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Ribotyping of *Salmonella* Enteritidis strains reveals the spread of a single genotype in the Brazilian city of Ribeirão Preto

Ribotipagem de cepas de Salmonella *Enteritidis revela a propagação de um único genótipo na cidade de Ribeirão Preto*

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key words

Ribotyping

***Salmonella* Enteritidis**

abstract

Background: The approach generally employed in leading laboratories worldwide to identify strains of *Salmonella* is to detect epidemiological markers by serotyping and molecular techniques. These molecular methods are important in sanitary surveillance and lead to the source of infection. Objectives: The aim of the present study is to characterize *Salmonella* Enteritidis strains by ribotyping. Material and methods: Thirty-eight strains of *S.* Enteritidis were isolated from in patients at the university hospital of Universidade de São Paulo, Ribeirão Preto, SP, Brazil, between 1996 and 1998. These strains were isolated from stools (31 samples), blood (4 samples) and other body fluids (3 samples), using routine bacteriological methods, and were serotyped and ribotyped. Results: The 38 strains of serotype *S.* Enteritidis were shown by the ribotyping to belong to two ribotypes: A (94.7% of the samples) and B (5.3%). Discussion and conclusion: These results suggest that the majority of patients (94.7%) were infected by the same strain. This strain could be endemic in the Ribeirão Preto community, or these patients may have been exposed to a common source of infection.

unitermos

Ribotipagem

Salmonella *Enteritidis*

resumo

Introdução: Na identificação de cepas de Salmonella*, os métodos de sorotipagem e ribotipagem na detecção de marcadores epidemiológicos são os mais utilizados nos laboratórios de referência mundiais. Esses métodos moleculares são imprescindíveis na vigilância epidemiológica e permitem a detecção da fonte da infecção. Objetivos: O presente trabalho objetivou caracterizar as cepas de* Salmonella *Enteritidis pela ribotipagem. Material e métodos: Trinta e oito cepas de* S. *Enteritidis foram isoladas de pacientes atendidos no Hospital das Clínicas da Universidade de São Paulo, Ribeirão Preto, SP, entre os anos de 1996 e 1998. As cepas foram isoladas de fezes (31 amostras), sangue (quatro amostras) e outros fluidos (três amostras). As cepas de* Salmonella *foram isoladas utilizando-se métodos bacteriológicos de rotina, sorotipadas e ribotipadas. Resultados: As 38 cepas de* S. *Enteritidis apresentaram na ribotipagem a separação das cepas em dois ribotipos: A (94,7% das amostras) e B (5,3% das amostras). Discussão e conclusão: Esses dados sugerem que grande parte dos pacientes (94,7%) foi infectada pela mesma cepa. Essa cepa pode ser endêmica na comunidade de Ribeirão Preto ou os pacientes foram expostos a uma fonte comum de infecção.*

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Introduction

Non-typhoid salmonellosis is one of the commonest foodborne diseases and most frequently reported bacterial infections. The usual causative agent of this disease is *Salmonella* typhimurium or the emerging pathogen *S.* Enteritidis[1]. In the last decade the incidence of gastrointestinal infections caused by *S.* Enteritidis has increased[16, 21]; outbreaks have been reported in the United States and Europe since the 1970s[3]. Also, in many developing countries, such as Brazil, foodborne outbreaks and nosocomial infections caused by *Salmonella enterica* subsp. *enterica* serotype Enteritidis (*S.* Enteritidis) remain an important public health problem. In the emergency unit at the university hospital of Faculdade de Medicina de Ribeirão Preto of Universidade de São Paulo (FMRP/USP), during the years 1985-1999, *S.* Enteritidis exhibited a steady and significant rise, becoming the most frequently isolated *Salmonella* serotype in 1998[6]. This increase could be linked to a rapid growth of the international trade of food products, changes in the type of food consumed, food contamination and salmonellosis in acquired immunodeficiency syndrome patients.

The worldwide rise of *S.* Enteritidis nowadays constitutes a public health concern; it is a challenge to epidemiologist and clinical staff alike[22, 24]. The main approach taken to control the dissemination of salmonellosis is to employ molecular techniques. Methods based on DNA restriction analysis, such as ribotyping, can discriminate *Salmonella* strains involved in human infection and give information on the epidemiological and genetic relationship among serotypes. Hence, to differentiate serotypes such as *S.* Enteritidis, ribotyping has been employed in epidemiological investigation all over the world[2, 9, 10].

The aim of this study was to analyze the rRNA gene restriction pattern of the *S.* Enteritidis strains isolated from patients at the university hospital of FMRP/USP, between 1996 and 1998.

Material and methods

A total of 83 strains of *Salmonella* (9.5%) were isolated from 872 patients admitted to the university hospital of FMRP/USP, State of São Paulo, Brazil, between March 1996 and April 1998. *Salmonella* strains were identified by standard bacteriological methods (biochemical and agglutination methods)[7] and serotyped, as detailed in Popoff and Le Minor[17].

S. Enteritidis was the most frequently cultivated serotype from clinical samples, totalizing 38 strains (45.8%), listed in **Table 1**. *Salmonella* chromosomal DNA was extracted, purified, as described by Brenner *et al.*[4], and digested with *Sph*I enzyme in accordance to manufacturer's instructions (Pharmacia Biotech, Herts, United Kingdom). Previous findings[14] had shown that this enzyme was the best to discriminate strains of *S.* Enteritidis.

DNA fragments were separated by horizontal electrophoresis in 0.8% agarose gel (Sigma) with Tris-acetate EDTA as running buffer[23], transferred to nylon membranes probe obtained by reverse transcription from *E. coli* rRNA (Boerhinger Mannheim, Germany), and labeled with digoxigenin (DIG), as in Popovic *et al.*[18]. In this method, genes are arranged in seven to eight bands (**Figure 1**) and a single difference in the number or position of these bands is considered as a different ribotype. DNA fragment sizes were estimated using DNA STAR software (DNA STAR Computer System for Molecular Biology and Genetics, London, UK), and *Haemophilus aegyptius* 3031 *Eco*RI DNA digest as molecular marker.

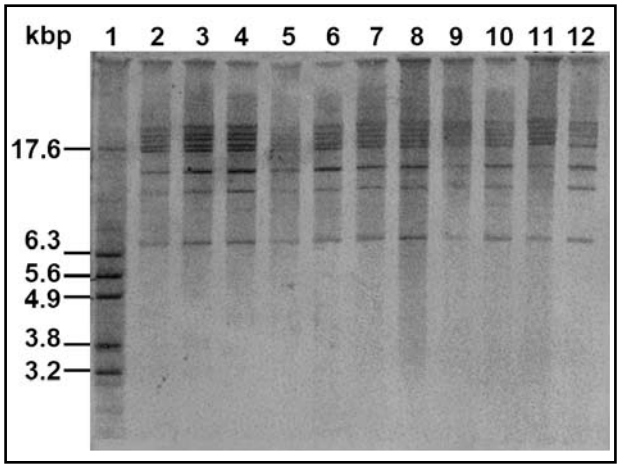

Figure 1 – *Ribotypes of S.* Enteritidis *after hybridization with DIG-labeled 16S+23S cDNA probe. Lane 1: molecular weight marker Haemophilus aegyptius 3031 EcoRI DNA digest. Lanes 2 to 11: ribotype A, after digestion with SphI. Lane 12: ribotype B, after digestion with SphI*

Results

The commonest occurrence of infection was in children in their first ten years. At the time of hospital admission, most patients presented acute gastroenteritis and fever. Some of the characteristics of the isolated strains, one from each patient, are displayed in Table 1.

In the ribotyping analysis following *Sph*I digestion, two ribotypes (A and B) were detected among the 38 *S.* Enteritidis strains (Figure 1). Thirty-six strains of *S.* Enteritidis (94.7%) had ribotype A and the other two (5.3%) had ribotype B, differing from the absence of one band at a position corresponding to the 17.6 Kbp marker.

Table 1 *Characteristics and results of the ribotyping test of the 38 S.* Enteritidis *isolates*

Date of isolate	Strain number	Clinical material	Age	Ribotype
5/3/1996	487	Feces	2y	A
5/27/1996	490	Bile	30y	A
6/17/1996	494	Blood	33y	B
7/29/1996	496	Feces	2y	A
8/6/1996	501	Blood	5y	A
9/6/1996	502	Feces	23y	A
9/27/1996	503	Feces	1y	A
9/30/1996	504	Feces	1y	A
10/15/1996	506	Synovial fluid	35y	A
10/30/1996	509	Feces	2m	A
10/31/1996	510	Feces	1y	A
11/4/1996	512	Feces	11y	A
11/5/1996	513	Feces	N	A
11/13/1996	514	Feces	10y	A
1/27/1997	524	Feces	34y	B
1/27/1997	529	Feces	N	A
2/8/1997	532	Feces	2y	A
3/31/1997	547	Feces	7m	A
4/29/1997	550	Feces	6m	A
5/5/1997	551	Feces	N	A
5/11/1997	552	Feces	4m	A
5/19/1997	553	Feces	7m	A
7/29/1997	558	Feces	6m	A
9/22/1997	566	Feces	N	A
10/25/1997	573	Feces	30y	A
11/17/1997	577	Feces	2y	A
12/4/1997	579	Feces	7m	A
12/23/1997	583	Feces	N	A
12/23/1997	584	Cerebrospinal fluid	27y	A
12/30/1997	587	Blood	49y	A
1/13/1998	592	Feces	1y	A
1/19/1998	605	Feces	3y	A
2/4/1998	597	Feces	2y	A
2/27/1998	600	Blood	42y	A
3/2/1998	601	Feces	29y	A
3/3/1998	602	Feces	41y	A
3/9/1998	606	Feces	1y	A
4/25/1998	611	Feces	3m	A

y: years; m: months; N: Newborn.

Discussion

Salmonellosis continues to be the major problem for food industries and the public health system[6, 13, 21]. Recent data report the increasing incidence of *S.* Enteritidis strains worldwide[13, 16, 21]. In the state of São Paulo, a remarkable rise in the number of patients infected by *S.* Enteritidis was observed in 1993. Since 1994, it has become the most frequent serotype responsible for foodborne outbreaks and sporadic cases of human disease. There has also been an increase in the frequency with which *S.* Enteritidis has been isolated from blood cultures, mainly in children[9, 24].

Laboratory characterization of this pathogen is epidemiologically important because it helps establish the connection between clinical cases and possible sources of infection. Investigators have shown that ribotyping can differentiate *Salmonella* serotypes according to source, regardless of host or geographic origin[8].

Ridley *et al.*[20] and Laconha *et al*[10] investigated the genotypic differences between strains of *Salmonella* by plasmid analysis, ribotyping and pulsed-field gel electrophoresis (PFGE). The results obtained by those researchers indicated that PFGE may offer a better level of discrimination of *S.* Enteritidis types than other genotypic methods. Conversely, other epidemiological studies of *S.* Enteritidis have demonstrated that PFGE methodology has a lower discriminatory capacity than ribotyping[11, 12, 15, 19, 25, 26].

Fernandes *et al.*[9] used rRNA gene restriction patterns to investigate the relatedness of *S.* Enteritidis strains isolated in São Paulo, from 1975 to 1995, and showed that ribotyping is a genomic profiling method that is reproducible and suitable for tracing the spread of *S.* Enteritidis. They found that the restriction endonuclease *Sph*I discriminated best between subtypes of this serotype. Thus, to discriminate *S.* Enteritidis strains in the present study, ribotyping was performed with the enzyme *Sph*I. This approach separated these *Salmonella* isolates into two ribotypes (A and B). Ribotype A was much the most prevalent and is probably endemic in the community of Ribeirão Preto. Otherwise, the infected patients were exposed to the same source of infection. This ribotype was the same as that most frequently identified among *S.* Enteritidis strains isolated in different geographic locations in São Paulo (and designated R11)[9].

We conclude that rRNA gene-restriction patterns were used effectively to identify subtypes among *Salmonella* Enteritidis strains, confirming the value of ribotyping as an epidemiological tool, as well as revealing that in Riberão Preto these strains display great clonal homogeneity.

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