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Salmonella SEROVARS ISOLATED FROM HUMANS IN SÃO PAULO STATE, BRAZIL, 1996-2003

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SUMMARY

Salmonellosis remains an important cause of diarrheal illness in humans in São Paulo State, Brazil. In this study were identified 3554 *Salmonella* isolates from human infections, during the period 1996-2003. Among 68 different serovars determined, *S.* Enteritidis was the most frequent one in gastrointestinal and extra-intestinal infections accounting for 67.4% of all isolates. *S*. Typhimurium and *S*. *enterica* subsp. *enterica* (4,5,12:i:-) were most frequently isolated from children aged < 1-4 year-old, in contrast, people with *S*. Enteritidis infections were most likely to be 20-50 year-old. In our geographic area the continued laboratorial surveillance of salmonellosis, including serotyping, has showed the trends in *Salmonella* serovars causing infections in humans throughout the time.

KEYWORDS: *Salmonella; S.* Enteritidis; Serovars.

INTRODUCTION

Salmonella spp. remains an important cause of diarrheal illness in humans in many parts of the world and also in São Paulo State, Brazil, despite the general improvement in sanitary conditions.

The *Salmonella* genus consists of only two species, *S. enterica* which is divided into six subspecies: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica*; and *S. bongori*. This nomenclature reflects the present *Salmonella* taxonomy[5,18]. Serovars belonging to *S. enterica* subsp. *enterica* are designated by a name usually related to the geographical place where those serovars were first isolated. This name is written in roman letters (not italicized) and the first letter is a capital letter. Serovars belonging to other subspecies are designated by their antigenic formulae, following the subspecies name.

The serotyping is based in a document called the Kauffmann-White Scheme[25] and consists in the characterization, generally by slide agglutination tests, of the somatic (O) and flagellar (H) antigens. Updating this scheme was responsibility of the WHO Collaborating Centre for Reference and Research on *Salmonella*, which is revised each five years. In the last revision of this scheme were described 2501 serovars[25] .

Serotyping is the epidemiological marker of choice for subdividing *Salmonella* spp. strains. Most of the information regarding the prevalence of salmonellae has been based on passive laboratory *Salmonella* surveillance. Reporting of animal and human salmonellosis has been substantially underestimated. However, the surveillance data allow broad comparisons and identify trends, reservoirs and routes of transmission of *Salmonella* serovars.

During the first six decades of the 1900s, the main issues with respect to salmonellosis were the occurrence of typhoid fever in humans, which declined dramatically in the following years in many parts of the world, as well in São Paulo State[23,32].

Since 1940, there has been a rapid increase in the isolation of non typhoidal serovars which are recognized as one of the major causes of food poisoning worldwide[4]. This was particularly the case of *S*. Typhimurium, which has been reported to be one of the most common causes of human salmonellosis[10,17,23,32,37] .

More recently, specific serovars have been linked with certain foods or exposures. In many countries, there has been an increased incidence of gastrointestinal infections caused by *S*. Enteritidis[27,28,30] and the outbreaks identified have been repeatedly associated with raw or undercooked eggs[2,3,9,22] .

This present report analyzed particularly the trends in *Salmonella* serovars causing infections in humans in São Paulo State, during 1996 to 2003.

METHODS

A total of 3554 *Salmonella* strains were isolated from human origin in different geographic locations from São Paulo State during the period

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of 1996-2003, which were received and analyzed at the Laboratory of Enteric Pathogens, Adolfo Lutz Institute, São Paulo. These strains were confirmed to be *Salmonella* genus on the basis of reduced number of conventional biochemical tests[11].

The serotyping of *Salmonella* strains were performed according to POPOFF[25] and POPOFF & LE MINOR[26] , by slide agglutination tests and the *Salmonella* antisera used were prepared in our laboratory by using standard guidelines[19]. The somatic (O) antigens were detected directly from growth bacterium on nutrient agar and the flagellar (H) antigens were identified by selecting the most mobile bacteria from cultivation on Sven-Gard semi-solid medium[14]. After identifying one of the flagellar phases, each strain was grown again on other Sven-Gard semi-solid medium plate containing the antiserum related to the previously detected flagellar phase, in order to identify the other phase.

Some serovars also required the subspecies level determination, which was done based on a small number of biochemical characteristics[25].

RESULTS

A total of 68 different serovars were identified among the 3554 strains isolated from humans, and the annual distribution of the most frequent ones is shown in Table 1. During the entire 8-year period, the top five reported *Salmonella* serovars: *S*. Enteritidis, *S*. Typhimurium, *S. enterica* subsp. *enterica* (4,5,12:i:-), *S.* Typhi and *S.* Dublin, accounted for 84.1% of all isolates.

In the studied period, *S*. Enteritidis was the most frequent serovar accounting for 2396 strains (67.4%), followed by S. Typhimurium, 185 (5.2%) (Table 1). The annual prevalence of *S*. Enteritidis remained high among the other 67 identified serovars.

A known clinical source was reported for 3509 (98.7%) isolates, which in majority 2922 (83.2%) were from stool samples, other 410 (11.5%) were from blood, 58 (1.6%) were from urine, 52 (1.4%) were from cerebrospinal fluid, and 67 (2.3%) were isolated from other sources (Table 2).

S. Enteritidis was the most frequently serovar identified in the different sites of *Salmonella* isolation (Table 2). Besides *S*. Enteritidis, 16 different serovars were identified among the strains isolated from blood being *S*. Typhi, *S*. Dublin, *S*. Typhimurium and *S. enterica* subsp. *enterica* (4,5,12:i:-) the prevalent ones.

The distribution of *Salmonella* serovars by age groups is shown in Table 3. Information on patient age was available for 80.7% of the reported isolates and most of them (52.6%) were isolated from people aged 20-59 year-old and other 20.5%, were from children aged < 1-4 year-old.

S. Typhimurium and *S. enterica* subsp. *enterica* (4,5,12:i:-) were most frequently isolated from children < 1-4 year-old, in contrast, people with *S*. Enteritidis infection were most likely to be 20-59 year-old (Fig. 1).

DISCUSSION

In this study we described 3554 culture confirmed *Salmonella* infections for 8-year period, 1996-2003, however the number of

Table 1
Annual number of *Salmonella* isolates from humans: 15 most frequent serovars, 1996-2003

Serovar	Year								TOTAL (%)
	1996	1997	1998	1999	2000	2001	2002	2003	
Enteritidis	352	262	268	331	309	316	262	296	2396 (67.4)
Typhimurium	17	3	35	24	22	19	37	28	185 (5.2)
4,5,12:i:-	55	5	31	25	7	19	26	16	184 (5.1)
Typhi	31	12	20	27	12	17	14	7	140 (4.0)
Dublin	7	8	19	5	9	11	13	14	86 (2.5)
Infantis	17	5	11	1	2	15	26	2	79 (2.3)
Agona	13	2	4	9	6	5	3	4	46 (1.4)
Panama	3	1	1	7	4	3	6	9	34 (1.0)
Oranienburg	4	4	5	3	2	2	7	4	31 (0.8)
Saintpaul	-	-	1	-	1	6	17	5	30 (0.8)
Newport	8	-	2	3	2	6	2	3	26 (0.7)
Hadar	7	3	2	-	3	-	1	6	22 (0.6)
Javiana	5	1	3	2	2	5	-	3	21 (0.5)
Mbandaka	1	-	1	1	-	2	7	2	14 (0.3)
Shwarzengrund	2	-	5	2	-	1	2	1	13 (0.4)
Other (53 Serovars)	37	21	33	26	29	31	29	41	247 (7.0)
Total	559	327	441	466	410	458	452	441	3554 (100.0)

Note: Data are No. of isolates with the serovars.

Table 2
Number of *Salmonella* isolates from humans, by source of isolation and serovar: 15 most frequent serovars, São Paulo, Brazil, 1996-2003

	Source						
Serovar	Stool	Blood	CSF*	Urine	Other**	Unknown	TOTAL
Enteritidis	2136 (91.2)	148 (6.3)	29 (1.2)	21 (0.9)	35 (14)	27	2396
Typhimurium	147 (79.4)	31 (16.8)	5 (2.8)	2 (1.0)	-	-	185
4,5,12:i:-	119 (65.7)	48 (26.5)	3 (1.7)	3 (1.7)	8 (4.4)	3	184
Typhi	45 (32.3)	91 (65.5)	-	3 (2.1)	3 (2.1)	1	140
Dublin	13 (15.2)	56 (65.2)	1 (1.1)	9 (10.4)	7 (8.1)	-	86
Infantis	75 (94.9)	-	1 (1.3)	3 (3.8)	-	-	79
Agona	41 (91.2)	3 (6.6)	1 (2.2)	-	-	1	46
Panama	26 (81.4)	1 (3.1)	2 (6.2)	2 (6.2)	1 (3.2)	2	34
Oranienburg	25 (80.6)	3 (9.7)	-	-	3 (9.7)	-	31
Saintpaul	28 (93.4)	2 (6.6)	-	-	-	-	30
Newport	22 (91.6)	1 (4.2)	-	1 (4.2)	-	2	26
Hadar	20 (91.0)	1 (4.5)	1 (4.5)	-	-	-	22
Javiana	19 (90.6)	1 (4.7)	1 (4.7)	-	-	-	21
Mbandaka	13 (92.8)	1 (7.2)	-	-	-	-	14
Shwarzengrund	10 (76.9)	2 (15.4)	-	-	1 (7.7)	-	13
Other (53 Serovars)	183 (81.1)	24 (104)	8 (3.5)	14 (6.1)	9 (3.9)	9	247
Total	2922 (83.3)	413 (11.8)	52 (1.5)	58 (1.6)	64 (1.8)	45	3554

Note: Data are No. (%) of isolates. Isolates for which source of isolation was unknown were excluded from percentage calculations. *CSF, cerebrospinal fluid. **Includes wound, sputum, abscess and unspecified other category.

Table 3
Distribution, by age group and serovar, of the 15 most frequently reported serovars of *Salmonella* isolates from humans, São Paulo State, Brazil, 1996-2003

	Age, years							
Serotype	< 1	1 – 4	5 – 14	15 – 19	20 – 59	≥ 60	Unknown	TOTAL
Enteritidis	110 (5.8)	118 (6.3)	293 (15.5)	200 (10.7)	1079 (57.3)	82 (4.4)	514	2396
Typhimurium	37 (22.6)	51 (31.2)	10 (6.2)	4 (2.5)	52 (31.9)	9 (5.6)	22	185
4,5,12:i:-	36 (22.9)	53 (33.7)	12 (7.7)	2 (1.3)	49 (31.3)	5 (3.1)	27	184
Typhi	1 (0.8)	5 (4.2)	17 (14.4)	12 (10.2)	78 (66.1)	5 (4.3)	22	140
Dublin	-	7 (8.5)	2 (2.5)	5 (6.0)	57 (69.5)	11 (13.4)	4	86
Infantis	10 (13.9)	4 (5.6)	6 (8.4)	5 (6.9)	42 (58.3)	5 (6.9)	7	79
Agona	18 (39.1)	8 (17.5)	5 (10.8)	1 (2.2)	14 (30.4)	-	-	46
Panama	2 (6.4)	8 (25.8)	2 (65)	1 (3.2)	18 (39.1)	-	3	34
Oranienburg	2 (6.5)	1 (3.3)	5 (16.1)	2 (6.4)	21 (67.7)	-	-	31
Saintpaul	3 (11.2)	14 (51.8)	-	1 (3.7)	9 (33.3)	-	3	30
Newport	1 (3.9)	6 (23.1)	1 (3.9)	-	17 (65.2)	1 (3.9)	-	26
Hadar	-	5 (22.8)	-	1 (4.5)	16 (72.7)	-	-	22
Javiana	4 (19.0)	3 (14.2)	2 (9.5)	1 (4.7)	9 (42.8)	2 (9.5)	-	21
Mbandaka	-	1 (7.1)	7 (50.1)	-	3 (21.4)	3 (21.4)	-	14
Shwarzengrund	4 (30.7)	3 (23.0)	-	1 (7.8)	5 (38.5)	-	-	13
Other (53 Serovars)	39 (23.7)	33 (20.1)	14 (8.5)	16 (9.7)	41 (25.1)	18 (10.9)	83	247
Total	270 (9.5)	320 (11.1)	376 (13.1)	252 (8.8)	1510 (52.6)	141 (4.9)	685	3554

Note: Data are No. (%) of isolates. Persons for whom age was unknown were excluded from percentage calculations.

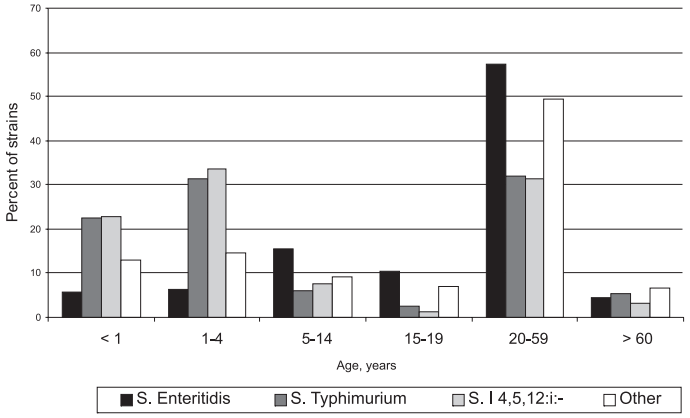

Fig. 1 - Percentage of distribution by age of patients for *Salmonella* serovars in São Paulo State, 1993-2003.

infection that goes unreported has been estimated to be much greater than the number of reported infections.

Characterization of *Salmonella* serovars in São Paulo State began in 1950 and has been conducted by Laboratory of Enteric Pathogens in Adolfo Lutz Institute. These laboratorial surveillance data are periodically summarized and published[32,33,34]. By this way, it has been detected the changing patterns of *Salmonella* serovars caused by the trend increase of *S*. Enteritidis isolated from human and non human sources, since 1993[33].

There have been significant changes in the epidemiology of salmonellosis in São Paulo, Brazil, over the past 54 years, by comparing these results with the previous reports.

S. Typhi showed a striking declining trend after late 70's[32], although typhoid fever is still a public health concern, due to poor sanitary conditions and unsatisfactory personal hygiene of people having a low standard of living. This serovar represented the fourth most prevalent among the identified isolates (Table 1) and the second one among strains isolated from blood (Table 2).

In São Paulo State, a substantial rise in *S.* Typhimurium changed the patterns in the 70's accounting for 77.7% of all serovars of clinical sources, and presented an epidemic feature[32]. The following years (1977-90) were characterized by a declining trend of *S.* Typhimurium, a significant rise of the isolation of other serovars including *S.* Agona[32]. During the period 1991-95, it was reported the changing patterns of *Salmonella* serovars in São Paulo State caused by the world trend increase of *S*. Enteritidis[33].

During the last decades *S*. Enteritidis and *S*. Typhimurium have emerged as the predominant serovars in the majority of countries disposing this information[27,29,36]. Our findings indicate the general steady high prevalence in *S*. Enteritidis infections during the reporting period (Table 1), that is consistent with previous studies in São Paulo State, Brazil[33].

In São Paulo State, the remarkable increase in the incidence of human *S*. Enteritidis isolates began to be noticed in 1993 (10.1%) and since 1994, accounted for 43.3% of all isolates. It became the most frequent serovar responsible for foodborne outbreaks and sporadic cases of human gastrointestinal diseases[33]. Many of the outbreaks reported have been epidemiologically linked to the consumption of raw or undercooked eggs, to a lesser extent, chicken as vehicles for transmission[2,3,4,16,24]. The frequent occurrence of this serovar in chickens suggests that poultry may be an important reservoir, a finding that is consistent with almost all other studies in other countries. The increase of *S.* Enteritidis in our State, is clearly related to the progression of phage type 4 (PT-4) strains, as previously detected[12,15], which has also emerged as the predominant phage type in Europe[36]. Thus, São Paulo State has also been part of the global pandemic of S. Enteritidis observed in the late 1980s[28].

Besides the prevalence of *S*. Enteritidis, other serovars like *S*. Typhimurium, *S*. Agona, *S*. Typhi, and *S*. *enterica* subsp. *enterica* (4,5,12:i:-) were among the most frequent ones (Table 1). *S*. *enterica* subsp. *enterica* (4,5,12:i:-), the atypical and monophasic serovar, firstly identified in 70's in São Paulo[7], has been among the most prevalent serovars during the last decade, including in other countries[10]. It shares almost all antigenic factors with *S*. Typhimurium and some studies on phenotypic and molecular characterization of these two serovars have been carried out, including in our laboratory[35], in order to confirm their relationship.

There has not been detected any significant change in the prevalence of the main serovars isolated from humans, by comparing with those ones reported in the last period, 1991-1995[33]. The isolation rates of the most common *Salmonella* serovars from human infections, especially *S*. Enteritidis, generally showed the same trends in magnitude as those *Salmonella* serovars isolated from non human sources and identified during 1996 to 2000[34].

However as it happens almost every year, the list of different *Salmonella* serovars detected in an specific region, increases by the addition of some serovars that had not been previously detected. In this way, *S*. Businga, *S*. *enterica* subsp. *houtenae* 40:z_4,z_{24}:-, *S*. Weltevreden, *S*. IIIb 50:r:z (indole-positive), *S*. IIIb 61:c:-, *S*. Corvallis, and *S*. Cotia were detected for the first time in human infections in São Paulo State, during the studied period. Among these serovars, *S*. Corvallis was firstly isolated in 1993 from human stool and it was observed that its prevalence has been increasing in cases of human infection and in poultry isolates (recent data from our Enteric Pathogens Laboratory).

As regards the samples from human origin, the great majority of strains (60.1%) were isolated from gastrointestinal infections, which are related to the significant number of foodborne outbreaks occurred during that period[1,16,24]. Among the stool isolates were identified 52 different serovars with the total predominance of *S*. Enteritidis. However, it was detected a very significant percentage of *Salmonella* strains, 587 (12.0%), associated with extra-intestinal infections. Several serovars with somatic antigens belonging to B, C1, and D1 groups, more commonly cause invasive infection and are frequently isolated from blood. Although *Salmonella* virulence factors are not well understood, differences in the somatic antigen-side chain of the lipopolysaccharide seem to be important[37].

Serotyping of *Salmonella* strains detected 18 different serovars from blood isolates, 12 from cerebrospinal isolates, 10 from fluids and nine from urine isolates (data not shown). However, *S*. Enteritidis was the most common serovar isolated from extra-intestinal sources, followed by *S*. Typhi, *S*. Dublin, *S*. *enterica* subsp. *enterica* (4,5,12:i:-), and *S*. Typhimurium (Table 2), findings which were similar to other studies in different countries[8,20,23].

Extra-intestinal salmonellosis is commonly observed in children, immunocompromised patients, and elderly people. In adult patients with acquired immunodeficiency syndrome (AIDS) several infections were described, predominantly caused by *S*. Enteritidis and *S*. Dublin[8,13,20,21]. In this study the prevalence of *S*. Enteritidis (52.0%) and *S*. Dublin (36.0%) among patients with AIDS was also observed.

Salmonella isolation rates were considered high for infants up to 4 year-old (Table 3), mainly from extra-intestinal sites and the reasons for this are unknown but may include host susceptibility, exposure differences and sample from ill infants were cultured. An important difference in the prevalence of *Salmonella* serovar in different age groups was observed, as *S*. Typhimurium and *S*. *enterica* subsp. *enterica* (4,5,12:i:-) for infants up to 4 year-old (Fig.1). Similar results have been reported by other countries, although with higher incidence rates for infants, specially < 1 year-old[23,29].

S. Typhi was consistently the most common extra-intestinal serovar isolated from adults. However among 118 culture-positive typhoid fever cases with known age, six (5.0%) were in children aged under five year-old and 29 (24.5%) 5-19 year-old (Table 3). The incidence of typhoid fever in these groups was considered significant, as well as observed by other reports [6,31].

The high prevalence of *S*. Enteritidis infection among adults (20-59 year-old) which are more exposed to the consumption of all kinds of food, it is more likely associated to greater general outbreaks of gastroenteritis including familiar outbreaks.

These data have several limitations considering that only people who were ill, sought care, had a stool culture, and had the *Salmonella* isolate forwarded to be serotyped could be included for the reported data. For these reasons, it is recognized that the burden of illness caused by salmonellosis is greatly undereported in São Paulo State.

Serotyping remains as an important laboratory tool that helps public health researchers to better understand and define the epidemiology of salmonellosis in a geographic area. Measuring trends in serovars over time can provide information about emerging serotypes and about the efficacy of prevention and control measures.

RESUMO

Sorovares de *Salmonella* isolados de humanos no Estado de São Paulo, Brasil, 1996-2003

A salmonelose permanece uma importante causa de doença diarréica em humanos no Estado de São Paulo, Brasil. Nesse estudo foram identificados 3554 isolados de *Salmonella* de infecções humanas, durante o período 1996-2003. Entre os 68 diferentes sorovares determinados, *S*. Enteritidis foi predominante em infecções gastrointestinais e extra-intestinais, responsável por 67.4% de todos os isolados. *S*. Typhimurium e *S*. *enterica* subsp. *enterica* (4,5,12:i:-) foram mais freqüentemente isolados de crianças com idade de < 1 a 4 anos, em contraste, infecções por *S*. Enteritidis foram predominantes em pessoas entre 20 e 50 anos. A contínua vigilância laboratorial das salmoneloses, incluindo a sorotipagem, tem evidenciado os sorovares de *Salmonella* causando infecções humanas em nossa área geográfica ao longo do tempo.

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