., 2011; Shah et al., 2011; Zou et al., 2012). Moreover, there are virulence-associated plasmids that have the *spv* operon, which consists of five genes (*spvRABCD*), are associated with *Salmonella* survival and growth in macrophages (Rychlík et al., 2006).

Molecular typing methods, such as: pulsed-field gel electrophoresis (PFGE), repetitive sequence-based PCR (rep-PCR) and Multilocus Sequence Typing (MLST), have been used successfully in *Salmonella* epidemiological and phylogenetic studies (Albufera et al., 2009; Campioni et al., 2012; Fonseca et al., 2006; Harbottle et al., 2006; Hauser et al., 2012; Johnson et al., 2001; Kotetishvili et al., 2002; Merino et al., 2003; Ross and Heuzenroeder, 2008).

There is very limited data available on the molecular characteristics of *S.* Infantis isolated in Brazil, despite its clinical importance

* Corresponding author. Address: Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Universidade de São Paulo, Av. do Café s/n, 14040-903 Ribeirão Preto, SP, Brazil. Tel.: +55 16 36024896; fax: +55 16 36024725.
E-mail address: jufalcao@fcfrp.usp.br (J.P. Falcão).

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.07.004

(Fonseca et al., 2006). In Brazil, *S.* Infantis has been isolated from human sources, hospital outbreaks and food items, including mayonnaise, poultry meat, among others (Fonseca et al., 2006; Hofer and Reis, 1994; Moraes et al., 2000; Pessoa-Silva et al., 2002; Tavechio et al., 1996, 2002). From 1950 to 2003, *S.* Infantis was among the sixth most isolated serovars from human and non-human sources in São Paulo State, Brazil, behind Enteritidis, Typhimurium and other common serovars (Fernandes et al., 2006; Taunay et al., 1996; Tavechio et al., 2002).

Therefore, molecular typing studies with the strains of this serovar, isolated from food and human sources, may improve the understanding of the epidemiology of *S.* Infantis in Brazil. It should be emphasized that according to the available published data, there are currently no MLST studies on *Salmonella* isolated from Brazil.

Thus, the aims of this study were to type *S.* Infantis strains isolated from human sources and food items over a 25-year period in Brazil using enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR), PFGE and MLST, and to investigate the presence of some virulence markers. These data were analyzed to gain a deeper understanding of the epidemiology and pathogenic potential of *S.* Infantis strains isolated for more than two decades in this country.

2. Material and methods

2.1. Bacterial strains

A total of 35 *Salmonella* Infantis strains were studied; these strains were isolated from human feces (25) and food items (10) from sporadic cases from various cities in the São Paulo State, Brazil between 1984 and 2009. These strains were selected from a collection of strains available at the Adolfo Lutz Institute of Ribeirão Preto in Brazil and represent strains isolated during a 25 year period in this state. Table 1 lists the year, source of isolation and city of origin of the 35 *S.* Infantis strains used in this study.

2.2. Detection of virulence markers by PCR

The genomic DNA of the 35 strains listed in Table 1 was extracted as described by Falcão et al. (2006), and the DNA concentration was determined as described in Sambrook and Russell (2001). The general PCR procedure was performed according to the method described in Falcão et al. (2006). The 11 virulence markers investigated were *invA*, *sopB*, *sopD*, *sipA*, *sipD*, *flgK*, *fljB*, *flgL*, *ssaR*, *sifA*, and *spvB*. The PCR conditions and the primers used were described in the following studies: Hur et al. (2011) for *invA*, *sopB*, *sopD*, *ssaR* and *sifA* genes; Shah et al. (2011) for *sipA*, *sipD*, *flgK*, *fljB* and *flgL* genes; and Rychlík et al. (2008) for *spv*B. A template PCR without DNA was used as the negative control.

To evaluate the reproducibility of the experiments, the PCR reactions were repeated twice for some strains. The PCR products were analyzed by agarose gel electrophoresis and visualized under UV light after staining the gel with ethidium bromide ($1.0\ \mu g\ mL^{-1}$).

2.3. ERIC-PCR typing and analysis

Genomic DNA was extracted as described above. The ERIC-PCR assay was performed for all 35 strains presented in Table 1, and the

Table 1
Source and year of isolation, city of origin, virulence markers, ERIC and PFGE profiles of 35 *Salmonella* Infantis strains studied.

Strains	Source	Year	City	ERIC profile	PFGE profile	Presence of virulence markers
SI 01	Human diarrheic feces	1984	Ribeirao Preto	ERIC-6	PFGE-3	Profile 1
SI 02	Human diarrheic feces	1985	Ribeirao Preto	ERIC-6	PFGE-9	Profile 2
SI 03	Meat	1986	Ribeirao Preto	ERIC-6	PFGE-2	Profile 1
SI 04	Human diarrheic feces	1986	Ribeirao Preto	ERIC-6	PFGE-22	Profile 1
SI 05	Human diarrheic feces	1986	Ribeirao Preto	ERIC-6	PFGE-3	Profile 3
SI 06	Human diarrheic feces	1986	Ribeirao Preto	ERIC-6	PFGE-3	Profile 4
SI 07	Human diarrheic feces	1986	Ribeirao Preto	ERIC-7	PFGE-23	Profile 1
SI 08	Human diarrheic feces	1986	Cravinhos	ERIC-6	PFGE-8	Profile 1
SI 09	Human diarrheic feces	1986	Ribeirao Preto	ERIC-6	PFGE-8	Profile 1
SI 10	Human diarrheic feces	1988	Ribeirao Preto	ERIC-7	PFGE-2	Profile 1
SI 11	Human diarrheic feces	1989	Ribeirao Preto	ERIC-5	PFGE-17	Profile 1
SI 12	Human diarrheic feces	1989	Ribeirao Preto	ERIC-6	PFGE-3	Profile 1
SI 13	Human diarrheic feces	1990	Ribeirao Preto	ERIC-6	PFGE-4	Profile 1
SI 14	Human diarrheic feces	1990	Ribeirao Preto	ERIC-7	PFGE-4	Profile 1
SI 15	Human diarrheic feces	1990	Ribeirao Preto	ERIC-7	PFGE-6	Profile 1
SI 16	Human diarrheic feces	1991	Ribeirao Preto	ERIC-6	PFGE-2	Profile 1
SI 17	Human diarrheic feces	1991	Ribeirao Preto	ERIC-6	PFGE-10	Profile 1
SI 18	Human diarrheic feces	1992	Ribeirao Preto	ERIC-7	PFGE-7	Profile 1
SI 19	Human diarrheic feces	1993	Ribeirao Preto	ERIC-2	PFGE-10	Profile 1
SI 20	Human diarrheic feces	1993	Jardinopolis	ERIC-1	PFGE-1	Profile 1
SI 21	Meat	1995	Ribeirao Preto	ERIC-1	PFGE-5	Profile 1
SI 22	Chicken	1995	F. I. S.[a]	ERIC-1	PFGE-15	Profile 1
SI 23	Human diarrheic feces	1995	Ribeirão Preto	ERIC-6	PFGE-13	Profile 1
SI 24	Human diarrheic feces	1995	Ribeirão Preto	ERIC-6	PFGE-19	Profile 1
SI 25	Human diarrheic feces	1995	Ribeirão Preto	ERIC-2	PFGE-20	Profile 1
SI 26	Human diarrheic feces	1995	Ribeirão Preto	ERIC-3	PFGE-14	Profile 1
SI 27	Chicken	1996	F. I. S.[a]	ERIC-3	PFGE-15	Profile 1
SI 28	Chicken	1997	F. I. S.[a]	ERIC-4	PFGE-16	Profile 1
SI 29	Vegetable	1997	Ribeirao Preto	ERIC-5	PFGE-18	Profile 1
SI 30	Tuscan raw sausage	1998	F. I. S.[a]	ERIC-8	PFGE-11	Profile 1
SI 31	Pre-ground meat	2000	Ribeirao Preto	ERIC-3	PFGE-12	Profile 1
SI 32	Raw kebab	2001	Ribeirao Preto	ERIC-6	PFGE-1	Profile 1
SI 33	Human diarrheic feces	2001	Ribeirao Preto	ERIC-6	PFGE-2	Profile 1
SI 34	Raw ground meat	2003	Orlandia	ERIC-6	PFGE-2	Profile 1
SI 35	Human diarrheic feces	2009	Serra Azul	ERIC-6	PFGE-21	Profile 1

F. I. S.: Federal Inspection Service.
[a] From various cities of Sao Paulo State. Profile 1 = *sipA*, *sipD*, *sopB*, *sopD*, *invA*, *flgK*, *fljB*, *flgL*, *sifA*, *ssaR*. Profile 2 = *sipA*, *sipD*, *sopB*, *invA*, *flgK*, *fljB*, *flgL*, *sifA*, *ssaR*. Profile 3 = *sipA*, *sipD*, *sopB*, *sopD*, *invA*, *flgK*, *fljB*, *flgL*, *sifA*. Profile 4 = *sopB*, *sopD*, *flgK*, *fljB*, *flgL*, *invA*, *sifA*.

results were analyzed as described by Souza et al. (2010), with a few modifications. All amplifications were performed in a total volume of 50 μL with 100 ng of DNA template. The PCR reaction mixture also contained 1.25 mM of each deoxyribonucleotide (Invitrogen – Life Technologies), 5 mM $MgCl_2$ (Invitrogen – Life Technologies), 1.0 U of Klentaq™ DNA polymerase (Ab peptides), 1× PCR buffer (Invitrogen – Life Technologies) and 50 pmol of each primer synthesized by IDT – Integrated DNA technologies (United States). PCR primers and cycle conditions were previously described by Versalovic et al. (1991). The ERIC-PCR reaction was repeated at least twice for each strain to verify the reproducibility of the experiment. Reaction mixtures without the DNA template were used as negative controls. The PCR was performed in a DNA Engine® Peltier Thermal Cycler (Bio-Rad). Using 1.5% agarose gel electrophoresis, the ERIC-PCR amplicons were resolved into bands which were stained with ethidium bromide (0.5 $\mu g\ mL^{-1}$) and visualized under UV light.

Data were analyzed using the software package BioNumerics 5.1 (Applied Maths, Keistraat, Belgium). Only bands that represented amplicons between 298 and 4072 bp in size were included in the analysis. A similarity dendrogram was constructed by the unweighted-pair group method (UPGMA) using the Dice similarity coefficient and a position tolerance of 1.5%. A standard molecular weight ladder (1 kb Plus DNA Ladder from Invitrogen – Life Technologies) was included three times on each gel to normalize the images and allow for valid comparisons of the generated fingerprints between different gels.

2.4. PFGE typing and analysis

The genomic DNAs of the all isolates were prepared in agarose plugs using the protocol described by Souza et al. (2010) with only one modification: pure cultures of each bacterial strain were grown in BHI broth (HiMedia Lab, India) overnight at 37 °C. The plugs were digested with 40 U of *Xba*I (Invitrogen – Life Technologies) for 12–18 h.

Macrorestriction fragments were resolved by counter-clamped homogeneous electric field electrophoresis in a CHEF-DR III apparatus (Bio-Rad Laboratories) as described by Ribot et al. (2006).

A standard molecular weight ladder (Lambda Ladder PFG Marker – New England BioLabs) was included three times on each gel to compare the fingerprinting over several gels. The gels were stained with ethidium bromide (0.5 $\mu g\ mL^{-1}$) for 30 min and destained in distilled water for 20 min. The restriction fragments were viewed under UV light.

The similarities of the PFGE profiles were analyzed using the software package BioNumerics 5.1 (AppliedMaths). PFGE analysis with *Xba*I only bands that represent fragments between 48.5 and 582.0 kb in size were included. A similarity dendrogram was constructed by the UPGMA method using the Dice similarity coefficient and a position tolerance of 1.5%.

2.5. MLST

A total of 16 *S.* Infantis strains were selected for MLST analysis: SI01, SI02, SI03, SI04, SI05, SI06, SI07, SI10, SI14, SI15, SI18, SI24, SI26, SI28, SI29 and SI34. These isolates were chosen based on the concatenated dendrogram constructed from the ERIC-PCR and PFGE results. The dendrogram presented two clusters. Representative isolates of these two clusters, as well as one strain that failed to cluster (SI07) were systematically chosen for the MLST technique (data not shown).

MLST was performed using seven conserved housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*). A detailed protocol of the MLST procedure, including the housekeeping gene's amplification primers and the annealing temperatures, is available in the MLST database (http://www.mlst.ucc.i.e./mlst/dbs/Senterica/documents/primersEnterica_html). All amplifications were performed in a total volume of 50 μL as described by Souza et al. (2010). A sample of the complete mix, without the DNA, was used as a negative control in all runs. The PCR reactions were performed in a DNAEngine® Peltier Thermal Cycler (Bio-Rad). Automated DNA sequencing was performed with a MegaBACE 1000 DNA Sequencer (GE HealthCare) using the primer set described in the MLST database. Each forward and reverse strand was sequenced at least three times.

2.6. Discrimination index (DI)

The discrimination index of ERIC-PCR and PFGE was evaluated using the Hunter and Gaston (1988) formula:

$$DI = 1 - [1/N(N-1)]\Sigma nj(nj-1),$$

where N is the total number of isolates in this population, and nj is the number of isolates representing each type. This approach is based on the probability that two unrelated strains from the test population will be placed into different groups.

3. Results

3.1. Virulence markers

All 35 *S.* Infantis strains carried the *flgK*, *fljB*, *flgL*, *invA*, *sopB* and *sifA* genes. All isolates, with the exception of the SI06 strain, carried *sipA* and *sipD*. All isolates, with the exception of the SI02 strain, carried *sopD*. All 35 isolates, with the exception of the SI05 and SI06 strains, carried *ssaR*. However, none of the 35 isolates carried the *spvB* gene. These data are presented in Table 1.

3.2. ERIC-PCR

The dendrogram based on the ERIC-PCR fingerprint analysis showed a high similarity among the majority of the strains. Of the 35 strains, 34 (97.1%) strains presented a similarly above 93.7%. The strains were grouped in three main clusters, denominated as A, B and C. In the A cluster, nine (25.7%) strains, which were isolated from human sources and food items between 1993 and 2000, exhibited a similarity above 94.0%. In the B cluster, 25 (71.4%) strains, which were isolated from human sources and food items between 1984 and 2009, exhibited a similarity above 95.4%. Only one representative (SI30), which was isolated from Tuscan raw sausage in 1998, was grouped in the C cluster, and it had a similarity above 87.7% when compared with the A and B clusters (Fig. 1).

ERIC-PCR revealed 8 different ERIC profiles. The main ERIC profile was ERIC-6, which was found in 18 strains isolated from human sources and food items between 1984 and 2009 (Fig. 1).

3.3. PFGE

Of the 35 strains, 32 strains (91.4%), isolated between 1984 and 2003, from food items and human sources, exhibited a similarity above 80.6%. PFGE revealed 23 different PFGE profiles. The main PFGE profile was PFGE-2, which was found in 5 strains and was isolated from food items and human sources, between 1986 and 2003 (Fig. 2). The grouping of strains in main clusters was not observed.

3.4. MLST

For the 16 *S.* Infantis strains selected and typed by MLST, no nucleotide differences were detected in the seven housekeeping

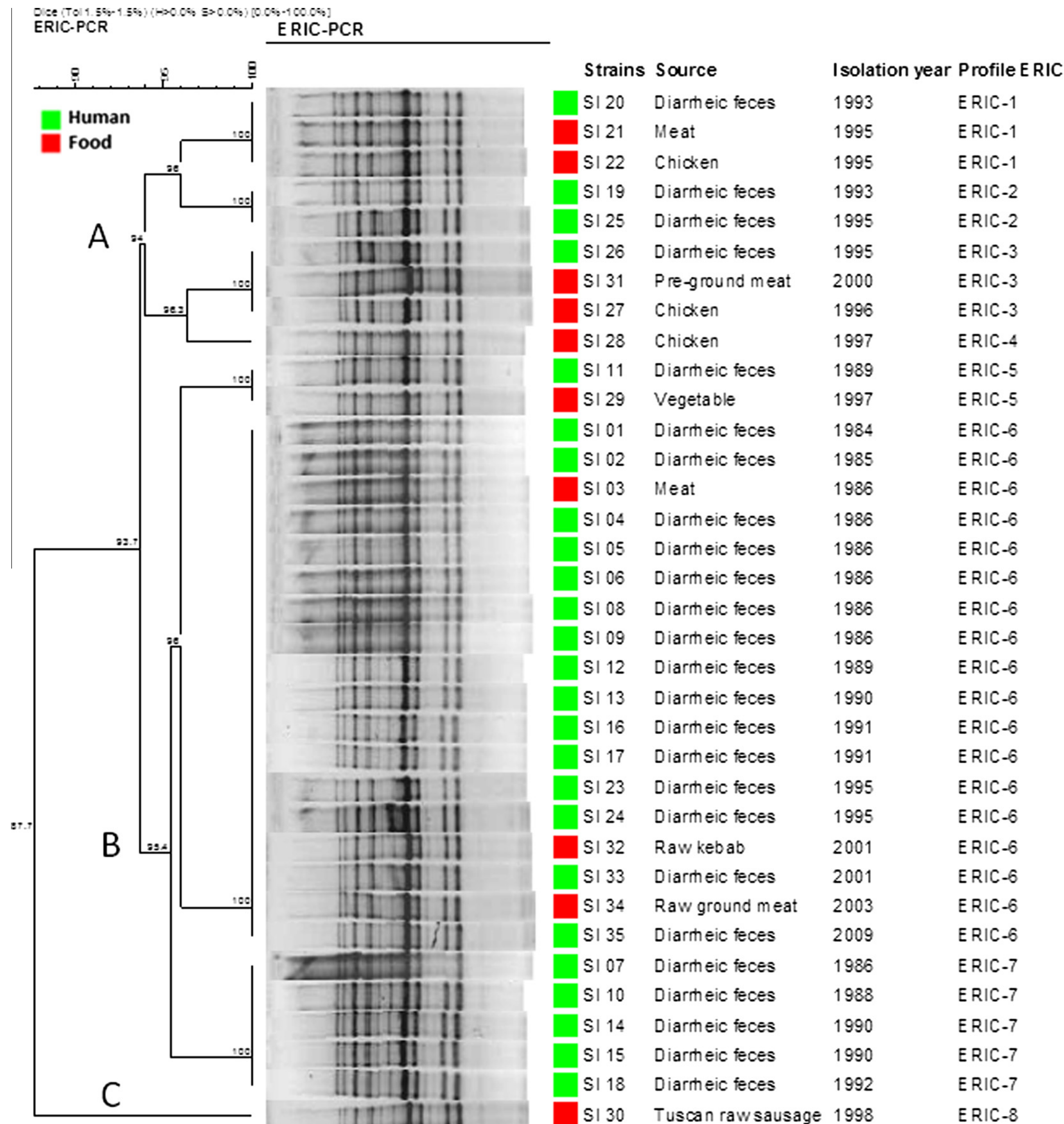

Fig. 1. Dendrogram representing genetic relationships among 35 *Salmonella* Infantis strains based on ERIC-PCR fingerprints. Similarity (%) between patterns was calculated by using the Dice index and is represented by the numbers beside the nodes. The data were sorted by the UPGMA method.

genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*) that were analyzed. The 16 *S.* Infantis strains that were studied were characterized as carrying the following alleles: 17 (*aro*C), 18 (*dna*N), 22 (*hem*D), 17 (*his*D), 5 (*pur*E), 21 (*suc*A) and 19 (*thr*A). This numerical sequence resulted as ST32. The sequence data for each allele are available in the publically available MLST database (http://www.mlst.ucc.i.e./mlst/dbs/Senterica/Downloads_HTML).

3.5. Discrimination index

The DI was 0.71 and 0.965 for ERIC-PCR and PFGE, respectively.

4. Discussion and conclusion

This study examined the molecular epidemiology of 35 *S.* Infantis strains isolated from human sources and food items in sporadic cases between 1984 and 2009 from various cities in São Paulo State, an important metropolitan region in Brazil, using ERIC-PCR, PFGE and MLST techniques. Moreover, the presence of some virulence markers, including SPI-1, SPI-2 and virulence-associated plasmids, were studied.

All strains were positive for most of the virulence markers located in SPI-1 and SPI-2 (Table 1). However, *spvB*, located in virulence-associated plasmids, was absent in all 35 *S.* Infantis strains. One explanation for the absence of *spvB* containing plasmids is that some serovars, such as Typhi, Paratyphi, Hadar and Infantis, are usually plasmid free. This characteristic is not true for some serovars that are frequently associated with infections in humans and farm animals, which includes the following serovars: Enteritidis, Typhimurium, Dublin, Choleraesuis, Gallinarum, Pullorum and Abortus-ovis. These serovars usually harbor a virulence associated-plasmid (Rychlík et al., 2006). However, *S.* Infantis strains harboring an antibiotic resistance plasmid have been reported in Brazil (Moraes et al., 2000).

The high prevalence of virulence markers that were investigated highlights the pathogenic potential of these *S.* Infantis strains that have been causing disease in humans and contaminating food for more than two decades in Brazil. Specifically in this country, *S.* Infantis was reported as the fourth most isolated serovar from human sources during the period of 1950–1990 and the sixth most isolated during 1996–2003 (Taunay et al., 1996; Fernandes et al., 2006). Furthermore, it was the most frequently isolated serovar from non-human sources during 1970–1990 and was the sixth

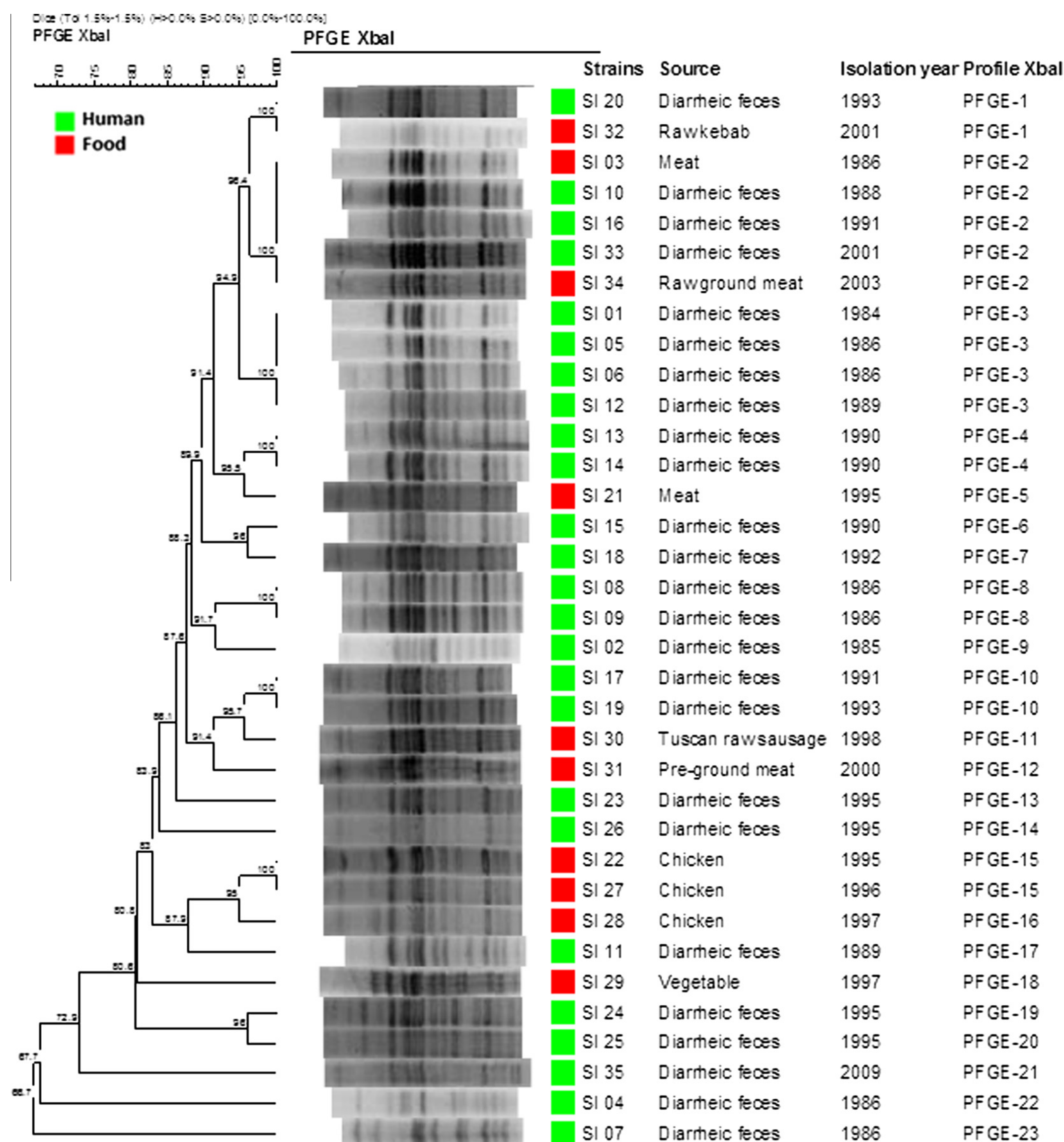

Fig. 2. Dendrogram representing genetic relationships among 35 *Salmonella* Infantis strains based on PFGE *Xba*I fingerprints. Similarity (%) between patterns was calculated by using the Dice index and is represented by the numbers beside the nodes. The data were sorted by the UPGMA method.

most isolated during 1996–2000 (Taunay et al., 1996; Tavechio et al., 2002). However, despite the importance of this serovar, few molecular typing studies have been performed in Brazil (Fonseca et al., 2006).

There are only a few published studies that used ERIC-PCR to type *S.* Infantis strains (Johnson et al., 2001; Merino et al., 2003; Weigel et al., 2004). In the study by Johnson et al. (2001), ERIC-PCR proved to be an adequate method to type *S.* Infantis strains, isolated from 1996 to 1997, and helped to retrospectively evaluate a hospital cafeteria-associated outbreak of gastroenteritis caused by strains of this serovar. In contrast, Merino et al. (2003) could not differentiate 15 *S.* Infantis strains isolated from 1997 to 1998 because the patterns contained few clearly visible bands. Although Weigel et al. (2004) concluded that Rep-PCR, including ERIC-PCR, could greatly discriminate 68 *Salmonella* of different serovars, only four *S.* Infantis strains were studied, making it difficult to draw conclusions on the genetic diversity of this serovar.

In the present study, 34 out of 35 *S.* Infantis strains investigated by ERIC-PCR exhibited a genetic similarity higher than 93.7% and were discriminated into only eight ERIC-types (Fig. 1). Similarly, this high genomic similarity was observed by PFGE where 32 of 35 strains presented a similarity over 80.6% and were discriminated into 23 PFGE-types (Fig. 2). Although both techniques gave similar results, indicating a high similarity among *S.* Infantis strains isolated from human sources and food items during 25 years in Brazil, the PFGE methodology could discriminate *S.* Infantis more efficiently than could ERIC-PCR.

Despite the development of new molecular typing methods, PFGE remains as the preferred method for typing *Salmonella* strains and other important foodborne pathogens (Heir et al., 2002). Specifically, PFGE has been used successfully to type *S.* Infantis strains isolated from different sources worldwide, elucidating outbreaks and their epidemiology (Hauser et al., 2012; Lindqvist and Pelkonen, 2007; Nógrády et al., 2007, 2008, 2012; Rivoal et al., 2009). However, despite the abundance of research on strains of this serovar worldwide, there is only one previously published study using PFGE to type 35 *S.* Infantis strains in Brazil. These strains were isolated from children admitted to four different hospitals in Rio de Janeiro between 1996 and 2001, and PFGE results indicated that 33 strains came from the same genetic lineage (Fonseca et al.,

2006). Similar to the results presented in this study, Gal-Mor et al. (2010) found high genetic similarity among 87 *S.* Infantis strains isolated between 1970 and 2009 in Israel.

Our results, obtained by ERIC-PCR and PFGE, showed a high genotypic similarity among the strains isolated from 1984 to 2009, suggesting that the majority of *S.* Infantis strains studied may have descended from a common ancestor that has differed little and is responsible for the contamination of human population and food items in Brazil for 25 years.

It is important to note that both ERIC-PCR and PFGE were found to be adequate tools for long-term epidemiological investigations. However, based on the results obtained in this work, PFGE remains the preferred method for typing *Salmonella* strains for some reasons. First, PFGE presented a higher discrimination power in comparison to ERIC-PCR. This finding may be explained by the fact that only inter-ERIC regions are included in ERIC-PCR analyses compared the PFGE method, which allows for the analysis of nearly the entire genome. Second, the PFGE furnished more consistent epidemiological information than ERIC-PCR. For example, strains SI07, SI10, SI14, SI15 and SI18 that were unlikely to be epidemiologically related were indistinguishable by ERIC-PCR (ERIC-7), but PFGE showed that these strains were differentiated and actually epidemiologically unrelated.

All *S.* Infantis strains studied in this work were found to be ST32 by MLST. Interestingly, this is consistent with previous results generated from *S.* Infantis strains collected from different sources, countries and years (Hauser et al., 2012; Liu et al., 2011; Torpdahl et al., 2005). Therefore, ST32 may be the main allelic profile for this serovar. The MLST results generated in this study contributed with additional typing information from *S.* Infantis isolated in Brazil, which could be compared with other strains isolated worldwide.

The results in this study provide additional information on the epidemiology and genetic diversity of *S.* Infantis isolated from human sources and food items over many years in Brazil.

In conclusion, the *S.* Infantis strains studied were genetically similar, suggesting that a prevalent subtype may have been causing disease and contaminating food for 25 years in São Paulo State, an important metropolitan region in Brazil. Furthermore, the contamination between strains from food items and sick individuals indicates that better control measures for *S.* Infantis are needed in this country.

Acknowledgements

We thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Processo 2008/57478-1) for financial support. During the course of this work, Almeida, F. was supported by a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Processo 135935/2009-1).

References

Albufera, U., Bhugaloo-Vial, P., Issack, M.I., Jaufeerally-Fakim, Y., 2009. Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD analysis. Infect. Genet. Evol. 9, 322–327.

Campioni, F., Bergamini, A.M.M., Falcao, J.P., 2012. Genetic diversity, virulence genes and microbial resistance of *Salmonella* Enteritidis isolated from food and humans over a 24-year period in Brazil. Food Microbiol. 32, 254–264.

Dunowska, M., Morley, P.S., Traub-Dargatz, J.L., Davis, M.A., Patterson, G., Frye, J.G., Hyatt, D.R., Dargatz, D.A., 2007. Comparison of *Salmonella enterica* serotype Infantis isolates from a veterinary teaching hospital. J. Appl. Microbiol. 102, 1527–1536.

Falcão, J.P., Falcão, D.P., Pitondo-Silva, A., Malaspina, A.C., Brocchi, M., 2006. Molecular typing and virulence markers of Yersinia enterocolitica strains from human, animal and food origins isolated during 1968–2000 in Brazil. J. Med. Microbiol. 55, 1539–1548.

Fernandes, S.A., Tavechio, A.T., Ghilardi, A.C.R., Dias, A.M.G., Almeida, I.A.Z.C., Melo, L.C.V., 2006. *Salmonella* serovars isolated from humans in São Paulo State, Brazil, 1996–2003. Rev. Inst. Med. Trop. Sao Paulo 48, 179–184.

Fonseca, E.L., Mykytczuk, O.L., Asensi, M.D., Reis, E.M.F., Ferraz, L.R., Paula, F.L., Ng, L.K., Rodrigues, D.P., 2006. Clonality and antimicrobial resistance gene profiles of multidrug-resistant *Salmonella enterica* serovar Infantis isolates from four public hospital in Rio de Janeiro, Brazil. J. Clin. Microb. 44, 2767–2772.

Gal-Mor, O., Valinsky, L., Weinberger, M., Guy, S., Jaffe, J., Schorr, Y.I., Raisfeld, A., Agmon, V., Nissan, I., 2010. Multidrug-resistant *Salmonella enterica* serovar Infantis, Israel. Emerg. Infect. Dis. 16, 1754–1757.

Giacomodonato, M.N., Uzzau, S., Bacciu, D., Caccuri, R., Sarnacki, S.H., Rubino, S., Cerquetti, M.C., 2007. SipA, SopA, SopB, SopD and SopE2 effector proteins of *Salmonella enterica* serovar Typhimurium are synthesized at late stages of infection in mice. Microbiology 153, 1221–1228.

Guibourdenche, M., Roggentin, P., Mikoleit, M., Fields, P.I., Bockemühl, J., Grimont, P.A.D., Weill, F.X., 2010. Supplement 2003–2007 (No. 47) to the White-Kauffmann-Le Minor scheme. Res. Microbiol. 161, 26–29.

Harbottle, H., White, D.G., Mcdermott, P.F., Walker, R.D., Zhao, S., 2006. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of *Salmonella enterica* serotype newport isolates. J. Clin. Microbiol. 44, 2449–2457.

Hauser, E., Tietze, E., Helmuth, R., Junker, E., Prager, R., Schroeter, A., Rabsch, W., Fruth, A., Toboldt, A., Malorny, B., 2012. Clonal dissemination of *Salmonella enterica* serovar Infantis in Germany. Foodborne Pathog. Dis. 9, 352–360.

Heir, E., Lindstedt, B.A., Nygård, I., Vardund, T., Hasseltvedt, V., Kapperud, G., 2002. Molecular epidemiology of *Salmonella* Typhimurium isolates from human sporadic and outbreak cases. Epidemiol. Infect. 128, 373–382.

Hendriksen, R.S., Vieira, A.R., Karlsmose, S., Wong, D.M.A.L.F., Jensen, A.B., Wegener, H.C., Aarestrup, F.M., 2011. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country data bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog. Dis. 8, 887–899.

Hofer, E., Reis, E.M.F., 1994. *Salmonella* serovars in food poisoning episodes recorded in Brazil from 1982 to 1991. Rev. Inst. Med. Trop. Sao Paulo 36, 7–9.

Hunter, P.R., Gaston, M.A., 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. 26, 2465–2466.

Hur, J., Choi, Y.Y., Park, J.H., Jeon, B.W., Lee, H.S., Kim, A.R., Lee, J.H., 2011. Antimicrobial resistance, virulence-associated genes, and pulsed-field gel electrophoresis profiles of *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolated from piglets with diarrhea in Korea. Can. J. Vet. Res. 75, 49–56.

Johnson, J.R., Clabots, C., Azar, M., Boxrud, D.J., Besser, J.M., Thurn, J.R., 2001. Molecular analysis of a hospital cafeteria-associated salmonellosis outbreak using modified repetitive element PCR fingerprinting. J. Clin. Microbiol. 39, 3452–3460.

Kotetishvili, M., Stine, O.C., Kreger, A., Morris Jr., J.G., Sulakvelidze, A., 2002. Multilocus sequence typing for characterization of clinical and environmental *Salmonella* strains. J. Clin. Microbiol. 40, 1626–1635.

Lindqvist, N., Pelkonen, S., 2007. Genetic surveillance of endemic bovine *Salmonella* Infantis infection. Acta Vet. Scand. 49, 1–9.

Liu, W.B., Liu, B., Zhu, X.N., Yu, S.J., Shi, X.M., 2011. Diversity of *Salmonella* isolates using serotyping and multilocus sequence typing. Food Microbiol. 28, 1182–1189.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., O'brien, S., Jones, T.F., Fazil, A., Hoekstra, R.M., 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin. Infect. Dis. 50, 882–889.

Merino, L.A., Ronconi, M.C., Navia, M.M., Ruiz, J., Sierra, J.M., Cech, N.B., Lodeiro, N.S., Vila, J., 2003. Analysis of the clonal relationship among clinical isolates of *Salmonella enterica* serovar Infantis by different typing methods. Rev. Inst. Med. Trop. Sao Paulo 45, 119–123.

Moraes, B.A., Cravo, C.A.N., Loureiro, M.M., Solari, C.A., Asensi, M.D., 2000. Epidemiological analysis of bacterial strains involved in hospital infection in a University Hospital from Brazil. Rev. Inst. Med. Trop. Sao Paulo 42, 201–207.

Nógrády, N., Tóth, Á., Kostyák, Á., Pászti, J., Nagy, B., 2007. Emergence of multidrug-resistant clones of *Salmonella* Infantis in broiler chickens and humans in Hungary. J. Antimicrob. Chemother. 60, 645–648.

Nógrády, N., Kardos, G., Bistyák, A., Turcsányi, I., Mészáros, J., Galántai, Zs., Juhász, Á., Samu, P., Kaszanyitzky, J.É., Pászti, J., Kiss, I., 2008. Prevalence and characterization of *Salmonella* Infantis isolates originating from different points of the broiler chicken-human food chain in Hungary. Int. J. Food Microbiol. 127, 162–167.

Nógrády, N., Király, M., Davies, R., Nagy, B., 2012. Multidrug resistant clones of *Salmonella* Infantis of broiler origin in Europe. Int. J. Food Microbiol. 157, 108–112.

Pessoa-Silva, C.L., Toscano, C.M., Moreira, B.M., Santos, A.L., Frota, A.C., Solari, C.A., Amorim, E.L., Carvalho, M.G., Teixeira, L.M., Jarvis, W.R., 2002. Infection due to extended-spectrum β-lactamase producing *Salmonella enterica* subsp. *enterica* serotype Infantis in a neonatal unit. J. Pediatr. 141, 381–387.

Ribot, E.M., Fair, M.A., Gautom, R., Cameron, D.N., Hunter, S.B., Swaminathan, B., Barrett, T.J., 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog. Dis. 3, 59–67.

Rivoal, K., Protais, J., Quéguiner, S., Boscher, E., Chidaine, B., Rose, V., Gautier, M., Baron, F., Grosset, N., Ermel, G., Salvat, G., 2009. Use of pulsed-field gel electrophoresis to characterize the heterogeneity and clonality of *Salmonella* serotype Enteritidis, Typhimurium and Infantis isolates obtained from whole liquid eggs. Int. J. Food Microbiol. 129, 180–188.

Ross, I.L., Heuzenroeder, M.W., 2008. A comparison of three molecular typing methods for the discrimination of *Salmonella enterica* serovar Infantis. FEMS Immunol. Med. Microbiol. 53, 375–384.

Rychlík, I., Gregorova, D., Hradecka, H., 2006. Distribution and function of plasmids in *Salmonella enterica*. Vet. Microbiol. 112, 1–10.

Rychlík, I., Hradecka, H., Malcova, M., 2008. *Salmonella enterica* serovar Typhimurium typing by prophage-specific PCR. Microbiology 154, 1384–1389.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: a Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Shah, D.H., Zhou, X., Addwebi, T., Davis, M.A., Orfe, L., Call, D.R., Guard, J., Besser, T.E., 2011. Cell invasion of poultry-associated *Salmonella enterica* serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system. Microbiology 157, 1428–1445.

Shahada, F., Chuma, T., Tobata, T., Okamoto, K., Sueyoshi, M., Takase, K., 2006. Molecular epidemiology of antimicrobial resistance among *Salmonella enterica* serovar Infantis from poultry in Kagoshima, Japan. Int. J. Antimicrob. Agents 28, 302–307.

Souza, R.A., Pitondo-Silva, A., Falcão, D.P., Falcão, J.P., 2010. Evaluation of four molecular typing methodologies as tools for determining taxonomy relations and for identifying species among *Yersinia* isolates. J. Microbiol. Methods. 82, 141–150.

Taunay, A.E., Fernandes, S.A., Tavechio, A.T., Neves, B.C., Dias, A.M.G., Irino, K., 1996. The role of public health laboratory in the problem of Salmonellosis in São Paulo, Brazil. Rev. Inst. Med. Trop. Sao Paulo 38, 119–127.

Tavechio, A.T., Fernandes, S.A., Neves, B.C., Dias, A.M.G., Irino, K., 1996. Changing patterns of *Salmonella* serovars: increase of *Salmonella* Enteritidis in São Paulo, Brazil. Rev. Inst. Med. Trop. Sao Paulo 38, 315–322.

Tavechio, A.T., Ghilardi, A.C.R., Peresi, J.T.M., Fuzihara, T.O., Yonamine, E.K., Jakabi, M., Fernandes, S.A., 2002. *Salmonella* serotypes isolated from nonhuman sources in São Paulo, Brazil, from 1996 through 2000. J. Food Prot. 65, 1041–1044.

Torpdahl, M., Skov, M.N., Sandvang, D., Baggesen, D.L., 2005. Genotypic characterization of *Salmonella* by multilocus sequence typing, pulsed-field gel electrophoresis and amplified fragment length polymorphism. J. Microbiol. Methods. 63, 173–184.

Versalovic, J., Koeuth, T., Lupski, J.R., 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprint of bacterial genomes. Nucleic Acids Res. 19, 6823–6831.

Weigel, R.M., Qiao, B., Teferedegne, B., Suh, D.K., Barber, D.A., Isaacson, R.E., White, B.A., 2004. Comparison of pulsed field gel electrophoresis and repetitive sequence polymerase chain reaction as genotyping methods for detection of genetic diversity and inferring transmission of *Salmonella*. Vet. Microbiol. 100, 205–217.

Zou, M., Keelara, S., Thakur, S., 2012. Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from humans by antimicrobial resistance, virulence genes, and pulsed-field gel electrophoresis. Foodborne Pathog. Dis. 9, 1–7.