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Molecular typing of *Salmonella* from Sergipe, Northeastern Brazil, showing the epidemiological relationship between poultry and human infection

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ABSTRACT. Randomly amplified polymorphic DNA (RAPD) has been widely used for epidemiological and phylogenetic purposes owing to its rapidity and efficiency. The aim of this study was to perform genome typing of *Salmonella* samples isolated from different sources by RAPD profiling. Thirty-three *Salmonella* samples from the bacterial collection of the Laboratório de Virologia Comparada, Departamento de Morfologia, Universidade Federal de Sergipe, Brazil, and two standard samples were used. RAPD profiling was conducted using six primers of the Ready-To-Go RAPD system. The amplified products were electrophoresed on 5% polyacrylamide gel and silver-stained. RAPD analysis resulted in reproducible and stable banding patterns and showed high genetic diversity among the isolated strains. The Primer P1-generated dendrogram showed an epidemiologic relationship between the human

and poultry isolated samples, highlighting the usefulness of RAPD for molecular typing and epidemiological studies.

Key words: *Salmonella*; Randomly amplified polymorphic DNA; Randomly amplified polymorphic DNA; Epidemiology; Molecular typing

INTRODUCTION

Members of the genus *Salmonella*, of the family Enterobacteriaceae, are important foodborne pathogens. They are among the most important causes of enteric pathologies in the world, and are therefore relevant to public health owing to their high rates of endemicity and morbidity (Chansiripornchai et al., 2000; Ruiz et al., 2003).

The *Salmonella* genome is characterized by the presence of a large number of genetic elements (*Salmonella* pathogenicity islands) that can be acquired by horizontal transmission. These pathogenicity islands allow bacteria to rapidly gain complex virulence and enable the emergence of new antibiotic-resistant epidemic strains (Yan et al. 2004), resulting in great genetic diversity.

With the advent of molecular biology, many DNA-based methods have emerged that allow for the detection of genetic variations present in isolated samples, as well as inter- and intra-serotype differentiation (Lin et al., 1996; Soto et al., 1999; Nath et al., 2010). Molecular typing of *Salmonella* is very important in epidemiological studies because it reflects the genetic link between samples, which can be used to identify the sources of microorganisms, trace the transmission and environment diffusion chain, and monitor the tendency to antimicrobial resistance. Use of these modern molecular typing methods could thus lead to the establishment of more efficient public health policies, permitting better sanitary control of the products offered to the population, and could even serve as a complement to the conventional techniques used for biotyping (Laconcha et al., 1998; De Cesare et al., 2001).

Randomly amplified polymorphic DNA (RAPD), also known as arbitrarily primed-polymerase chain reaction (PCR), amplifies DNA fragments through random primers. It does not require any previous knowledge of the DNA sequence of the target organisms, and results in simpler, low-cost, and rapid detection of the genetic variations present in isolated samples (Colauto et al., 2002; Ruiz et al., 2003; Santos and Bäumler, 2004). RAPD has been used as a tool to trace *Salmonella* epidemiologically, to distinguish *Salmonella* strains from different geographical origins, and for complementing serotyping and phage typing methods (Soto et al., 1999; De Cesare et al., 2001; Quintaes et al., 2002; Lim et al., 2005; Smith et al., 2011; Rezk et al., 2012).

The aim of the present study was to assess the inter- and intra-serotype diversity of *Salmonella* spp and verify a possible epidemiologic relationship using the RAPD technique.

MATERIAL AND METHODS

Bacterial strains

Thirty-three *Salmonella* spp strains isolated from different sources (poultry, oysters, environmental water, human feces, and human blood, Table 1) from the state of Sergipe, Northeastern Brazil, were utilized in this study. These epidemiologically unrelated sporadic

isolates belonged to ten different serotypes: nine *Salmonella enterica* serotype Agona; three *S. enterica* subsp *enterica* (8,20:Z4,Z23:-); one *S. enterica* subsp *enterica* (068:Z4,Z23:-); nine *S*. Enteritidis; two *S*. Houtenae; one *S*. Newport; three *S.* Panama; two *S.* Schwarzengrund; one *S*. Tennessee; and two *S.* Typhimurium. Two international standard strains, ATCC 13076 and ATCC 13311, were also included.

Table 1. Randomly amplified polymorphic DNA (RAPD) profile of *Salmonella* spp samples isolated from different sources in the state of Sergipe, Brazil.

No. of type	Serotype	Source	Primer P1	Primer P2	Primer P3	Primer P4	Primer P5	Primer P6
7	*S.* Enteritidis	ATCC 13 076	A1	B1	C1	D1	E1	F1
5	*S.* Enteritidis	Human feces	A1	B2	C2	D2	E2	F2
9	*S.* Enteritidis	Human feces	A2	B3	C3	D3	E3	F3
11	*S.* Enteritidis	Human feces	A3	B4	C4	D4	E3	F4
12	*S.* Enteritidis	Human feces	A3	B3	C3	D1	E4	F3
17	*S.* Enteritidis	Human feces	A2	B5	C5	D5	E5	F5
31	*S.* Enteritidis	Human feces	A4	B6	C6	D6	E6	F6
32	*S.* Enteritidis	Human feces	A5	B7	C7	D7	E7	F7
37	*S.* Enteritidis	Human blood	A6	B8	C8	D7	E8	F8
18	*S.* Enteritidis	Poultry	A7	B9	C8	D1	E9	F3
24	*S.* Agona	Poultry	A8	B10	C9	D8	E10	F9
25	*S.* Agona	Poultry	A9	B11	C10	D9	E11	F9
26	*S.* Agona	Poultry	A10	B11	C10	D10	E12	F9
27	*S.* Agona	Poultry	A11	B12	C10	D11	E13	F10
28	*S.* Agona	Poultry	A12	B13	C10	D12	E13	F11
4	*S.* Agona	Env. water‡	A13	B14	C11	D13	E14	F12
6	*S.* Agona	Env. water‡	A14	B15	C12	D14	E15	F13
8	*S.* Agona	Env. water‡	A15	B16	C13	D15	E16	F14
22	*S.* Agona	Env. water‡	A16	B17	C14	D16	E17	F15
10	*S.* Typhimurium	ATCC 13 311	A17	B18	C15	D17	E18	F16
16	*S.* Typhimurium	Human blood	A18	B19	C16	D17	E19	F17
36	*S.* Typhimurium	Human blood	A19	B20	C17	D18	E20	F18
34	*S. enterica* subsp *enterica**	Human blood	A6	B21	C18	D19	E21	F19
35	*S. enterica* subsp *enterica**	Human blood	A20	B22	C19	D20	E22	F19
19	*S. enterica* subsp *enterica**	Poultry	A7	B23	C20	D21	E23	F20
23	*S. enterica* subsp *enterica*†	Poultry	A6	B22	C21	D22	E24	F19
33	*S.* Panama	Poultry	A21	B24	C10	D7	E25	F21
20	*S.* Panama	Env. water‡	A21	B25	C18	D23	E26	F21
21	*S.* Panama	Env. water‡	A22	B26	C18	D24	E27	F21
29	*S.* Schwarzengrund	Poultry	A23	B27	C22	D25	E28	F22
30	*S.* Schwarzengrund	Poultry	A23	B28	C23	D25	E28	F22
2	*S.* Houtenae	Env. water‡	A24	B29	C24	D26	E29	F23
3	*S.* Houtenae	Env. water‡	A24	B30	C11	D26	E29	F12
13	*S.* Newport	Human feces	A25	B31	C25	D27	E30	F24
1	*S.* Tennessee	Oysters	A26	B32	C26	D28	E31	F25

A1-A26, B1-B32, C1-C26, D1-D28, E1-E31, F1-F25 represent the different RAPD profiles relating to each primer **S. enterica* subsp enterica (8,20:64,Z23:-); †*S. enterica* subsp. enterica (068:64,Z23:-); ‡Environmental water.

DNA extraction

DNA extraction was carried out according to Sambrook et al. (2001). Samples were grown in 1L of Luria-Bertani broth, at 37°C with agitation (120 rpm) for 12 h and then centrifuged at 5000 *g* for 20 min at 4°C. Pellets were resuspended in phosphate-buffered saline (PBS, pH 7.2). To extract total DNA, bacterial suspensions in PBS were centrifuged at 8000 *g* for 10 min and resuspended in 450 µL Tris-ethylenediaminetetraacetic acid, pH 8.0, and incubated at 56°C for 3 h together with 20 µL 20 mg/mL Proteinase K, 2 µL 10 mg/mL RNAse, and 30 µL 10% sodium dodecyl sulfate. They were then inactivated at 70°C for 10 min and

centrifuged at 3000 *g* for 5 min. The supernatant was transferred to a new tube and extracted with 1:1 phenol:chloroform four times at 3000 *g* for 15 min. Later, the samples were precipitated with 95% ethanol after addition of 1/10 (v/v) potassium acetate. Samples were then left at -20°C for 20 min and centrifuged at 11,000 *g* for 20 min. The DNA pellet was dried at room temperature and resuspended in 50 µL ultra-pure water.

RAPD

Six Ready-To-Go system (Amersham Biosciences, NJ, USA) primers were used to carry out the RAPD-PCR: P1 (5'-GGTGCGGGAA-3'), P2 (5'-GTTTCGCTCC-3'), P3 (5'-GTAGACCCGT-3'), P4 (5'-AAGAGCCCGT-3'), P5 (5'-AACGCGCAAC-3'), and P6 (5'-CCCGTCAGCA-3'). The mix consisted of 2.5 µL PCR buffer (30 mM KCl and 10 mM Tris-HCl, pH 8.3), 0.75 µL $MgCl_2$ (3 mM), 16.9 µL diethylpyrocarbonate-treated water, 3 µL primer (15 pmol), 0.6 µL dNTPs (0.4 mM), 0.25 µL Taq DNA polymerase (0.25 U), and 1 µL DNA. Tubes were heated at 95°C in a thermo cycle (Thermo Hybaid PCR Sprint) for 5 min, before the addition of 0.25 µL Taq DNA polymerase, followed by 40 cycles at 94°C for 30 s, 35°C for 1 min, and 72°C for 2 min. After 40 cycles, a final extension at 72°C was carried out for 5 min. The amplified products were separated by electrophoresis on a 5% polyacrylamide gel at 112 V for 3 h and visualized according to the PlusOne DNA Silver Staining Kit™ (Amersham Biosciences). PCR and gel electrophoresis were repeated at least three times in independent experiments to verify the reproducibility of the results. Optimization of the amplification conditions for PCR was carried out by varying the amounts of primer (3-5 µL) and the annealing temperatures (35-40°C), using the references *Escherichia coli* BL21, C1a, ATCC 13 076, and ATCC 13 311.

Data analysis

The RAPD patterns of individual strains were scored as discrete variables in a binary manner based on band presence or absence: 1 was used to indicate presence and 0 to indicate absence of a band in the profile. Diversity and genetic relationship among the strains were obtained using unweighted pair-group method with arithmetic average (UPGMA) and the NTSYS-pc 1.7 program (Rohlf, 1992). The discrimination index was calculated using Simpson's index of diversity (Hunter and Gaston, 1988).

RESULTS AND DISCUSSION

PCR-based fingerprinting using RAPD has been used widely for genome identification, and the RAPD technique using up to six primers has been reported to be a good tool for molecular typing, permitting the execution of public health measures necessary to control infection (Chansiripornchai et al., 2000; De Cesare et al., 2001; Khoodoo et al., 2002; Quintaes et al., 2002). During this study, we found that the best amplification conditions for RAPD-PCR were an annealing temperature of 35ºC with 3 µL primer. No loss in band numbers or shift in the position of PCR bands was observed in the reproducibility tests. Also, no PCR products were observed in reactions performed in the absence of primer or in the absence of DNA template.

The six primers utilized in this study showed different discriminatory powers, producing a variety of PCR profiles (Table 1). It was possible to discriminate all 35 samples of

Salmonella, showing that they were all genetically different. No primer alone was able to discriminate all isolates. However, the use of any two primers was enough to differentiate all 35 samples (Table 2). The samples that showed at least one polymorphic band were considered sufficiently divergent. Primer P2 produced the maximum number of RAPD profiles and thus has better discriminatory power than the others. The six RAPD-PCR 10-mer primers generated a total of 178 distinct RAPD profiles (Table 1), and proved to be useful for differentiating all the isolated samples, as mentioned above.

Table 2. Discrimination indices for different primers.

Primer	No. of types	Discrimination index
RAPD analysis P1	26	0.983
RAPD analysis P2	32	0.995
RAPD analysis P3	26	0.973
RAPD analysis P4	28	0.985
RAPD analysis P5	31	0.993
RAPD analysis P6	25	0.977

RAPD = randomly amplified polymorphic DNA.

Using these primers, a good Simpson's index of discrimination was obtained for *Salmonella* isolates. These high index values indicate the potential ability of the technique to differentiate between different isolates (Table 2). The discriminatory power is determined by the number of types defined and their relative frequencies. Thus, despite the fact that both primers P1 and P3 can differentiate 26 types, P1 is more discriminatory than P3 (Table 2). The above results show the presence of a great genetic diversity within the samples found in the state of Sergipe, Brazil. Such diversity may determine new genetic combinations, consequently improving virulence factors and resistant genes present in different samples.

Seven different RAPD profiles from 10 samples of *S.* Enteritidis were obtained with primer P1 (Table 1). Samples 5 and 7, samples 9 and 17, and samples 11 and 12 showed identical profiles (Table 1), all from human feces. Samples 18, 31, 32, and 37 produced individual profiles. Similarly, samples 37 (*S.* Enteritidis) and 34 [*S. enterica* subsp *enterica* (8,20:64,Z23:-)], both isolated from human blood, presented the same profile as sample 23 [*S. enterica* subsp *enterica* (068:64,Z23:-)], isolated from poultry; moreover, sample 19 [*S. enterica* subsp *enterica* (8,20:64,Z23:-)] was identical to sample 18 (*S*. Enteritidis).

A dendrogram using the RAPD results produced by primer P1 grouped together Enteritidis and *enterica* isolated from human blood, human feces, and poultry, showing a possible epidemiological relationship between these samples (Figure 1). The samples isolated from human feces and human blood were collected from patients showing diarrhea and septicemia symptoms. The poultry for sample isolation were obtained from a street market, probably with unhygienic conditions. The grouping of these samples close together indicates that the infection in humans probably began with ingestion of the contaminated poultry. Poultry are an important source of protein in the Brazilian diet and are often bought in open street markets. The results of this study reinforce the need for better sanitary control measures for this product.

As the RAPD technique is simple, rapid, and rather cheap, we suggest that it can serve as an additional tool for studying the genetic diversity and molecular epidemiology of *Salmonella* species, both inter- and intra-serovars. The isolation of various serovars of *Salmonella* from different sources indicates the presence and the wide distribution of *Salmonella* in the

state of Sergipe, Northeastern Brazil. Knowledge of genetic variability of subtypes of different serovars and the sources of *Salmonella* may provide valuable additional information for research, risk management, and public health strategies.

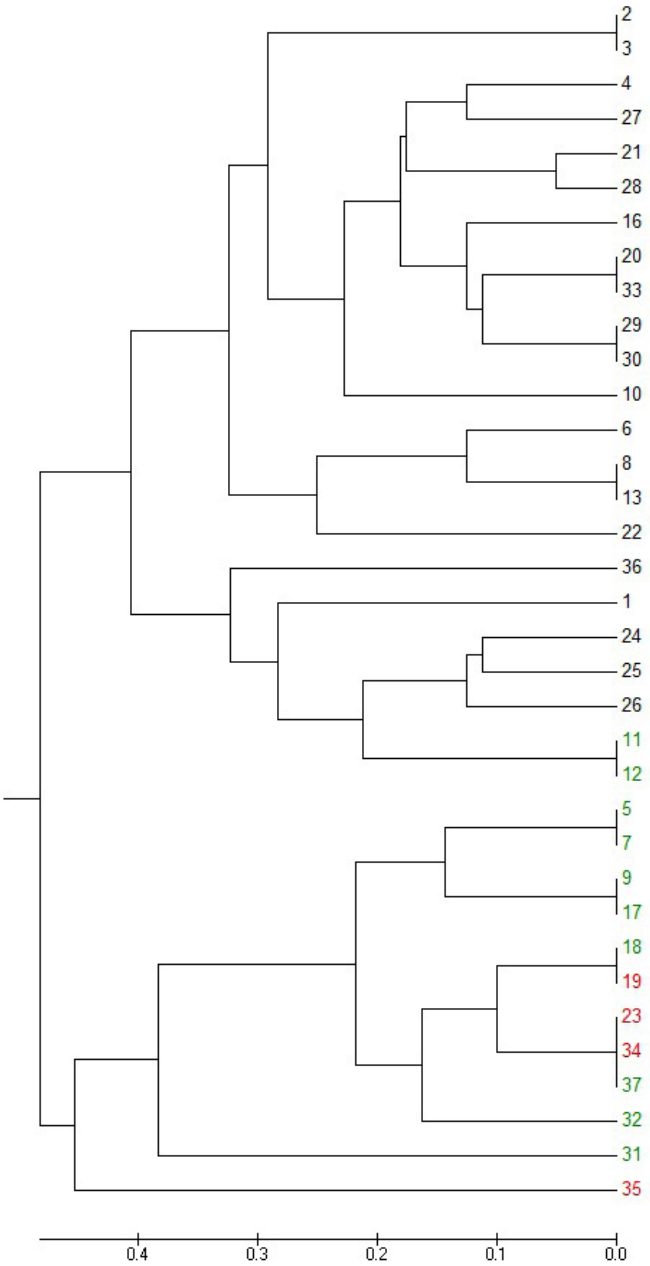

Figure 1. Dendrogram of randomly amplified polymorphic DNA (RAPD) profiles using primer P1. *Salmonella* Enteritidis and *S. enterica* isolates are marked in green and red, respectively.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Chansiripornchai N, Ramasoota P, Bangtrakulnonth A, Sasipreeyajan J, et al. (2000). Application of randomly amplified polymorphic DNA (RAPD) analysis for typing avian *Salmonella enterica* subsp. enterica. *FEMS. Immunol. Med. Microbiol*. 29: 221-225.

Colauto NB, Dias ES, Gimenes MA and Eira AF (2002). Genetic characterization of isolates of the basidiomycete *Agaricus blazei* by RAPD. *Braz. J. Microbiol*. 33: 131-133.

De Cesare A, Manfreda G, Dambaugh TR, Guerzoni ME, et al. (2001). Automated ribotyping and random amplified polymorphic DNA analysis for molecular typing of *Salmonella* Enteritidis and *Salmonella* Typhimurium strains isolated in Italy. *J. Appl. Microbiol*. 91: 780-785.

Hunter PR and Gaston MA (1988). Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol*. 26: 2465-2466.

Khoodoo MH, Issack MI and Jaufeerally-Fakim Y (2002). Serotyping and RAPD profiles of *Salmonella enterica* isolates from Mauritius. *Lett. Appl. Microbiol*. 35: 146-152.

Laconcha I, López-Molina N, Rementeria A, Audicana A, et al. (1998). Phage typing combined with pulsed-field gel electrophoresis and random amplified polymorphic DNA increases discrimination in the epidemiological analysis of *Salmonella* Enteritidis strains. *Int. J. Food. Microbiol*. 40: 27-34.

Lim H, Lee KH, Hong CH, Bahk GJ, et al. (2005). Comparison of four molecular typing methods for the differentiation of *Salmonella* spp. *Int. J. Food. Microbiol*. 105: 411-418.

Lin AW, Usera MA, Barrett TJ and Goldsby RA (1996). Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella* Enteritidis. *J. Clin. Microbiol.* 34: 870-876.

Nath G, Maurya P and Gulati AK (2010). ERIC PCR and RAPD based fingerprinting of *Salmonella* Typhi strains isolated over a period of two decades. *Infect. Genet. Evol*. 10: 530-536.

Quintaes BR, Leal NC, Reis EM, Fonseca EL, et al. (2002). Conventional and molecular typing of *Salmonella* Typhi strains from Brazil. *Rev. Inst. Med. Trop. Sao Paulo* 44: 315-319.

Rezk NA, Mansour H, Ghoneim NH and Rifaat MM (2012). Typing of *Salmonella* Typhi strains isolated from Egypt by RAPD PCR. *3 Biotech*. 2: 17-25.

Rohlf FJ (1992). NTSYS-PC version 1.7 numerical taxonomy and multivariate analysis system. Exeter Software Publisher, New York.

Ruiz M, Rodríguez JC, Sirvent E, Escribano I, et al. (2003). Usefulness of different techniques in the study of the epidemiology of salmonellosis. *APMIS.* 111: 848-856.

Sambrook J, Fritsch EF and Maniatis T (2001). Molecular cloning: a laboratory manual. 2nd edn. Cold Spring Harbor, New York.

Santos RL and Bäumler AJ (2004). Cell tropism of *Salmonella enterica*. *Int. J. Med. Microbiol.* 294: 225-233.

Smith SI, Fowora MA, Goodluck HA, Nwaokorie FO, et al. (2011). Molecular typing of *Salmonella* spp isolated from food handlers and animals in Nigeria. *Int. J. Mol. Epidemiol. Genet*. 2: 73-77.

Soto SM, Guerra B, González-Hevia MA and Mendoza MC (1999). Potential of three-Way randomly amplified polymorphic DNA analysis as a typing method for twelve *Salmonella* serotypes. *Appl. Env. Microbiol*. 65: 4830-4836.

Yan SS, Pendrak ML, Abela-Ridder B, Punderson JW, et al. (2004). An overview of *Salmonella* typing - Public health perspectives. *Clin. Appl. Immunol. Reviews*. 4: 189-204.