Prevalence and Antimicrobial Susceptibility of *Salmonella* Serotypes in Patients from Ribeirão Preto, São Paulo, Brazil, between 1985 and 1999

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***Salmonella* strains isolated from 1,138 samples representing 28,199 biological materials (stool, urine, blood and other fluids), collected between January 1985 and January 1999 at a reference University Hospital in Ribeirão Preto, São Paulo, Brazil, were studied. The most frequently detected serotypes were *Salmonella enterica* subspecies *enterica* serotype 4,5,12:i:- (*S.* I 4,5,12:i:) (21.2%), *S. agona* (15.8%) and *S. enteritidis* (11.3%). A changing pattern of *Salmonella* serotypes was observed between 1985-1999. *S. agona*, which represented 27% of *Salmonella* serotypes isolated from 1985-1989, declined to 4% during the period from 1995 to 1999. *S. enteritidis* isolation remained below 1% until 1989; rose to 5.9% between 1990 and 1994, and increased to 32.3% between 1995-1999. *S.* I 4,5,12:i:-; *S. enteritidis*; *S. typhimurium*; *S. dublin* and *S. infantis*, showed low to moderate resistance profiles to most antimicrobial drugs. Nalidixic acid and tetracycline were the most and the least effective drugs, respectively, in the disk diffusion tests. We encountered changes in salmonellosis epidemiology in this geographical region.**

Key Words: *Salmonella*, salmonellosis, serotypes, epidemiology and gastroenteritis.

In Brazil and in other developing countries human salmonellosis continues to be a major public health problem [1-8]. *Salmonella* is an enteropathogenic microorganism that causes infection accompanied by different clinical manifestations, most commonly gastroenteritis. Along with genuine systemic salmonellosis (typhoid and paratyphoid fever), salmonella infections in humans are mainly food-borne. More than 2,300 *Salmonella* serotypes have been described, but only some of them, such as *S. typhimurium*, *S. enteritidis*, *S. choleraesuis*, *S. hadar*, *S. virchow*, and *S. dublin* among others, play important epidemiological-epizootiological roles [9]. Although the mortality rate linked to salmonellosis is low, its high prevalence has significant economical, epidemiological and health implications [10-11].

Serotyping and molecular methods are important epidemiological tools for determining sources of infection, and serotyping has been used as an epidemiological marker for the study of *Salmonella* spp infection in a given population and location at a given time [12-15].

The aim of our study was to make a comprehensive analysis of the epidemiology of salmonellosis in the northeastern São Paulo state, Brazil. We determined the predominant serotypes and the resistance profile of *Salmonella* spp. strains isolated from patients attended over a period of 15 years (1985-1999) at the reference

Received on 19 April 2002; revised 27 August 2002.

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The Brazilian Journal of Infectious Diseases 2002;6(5):244-251

1413-8670

University Hospital of the School of Medicine of Ribeirão Preto of the University of São Paulo, Brazil, which receives patients from approximately 18 different cities of this region of São Paulo state.

Material and Methods

Study sample

A retrospective analysis was made of all records of *Salmonella* spp. isolations from the Microbiology Laboratory of the Emergency Unit of University Hospital, Ribeirão Preto School of Medicine, University of São Paulo – Brazil, taken from January 1985 to January 1999 . The 1,138 *Salmonella* strains, (Table 1), were isolated from 864 stool samples, 167 blood samples, 32 urine samples and 75 samples from other fluids (exudates, bile, cerebrospinal and synovial fluids), from 28,199 clinical samples.

Stool samples were routinely cultured on MacConkey agar, *Salmonella-Shigella* agar (SS) and Selenite F broth. Media were incubated overnight at 37°C and nonfermenting lactose colonies of different morphologic types growing on SS agar or MacConkey agar plates were identified on the basis of their biochemical properties. Specimens from other fluids (blood, urine and other fluids) were cultured using Brain Heart Infusion (BHI) – for blood, Cystine-Lactose-Electrolyte-Deficient agar (CLED) – for urine, and blood agar and chocolate agar – for other fluids. Inoculation and incubation were performed by standard procedures [16]. When morphological aspects suggested *Salmonella* spp., colonies were investigated by biochemical tests. The biochemical properties of isolated colonies were evaluated as described by Ewing [17]. At least three colonies having a typical appearance of *Salmonella* were chosen and applied in triplicate to triple sugar and iron and lysine iron agar slants, and incubated overnight at 37°C.

Agglutination tests

Colonies considered to be *Salmonella* as a result of biochemical testing, were investigated for O and H antigens using polyclonal antibodies and diagnostic anti-O and anti-H antisera (Probac, Brazil). These antigens were determined by the slide agglutination test.

Serotyping

All strains were serotyped according to the method described previously [18] at the Laboratory of Enteric Pathogens, Instituto Adolfo Lutz - São Paulo, Brazil.

Drug resistance assays

Antimicrobial susceptibility tests were done by the disk diffusion method by the standard methods outlined by the National Committee for Clinical Laboratory Standards (NCCLS) [19]. Three to five colonies were introduced into a tube containing trypticase soy broth, incubated overnight, and the broth cultures diluted until turbidity matched a 0.5 McFarland turbidity standard. Petri dishes with Mueller-Hinton agar were used for the sensitivity tests. Plates were dried for approximately 30 minutes prior to inoculation, and used within 1 day following preparation. Bacterial broth suspensions were streaked on the surface of media with a cotton swab. After the inoculum had dried, the antimicrobial disks were placed on the agar using flamed forceps, and gently pressed down to ensure contact. Disks with the following antibiotics (diffusible amounts) were provided from a commercial source (Ceccon): amikacin (30μg), sulfamethoxazole- trimethoprim (23.75 and 1.25 mg, respectively), ampicillin (10μg), tetracycline (30μg), tobramycin (10μg), cefoxitin (30μg), cephalothin (30μg), chloramphenicol (30 mg), nalidixic acid (30μg) and gentamicin (10μg).

Statistical analysis

Differences in *Salmonella* serotype prevalence over three, 5-year intervals (1985 to 1989; 1990 to 1994 and 1995 to 1999 periods), were compared using the *chi square* test [20]. The test was applied only for the most prevalent serotypes found in our study namely, *S.* I 4,5,12:i:-, *S. agona*, *S. enteritidis* and *S. typhimurium*. *Chi square* values were obtained using STATDISK (software by Password, Inc).

Results

From 1985 to 1999, 1,138 *Salmonella* spp strains were isolated from stool, blood, urine and other fluids (Table 1). Samples were obtained from 749 children (1 to 12 years) and 389 adults, totaling 593 (52.1%) male and 545 (47.9%) female individuals.

The *Salmonella* isolated were identified as belonging to 54 serotypes (Table 2). The serotypes most frequently isolated were *S.* I 4,5,12:i:-(21.2%), *S. agona* (15.8%), *S. enteritidis* (11.2%) and *S. typhimurium* (10.0%), isolated with different frequencies from specific biological materials (Table 1).

S. I 4,512:i:- showed a discrete trend to increased frequency from 1985 to 1999. *S. agona* was isolated at the highest percentage (26.9%) among all serotypes, between 1985 and 1989; its relative frequency declined to 13.6% from 1990-1994, and to 4% from 1995-1999. In contrast, the isolation of *S. enteritidis* increased progressively, and significantly, corresponding to respectively, < 0.1%, 5.9% and 32.3% over succeeding five year periods (Table 2). The statistical analysis confirming these data is shown in Table 3.

With the exception of tetracycline, the resistance profiles for *S.* I 4,5,12:i:-, *S. enteritidis*, *S. typhimurium*, *S. dublin*, and *S. infantis*, respectively, showed low resistance to the different antimicrobial substances tested. *S. agona* strains had comparatively high resistance to most of these drugs (Table 4).

Discussion

Salmonella serotypes have been isolated and identified as etiological agents of human infections in the State of São Paulo, Brazil since the late 1950s [6]. However, only after 1968 did some of them acquire significant epidemiological importance. We observed an expressive and significant increase in the prevalence of *S. enteritidis* between 1985 and 1999. These results agree with those of Tavechio et al. [15] who observed a change in the *Salmonella* serotype prevalence pattern from *S. agona* to *S. enteritidis* between 1991 and 1995, with a peak occurring in 1993. In our study, the most frequently isolated *Salmonella* serotypes in the northeastern region of the State of São Paulo, were *S.* I 4, 5, 12:i:-, *S. agona*, *S. enteritidis* and *S. typhimurium*.

Similarly, in a retrospective (1991-1995) study, Tavecchio et al. [15] showed that *S.* I 4, 5, 12:i:- was the second most prevalent serotype isolated from cases of salmonellosis. The reason for the high level of isolation of this serotype in Brazil has not yet been determined and speculations about its sources are difficult. *S.* I 4, 5, 12:i:- apparently was more invasive than other prevalent serotypes, but fortunately it has a low resistance profile.

According to Pessoa et al. [12], *S. typhimurium* predominated in the State of São Paulo, Brazil between 1970 and 1976, when it represented 77.7% of the serotypes isolated from clinical human samples. In other epidemiological studies, Calzada et al. [21] found that between 1977 and 1982, *S. typhimurium* still had a high (69.3%) isolation rate, followed by *S. agona* (16.1%), whereas at the end of the 1970s *S. agona* had been the predominant serotype [8].

In our study, *S. agona* was found to be the most prevalent serotype between 1985 and 1989, comprising 26.9% of the isolated strains; however, as shown in Tables 2 and 3, a large decrease in its isolation rate occurred from 1990 to 1999. An expressive and significant increase in the isolation of *S. enteritidis* from clinical samples was detected in the northeastern region of the State of São Paulo, Brazil between 1985 and 1999. This change agrees with the low percentage of *S. enteritidis* isolated during earlier decades [21, 22]. A retrospective analysis carried out in Belgium [23] between 1973 and 1992 showed a similar change in epidemiology, with Enteritidis predominating over *S. typhimurium* (43.8% *vs* 32.1%, respectively). Miller & Pegues [24], reported that in the USA, the isolation rate of *S. enteritidis* increased from 5%, recorded in 1974, to 26%, recorded in 1994. Similar findings were reported by Gutiérrez-Cogco et al. [25] who found a gradual increase in the isolation of the *S. enteritidis* serotype between 1990 and 1993 in Mexico. In 1994, *S. enteritidis* was considered to be an emergent pathogen in Chile [26], where increased numbers of clinical infection of adults and young children caused by this serotype were notified, comprising 478 cases

Table 1. Absolute figures and percentages of *Salmonella* serotypes isolated, according to their sources

Source	Serotypes					
	***S.* I 4,5,12: i: -**	***S. agona***	***S. enteritidis***	***S. typhimurium***	**Others**	**All**
Stool	156/ 64.7%	150/ 83.3%	107/ 83.6%	85/ 74.6%	366/ 77%	864/ 75.9%
Blood	46/ 19.1%	9/ 5.0%	18/ 14.0%	23/ 20.1%	71/ 14.9%	167/ 14.7%
Urine	10/ 4.1%	5/ 2.7%	2/ 1.6%	1/ 0.9%	14/ 2.9%	32/ 2.8%
Others	29/ 12.1%	16/ 9.0%	1/ 0.8%	5/ 4.4%	24/ 5%	75/ 6.6%
Total	241/100.0%	180/100.0%	128/100.0%	114/100.0%	475/100.0%	1,138/100.0%

in 1994 and 432 in 1995. According to Reis et al. [27], mapping of the geographic distribution of *Salmonella* serotypes in Brazil between 1992 and 1997 showed a more frequent occurrence of *S. enteritidis* and *S. typhimurium* in the southern region, of *S. agona* and *S. hadar* in the northeast, of *S. typhi* and *S. infantis* in the southeast and Panama in the north. These authors suggested that the prevalence of the *S. enteritidis* serotype in the southeast region is caused by a cosmopolitan phenomenon.

S. enteritidis was the only serotype that increased steadily during the period studied. It is possible that this increase is linked to the rapid growth of international trade in agricultural food products, facilitating the dissemination of new *Salmonella* serotypes, changes in types of food consumed, outbreaks of food poisoning, sporadic cases associated with family outbreaks and increased salmonellosis in acquired immunodeficiency syndrome patients [24, 28]. A possible worldwide increase of *S. enteritidis* in forthcoming years would be a cause of public health concern and a challenge to the epidemiologist and to clinical treatment, especially due to the eventual appearance of multi-resistant and invasive strains of these bacteria.

S. typhimurium, the most commonly reported pathogenic serotype of *Salmonella* in humans, was isolated at a low frequency (10%) over the three 5-year intervals examined in this study; however, when only the isolation from blood is considered, this percentage reaches 20%, which is a high percentage in developing countries. Nevertheless, the incidence of *S. typhimurium* remained relatively constant (Tables 2 and 3), probably because of the substantial rise in the isolation of Enteritidis [29]. It has been proposed that the *S. enteritidis* epidemy could be caused by the clonal expansion of a single, more virulent form of *S. enteritidis* [29].

Antimicrobial therapy is contraindicated in cases of non-complicated salmonella gastroenteritis. However, the current occurrence of multiresistant strains advises its use in high risk groups like newborns, the elderly, as well as in cancer, sickle cell disease, AIDS, transplanted and joint disease patients [24].

Although antimicrobial resistance among human nontyphoid salmonella isolates shows a worldwide trend towards increase, in our study the resistance profile of the majority of strains examined showed that they were sensitive to nearly all drugs tested. Low to moderate resistance rates were observed for serotypes *S.* I 4,5,12:i:-, *S. enteritidis*, *S. typhimurium*, *S. dublin* and *S. infantis* (Table 4); similar to the results obtained by Tavechio et al. [16]. *S. agona* showed greater resistance to most antibiotics when compared to the other serotypes; tetracycline was the least effective drug against *Salmonella in vitro*. A rather low incidence of antimicrobial resistance is generally observed for salmonella serotypes, except for serotypes *S. agona* and *S. infantis*; these data are unexpected in developing countries. It is conceivable that the low degree of resistance to antimicrobial drugs was caused

Table 2. *Salmonella* serotypes from human sources, isolated in Ribeirão Preto, SP, Brazil between 1985 and 1999

Strains/year	1985-1989		1990-1994		1995-1999		Total	
	Nº	%	Nº	%	Nº	%	Nº	%
S. I 4,5,12: i: -	69	17.2	100	22.7	72	24.2	241	21.2
S. agona	108	26.9	60	13.6	12	4.0	180	15.8
S. enteritidis	6	<0.1	26	5.9	96	32.3	128	11.3
S. typhimurium	35	8.7	53	12.0	26	8.8	114	10.0
S. dublin	18	4.5	32	7.3	8	2.7	58	5.1
S. infantis	27	6.7	15	3.4	12	4.0	54	4.8
S. oranienburg	20	5.0	19	4.3	3	1.0	42	3.7
S. hadar	13	3.2	23	5.2	5	1.7	41	3.6
S. typhi	13	3.2	10	2.2	2	0.7	25	2.1
S. panamá	6	1.5	14	3.2	3	1.0	23	2.0
S. I 6,7: -: 1,5	12	3.0	3	0.7	2	0.7	17	1.5
S. I 4,12: r: -	5	1.2	12	2.7	-	0	17	1.5
S. choleraesuis	9	2.2	5	1.1	1	0.3	15	1.3
S. anatum	1	0.2	6	1.4	5	1.7	12	1.1
S. I 4,12: i: -	4	1.0	-	0	7	2.4	11	1.0
S. brandenburg	5	1.2	2	0.5	4	2.0	11	1.0
S. give	4	1.0	5	1.1	1	0.3	10	0.9
S. derby	2	0.5	5	1.1	2	0.7	9	0.8
S. heidelberg	5	1.2	1	0.2	3	1.0	9	0.8
S. saintpaul	2	0.5	5	1.1	2	0.7	9	0.8
S. mbandaka	1	0.2	5	1.1	3	1.0	9	0.8
S. newport	3	0.7	3	0.7	2	0.7	8	0.7
S. bredeney	4	1.0	3	0.7	1	0.3	8	0.7
S. javiana	1	0.2	4	1.0	3	1.0	8	0.7
S. schwarzengrund	-	0	4	1.0	4	2.0	8	0.7
S. rissen	2	0.5	3	0.7	-	0	5	0.4
S. cerro	1	0.2	1	0.2	3	1.0	5	0.4
S. adelaide	3	0.7	2	0.5	-	0	5	0.4
S. ohio	2	0.5	3	0.7	-	0	5	0.4
S. emek	-	0	3	0.7	1	0.3	4	0.3
S. sandiego	-	0	1	0.2	3	1.0	4	0.3
S. inganda	-	0	3	0.7	1	0.3	4	0.3
S. albany	-	0	-	0	4	2.0	4	0.3
S. muenchen	2	0.5	1	0.2	-	0	3	0.2
S. berta	3	0.7	-	0	-	0	3	0.2
S. kingston	2	0.5	-	0	-	0	2	0.2
S. butantan	2	0.5	-	0	-	0	2	0.2
S. poona	1	0.2	1	0.2	-	0	2	0.2
S. I 6,8: e, h:	1	0.2	1	0.2	-	0	2	0.2
S. miami	2	0.5	-	0	-	0	2	0.2
S. I 6,8: z_{10}: -	2	0.5	-	0	-	0	2	0.2
S. braenderup	-	0	1	0.2	1	0.3	2	0.2
S. london	2	0.5	-	0	-	0	2	0.2
S. senftenberg	2	0.5	-	0	-	0	2	0.2
S. meleagridis	-	0	2	0.5	-	0	2	0.2
S. muenster	-	0	-	0	1	0.3	1	0.1
S. saphra	1	0.2	-	0	-	0	1	0.1
S. glostrup	-	0	1	0.2	-	0	1	0.1
S. abaetetuba	-	0	1	0.2	-	0	1	0.1
S. morehead	-	0	-	0	1	0.3	1	0.1
S. blockley	-	0	1	0.2	-	0	1	0.1
S. paratyphi B	-	0	-	0	1	0.3	1	0.1
S. montevideo	-	0	-	0	1	0.3	1	0.1
S. I 6,7: r: -	-	0	-	0	1	0.3	1	0.1
Total	**401**	**100.0**	**440**	**100.0**	**297**	**100.0**	**1,138**	**100.0**

Table 3. Chi square (χ^2) values for prevalent Salmonella serotypes isolated over three 5-year intervals (1985 to 1989,1990 to 1994, 1995 to 1999)

	Years				
Serotype	**1985-1989**	**1990-1994**	**1995-1999**	**Total**	**Values of χ^2**
S. I 4,5,12: i: -	69	100	72	241	6.09*
S. agona	108	60	12	180	69.72*
S. enteritidis	6	26	96	128	182.91*
S. typhimurium	35	53	26	114	3.27 ns

*p<0.05 by X^2 test for differences among the three five-year intervals; ns: not significant.

Table 4. Percent antimicrobial resistance values of prevalent *Salmonella* serotypes, isolated between 1985 and 1999

	Serotype					
Antibiotic	***S.*I 4,5,12:i:-**	***S. agona***	***S. enteritidis***	***S. typhimurium***	***S. dublin***	***S. infantis***
Ampicillin	8.7	41.2	0.8	13.2	5.2	13.0
Tetracycline	34.9	45.5	43.8	33.4	6.9	44.5
TMP-SMX#	7.0	39.5	8.6	15.0	3.5	11.2
Chloramphenicol	5.0	17.3	0.8	7.9	3.5	7.4
Cephalothin	2.5	13.9	2.4	7.0	3.5	7.4
Cefoxitin	0.4	5.6	1.3	0.9	3.5	11.2
Gentamicin	2.9	30.0	4.7	7.0	3.5	16.7
Amikacin	5.5	15.0	0.8	6.2	1.7	3.7
Tobramycin	0.8	31.2	2.4	7.9	1.7	18.5
Nalidixic acid*	0.8	0.6	0.8	0.9	0	0

TMP-SMX: trimethoprim-sulfamethoxazole. *Evaluated between 1991-1999.

by the fact that a large part of the salmonella infections recorded was acquired in the community rather than in the hospital. Ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol were less effective in the susceptibility test than nalidixic acid.

In conclusion, we found an increasing rate of *S. enteritidis* from 1985 to 1999 in northeastern São Paulo state. The resistance of *Salmonella* to antimicrobial agents, with the exception of tetracycline, was found to be low for drugs used in the treatment of infectious diarrhoea, apart from Agona, where strains were found to be more resistant to these drugs in comparison to strains of other *Salmonella* serotypes. The prevalence of S*almonella* serotypes isolated in our region over the last 15 years is epidemiologically important, since it discloses the dimension of the social and economic problem represented by this infection in our region, and demonstrates that salmonellosis is a public health problem in Ribeirão Preto, in the state of São Paulo, Brazil. This health problem is not exclusive to our region; salmonellosis has also been a public health problem in the Presidente Prudente region (in the State of São Paulo, Brazil) and in many other countries around the globe, such as the United States, France, Italy and Norway [30-35].

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