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CHANGING PATTERNS OF *SALMONELLA* SEROVARS: INCREASE OF *Salmonella* ENTERITIDIS IN SÃO PAULO, BRAZIL

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SUMMARY

Serovars of a total of 5,490 *Salmonella* strains isolated during the period of 1991-95, from human infections (2,254 strains) and from non-human materials (3,236 strains) were evaluated. In the studied period, 81 different serovars were determined among human isolates. *Salmonella* Enteritidis corresponded to 1.2% in 1991, 2% in 1992, 10.1% in 1993, 43.3% in 1994, and 64.9% in 1995 of all isolates. A significant rise on the isolation of this serovar was seen since 1993 linked to food poisoning outbreaks. It is reported also an increase on the isolation of *S.* Enteritidis from blood cultures, associated mainly with patients with immunodeficiency syndrome. *S.* Enteritidis was prevalent among one hundred and thirty different serovars isolated from non-human sources. Increasing number of isolation of this serovar was seen from shell eggs, breeding flocks and from environmental samples. It is also reported a contamination of commercial feed stuffs by *S.* Enteritidis which represents a major concern for Brazilian poultry industry.

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

INTRODUCTION

The problem of salmonellosis in São Paulo State, evaluated since the late 1940's from laboratory data, increased throughout decades representing a great public health concern[26].

A general picture of the distribution of different serovars and the introduction, predominance and changing patterns of *Salmonella* serovars of human and non-human origins, over the past 40 years (1950-90) in São Paulo State, Brazil, had been recently reported[26].

In the 1990's, is still only from laboratory records that the problem of *Salmonella* Enteritidis, witnessed by many countries in the 80's[5,10,11,12,19,21,24,28] could be evaluated, which stresses the important role of a Public Health Laboratory Service as a source of information, through which one can estimate the problem of salmonellosis in a geographic area[26].

The aim of this work is to report the changing patterns of *Salmonella* serovars during the period 1991-95 caused by the world trend increase of *S.* Enteritidis, and to evaluate the antimicrobial susceptibility patterns of *S.* Enteritidis.

MATERIAL AND METHODS

Bacterial strains: A total of 5,490 *Salmonella* strains isolated during the period of 1991-95, from human infections and from non-human materials were analysed. The strains have been isolated at the Central and Regional Public Health Laboratories, Hospital Laboratories, Laboratories of Animal Pathology, and at the Department of Microbiology and Food Microbiology of

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São Paulo University. All strains were serotyped according to POPOFF & LE MINOR[20] at the Laboratory of Enteric Pathogens, Instituto Adolfo Lutz, São Paulo, Brazil.

Antimicrobial susceptibility test - For the purpose of a retrospective study, a randomly selected 131 *S.* Enteritidis strains (92 from human sources and 39 from non-human sources) isolated during the period 1975-95 were submited to antimicrobial susceptibility tests by disk method[1]. The tested antimicrobial agents were: kanamycin (30μg), chloramphenicol (30μg), sulfametrim (25μg), nalidixic acid (30μg), sulfonamide (300μg), gentamicin (10μg), tetracycline (30μg), streptomycin (10μg), trimethoprim (5μg), ampicillin, cefalotin (30μg), ceftazidine (30μg), cefoperazone (75μg), carbenicillin (100μg).

RESULTS AND DISCUSSION

In spite of *S.* Enteritidis had been isolated in São Paulo, since 1950's[25], it represented for four decades less than 1% of the isolates[6,17,26]. A significant rise on its isolation is only seen since 1993. As presented in Table 1, among 81 different serovars identified among isolates of human sources, *S.* Enteritidis, is the only one that showed a rising trend over the studied period. Its progressive increase is clearly seen, corresponding to 1.2% in 1991, 2% in 1992, 10.1% in 1993, 43.3% in 1994, and 64.9% in 1995 of all isolates.

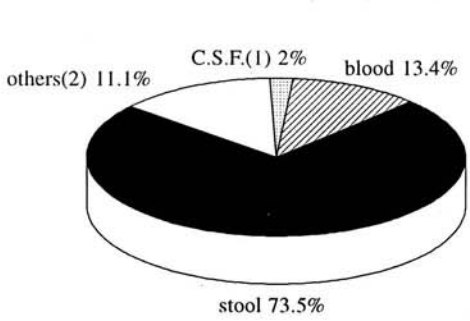
Among 668 strains of *S.* Enteritidis of human sources, isolated during the period 1991-95, almost 50% of them were linked to outbreaks, affecting adults in great majority. Taking into account that in our country there is no systematic reporting of food poisoning outbreaks[16], it is very likely that among sporadic cases, adults and patients with unknown ages with intestinal infections were associated with familiar outbreaks.

Although intestinal isolates represented by far the majority of strains, blood culture strains accounted for 13.4% of all isolates (Figure 1A). While the most prevalent non-typhoid serovars isolated from blood (S.I 4,5,12:i: -, *S.* Typhimurium, *S.* Dublin) presented either a declining trend or remained steady during the period 1991-95, *S.* Enteritidis showed an important increase in bacteremia cases. The increase of *S.* Enteritidis isolation from blood cultures, particulary in 1995, when this serovar corresponded to 35.3% of all serovars, is seen in Figure 2. Among blood isolates of *S.* Enteritidis, more than 50% of them were from adults, particularly due to the cases of salmonellosis in patients with acquired immunodeficiency syndrome.

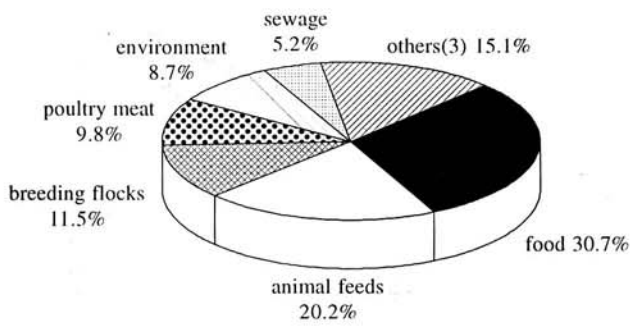
Taking into account that *Salmonella* bacteremia is a common manifestation of human immunodeficiency virus infection[7,9,11,15,23] which risk is affected by geographic prevalence and invasiveness of its serovars[11], it is very likely that patients with septicemia by *S.* Enteritidis may increase in the coming years, representing a special concern for public health, and a challenge to the treatment, particularly with multiresistant strains.

On the other hand, meningitis by *Salmonella*, especially among infants less than 1 year old, a serious complication of infection, with high mortality rate, is a major public health concern. Cases of meningitis caused by *S.* Enteritidis were seen mainly among children (Figure 3); although still by far comparable with the 1970's with *S.* Typhimurium[18], it should already be taken as an alert to introduce special care by all institutions that house these susceptible hosts.

As shown in Table 2, *S.* Enteritidis was also the prevalent serovar among non-human isolates; in the period 1991-95 it corresponded to 16.9% of all serovars followed by *S.* Havana (4.7%), *S.* Typhimurium (4.7%),



1A – human sources (2254 isolates)



1B – non-human sources (3236 isolates)

Fig. 1 – Sources of isolation of *Salmonella* during the period 1991-95. (1) – cerebrospinal fluid; (2) – urine, secretions, unknown; (3) – water, animals, shell eggs, unknown.

TABLE 1

Salmonella serovars isolated during the period of 1991-95 from human sources

	Period							Period					
Serovars	**1991**	**1992**	**1993**	**1994**	**1995**	**Total**	**Serovars**	**1991**	**1992**	**1993**	**1994**	**1995**	**Total**
S. Enteritidis	6	6	33	227	396	668	*S.* Paratyphi B	1	-	-	-	1	2
S. I 4, [5], 12:i:-	92	55	68	37	28	280	*S.* Bovismorbificans	1	-	-	-	1	2
S. Typhimurium	54	40	36	41	29	200	*S.* Senftenberg	-	1	-	-	1	2
S. Agona	78	38	28	19	22	185	*S.* Grumpensis	-	1	-	1	-	2
S. Infantis	84	11	9	23	17	144	*S.* Paratyphi A	-	1	1	-	-	2
S. Hadar	32	17	38	10	5	102	*S.* I 9,12:-:1,5	-	1	1	-	-	2
S. Dublin	30	15	23	7	11	86	*S.* Lille	-	-	-	1	1	2
S. Typhi	10	12	10	8	17	57	*S.* Thompson	1	-	-	-	-	1
S. Schwarzengrund	-	2	2	52	1	57	*S.* I 4,12:b:-	1	-	-	-	-	1
S. Saintpaul	5	31	4	4	3	47	*S.* I. 4,12:z10:-	1	-	-	-	-	1
S. Panama	13	11	7	7	7	45	*S.* Choleraesuis	1	-	-	-	-	1
S. Berta	19	5	3	1	-	28	*S.* Othmarschen	-	1	-	-	-	1
S. Mbandaka	4	6	7	4	3	24	*S.* Meleagridis	-	1	-	-	-	1
S. Newport	5	4	5	7	2	23	*S.* Abaetetuba	-	1	-	-	-	1
S. Anatum	6	2	5	5	2	20	*S.* IV 38:z4,z23:-	-	1	-	-	-	1
S. Brandenburg	4	1	1	6	8	20	*S.* Cerro	-	-	1	-	-	1
S. Oranienburg	4	5	4	1	6	20	*S.* Rubislaw	-	-	1	-	-	1
S. Ohio	2	2	4	7	4	19	*S.* Minnesota	-	-	1	-	-	1
S. Javiana	-	-	4	11	1	16	*S.* London	-	-	1	-	-	1
S. Bredeney	7	1	2	1	2	13	*S.* Coeln	-	-	1	-	-	1
S. Poona	-	6	6	1	-	13	*S.* Warragull	-	-	1	-	-	1
S. Muenchen	4	2	-	4	3	13	*S.* III 35:1,v:-	-	-	1	-	-	1
S. Give	4	6	1	-	1	12	*S.* I 4,12:d:-	-	-	1	-	-	1
S. Derby	1	6	2	1	-	10	*S.* I 6,7:-:1,5	-	-	1	-	-	1
S. Wien	-	-	-	9	-	9	*S.*Adelaide	-	-	-	1	-	1
S. I 6,7:r:-	-	2	-	-	7	9	*S.* I 4,12:-:-	-	-	-	1	-	1
S. I 4,5], 12:r:-	2	1	1	-	4	8	*S.* Jericho	-	-	-	1	-	1
S. Inganda	-	1	3	3	-	7	*S.* Carrau	-	-	-	1	-	1
S. Heidelberg	-	3	-	-	4	7	*S.* Alachua	-	-	-	1	-	1
S. Braenderup	1	-	-	1	4	6	*S.* Oslo	-	-	-	1	-	1
S. Sandiego	-	1	4	-	1	6	*S.* Kottbus	-	-	-	1	-	1
S. Albany	1	-	-	1	3	5	*S.* Worthington	-	-	-	1	-	1
S. Emek	3	-	-	1	-	4	*S.* I 16:z4,z23:-	-	-	-	1	-	1
S. Reading	1	-	-	3	-	4	*S.* I 6,7:r:-	-	-	-	1	-	1
S. Blockley	1	-	2	-	1	4	*S.* Haardt	-	-	-	-	1	1
S. Rissen	2	1	-	1	-	4	*S.* Stanleyville	-	-	-	-	1	1
S. I. 6,8:e,h:-	1	-	-	2	-	3	*S.* I 6,7:1,v:-	-	-	-	-	1	1
S. Glostrup	1	-	-	1	1	3	*S.* I 4,12:z:-	-	-	-	-	1	1
S. Belem	-	1	2	-	-	3	*S.* I 1,3,19:-:-	-	-	-	-	1	1
S. I 9,12:-:-	-	-	1	2	-	3	*S.* 4,5,12:y:-	-	-	-	-	1	1
S. Miami	-	2	-	1	-	3	*Salmonella* "R"	5	1	2	2	6	16
S. Banana	-	-	-	-	1	1	**Total**	**488**	**305**	**327**	**524**	**610**	**2,254**

TABLE 2

Salmonella serovars isolated from non-human sources during the period 1990-95

	Period							Period					
Serovars	**1991**	**1992**	**1993**	**1994**	**1995**	**Total**	**Serovars**	**1991**	**1992**	**1993**	**1994**	**1995**	**Total**
S. Enteriditis	-	-	16	116	414	546	*S.* Orion	-	-	7	2	2	11
S. Havana	1	3	136	7	7	154	*S.* Miami	1	4	-	-	6	11
S. Typhimurium	31	23	53	23	21	151	*S.*I 3,19: – : -	1	-	7	-	2	10
S. Infantis	9	35	9	26	41	120	*S.* Indiana	-	-	-	1	9	10
S. Hadar	8	34	23	14	37	116	*S.* Grumpensis	-	-	7	1	1	9
S. Agona	20	17	33	23	12	115	*S.* Javiana	-	5	2	1	1	9
S. Mbandaka	3	18	48	10	31	110	*S.* Rubislaw	-	5	3	1	-	9
S. Senftenberg	12	9	55	19	15	110	*S.*I 6,8: e, h: -	-	-	2	2	4	8
S. Anatum	18	31	27	11	15	102	*S.* London	2	-	2	4	-	8
S. Heidelberg	33	2	6	30	27	98	*S.* Irumu	7	-	-	-	-	7
S. Schwarzengrund	3	4	44	2	29	82	*S.* Blockley	-	-	2	5	-	7
S. Panama	2	40	17	11	11	81	*S.* Alachua	-	-	6	-	1	7
S. Montevideo	4	7	40	17	8	76	*S.* I 3,15: y: -	-	-	1	5	1	7
S. Newport	1	2	-	31	34	68	*S.* Sandiego	-	-	1	3	2	6
*S.*I 4, [5], 12: i: -	3	4	56	3	2	68	*S.*I 9,12: – : 1,5	-	-	5	1	-	6
S. Rissen	17	-	31	7	9	64	*S.* Paratyphi B	-	2	-	-	4	6
*S.*I 4, [5], 12: r: -	2	2	4	9	45	62	*S.* Abaetetuba	-	1	-	1	4	6
S. Cerro	3	5	40	6	7	61	*S.* Butantan	-	6	-	-	-	6
S. Give	9	10	8	15	9	51	*S.* I 8,20: z4, z23-	-	6	-	-	-	6
S. Ohio	2	8	34	1	4	49	*S.* Madelia	-	2	-	3	1	6
S. Oranienburg	5	35	2	3	4	49	*S.* Muenster	5	-	-	-	1	6
S. Brandenburg	28	10	4	2	5	49	*S.* Belem	5	-	-	-	-	5
S. Derby	1	30	-	8	4	43	*S.* Livingstone	-	1	3	-	1	5
S. Tennessee	-	8	25	2	6	41	*S.* Kiambu	-	5	-	-	-	5
S. Gallinarum	13	14	4	1	3	35	*S.* Kentucky	-	5	-	-	-	5
S. Bredeney	5	5	7	8	7	32	*S.* Reading	3	-	-	-	2	5
S. Worthington	2	1	2	14	12	31	*S.* I 3,10: – : 1,7	-	-	-	2	3	5
S. Saintpaul	4	2	2	2	19	29	*S.* I 6,7: z 10: -	-	-	4	-	1	5
S. Minnesota	-	3	13	1	9	26	*S.* Hull	-	4	-	-	-	4
S. Emek	1	8	1	10	3	23	*S.* I 3,19: i: -	-	4	-	-	-	4
*S.*I 6,7: r: -	2	6	2	6	7	23	*S.* Ealing	-	-	-	-	4	4
S. Pullorum	-	2	3	-	16	21	*S.* I 4,12: – : 1,7	-	-	-	2	2	4
S. Pomona	-	-	20	1	-	21	*S.* I 4,12: z : -	-	-	-	-	4	4
S. Adelaide	1	1	13	-	1	16	*S.* Braenderup	-	1	-	1	1	3
S. Albany	-	-	-	-	14	14	*S.* Poona	-	3	-	-	-	3
S. Dublin	1	1	3	3	5	13	*S.* IV 51: b: –	-	-	3	-	-	3
S. Glostrup	-	2	4	-	7	13	*S.* Lille	-	3	-	-	-	3
S. IV 40: z4, z23: -	11	-	1	-	-	12	*S.* Durban	3	-	-	-	-	3
S. Wettvreden	-	-	12	-	-	12	*S.* I 13,23: z: -	-	-	-	-	3	3
S. Meleagridis	-	10	2	-	-	12	*S.* Gloucester	-	-	-	-	3	3
S. Vinohrady	12	-	-	-	-	12	*S.* I 4,5,12: e,h: -	-	-	1	-	2	3
S. Berta	7	2	-	2	-	11	*S.* I 9,12: – : –	-	-	-	1	1	2
S. Thompson	-	-	-	11	-	11	*S.* Muenchen	1	-	1	-	-	2

TABLE 2 (cont.)

	Period							Period					
Serovars	**1991**	**1992**	**1993**	**1994**	**1995**	**Total**	**Serovars**	**1991**	**1992**	**1993**	**1994**	**1995**	**Total**
S. Morehead	-	1	1	-	-	2	*S.* Coeln	-	-	-	1	-	1
S. Gaminara	-	-	2	-	-	2	*S.* I 6,8: z10: -	-	-	-	1	-	1
S. IV 50: g, z51: -	1	-	1	-	-	2	*S.* Indiana	-	-	-	1	-	1
S. IV 11: z4, z23: -	1	-	-	-	1	2	*S.* Inganda	-	-	-	1	-	1
S. I 4,12: d: -	1	-	-	-	1	2	*S.* I 6,7: – : -	-	-	-	1	-	1
S. Takoradi	1	-	-	-	-	1	*S.* III 50: r: z	-	-	-	1	-	1
S. Stanley	1	-	-	-	-	1	*S.* I 6,7: e,h: -	-	-	-	1	-	1
S. Kande	1	-	-	-	-	1	*S.* Chester	-	-	-	-	1	1
S. I 6,7, 14: – : -	1	-	-	-	-	1	*S.* Yoruba	-	-	-	-	1	1
S. I 1,3,15,19: y: –	1	-	-	-	-	1	*S.* I 4,12: z 10: -	-	-	-	-	1	1
S. Kisangi	-	1	-	-	-	1	*S.* I 4,12: – : 1,2	-	-	-	-	1	1
S. Urbana	-	1	-	-	-	1	*S.* I 40: z4, z24: -	-	-	-	-	1	1
S. Typhi	-	1	-	-	-	1	*S.* I 54: – : -	-	-	-	-	1	1
S. I 3,15,34: y: -	-	1	-	-	-	1	*S.* Cubana	-	-	-	-	1	1
S. I 6,7: 1, v: -	-	1	-	-	-	1	*S.* I 28: – : -	-	-	-	-	1	1
S. I 1,3,15,19: – : -	-	-	1	-	-	1	*S.*I 6,7: e,h: -	-	-	-	-	1	1
S. Isangi	-	-	1	-	-	1	*S.*I 6,7: – : 1,5	-	-	-	-	1	1
S. Denver	-	-	1	-	-	1	*S.* Arechavaleta	-	-	-	-	1	1
S. IV 45: g, z51: -	-	-	1	-	-	1	*S.* I 6,7 : – : -	-	-	-	-	1	1
S. Johannesburg	-	-	1	-	-	1	*S.* I 4,12: g,s,t -	-	-	-	-	1	1
S. Saphra	-	-	1	-	-	1	*S.* I 21: b: -	-	-	-	-	1	1
S. Oslo	-	-	1	-	-	1	*Salmonella* "R"	2	5	47	31	27	112
S. Freetown	-	-	1	-	-	1	**Total**	**312**	**462**	**916**	**528**	**1,018**	**3,236**

S. Infantis (3.7%), *S.* Hadar (3.6%), *S.* Agona (3.5%), *S.* Mbandaka (3.4%), *S.* Senftenberg (3.4%), *S.* Anatum (3.1%), and *S.* Heidelberg (3.0%), the ten most frequent serovars among one hundred and thirty different serovars isolated from non-human sources.

Food was the main source of isolation of *Salmonella* among non-human origins followed by animal feeds, breeding flocks, environment, sewage and poultry meat (Figure 1B). While *S.* Enteritidis corresponded to 25% of all serovars isolated from shell eggs in 1994, in 1995 it represented 87% of all serovars.

The increase of *S.* Enteritidis in our State is related to the consumption of raw eggs as indicated by epidemiological investigations of outbreaks. The rise of *S.* Enteritidis isolation from eggs and food (mainly homemade mayonnaise, desserts as mousse and salad dressing), all of them containing raw eggs, emphasizes the important role of eggs as a vehicle of its transmission.

Commercial feed stuffs of animal origin and poultry feeds are known to be contaminated by *Salmonella* as reported by BERCHIERI et al.[2,3,4] and MIRANDA et al.[13], in surveys undertaken in São Paulo, Brazil. Despite the isolation of a number of many different *Salmonella* serovars, no *S.* Enteritidis had been isolated. However, the fact that, 15.3% of strains isolated in 1994 from animal feed corresponded to *S.* Enteritidis, and the high percentage, 32% in 1994, and 63.6% in 1995 of all isolates from internal organs of breeding flocks corresponded to *S.* Enteritidis, are great concerns to our poultry industry as chicken are major reservoir of this serovar acquiring it through contamined feed.

Contamination of poultry by *Salmonella* seems to be very high. Among all serovars recovered in 1995 from poultry meat, 50% of all them corresponded to *S.* Enteritidis. This fact arises a need of a systematic control of poultry flocks.

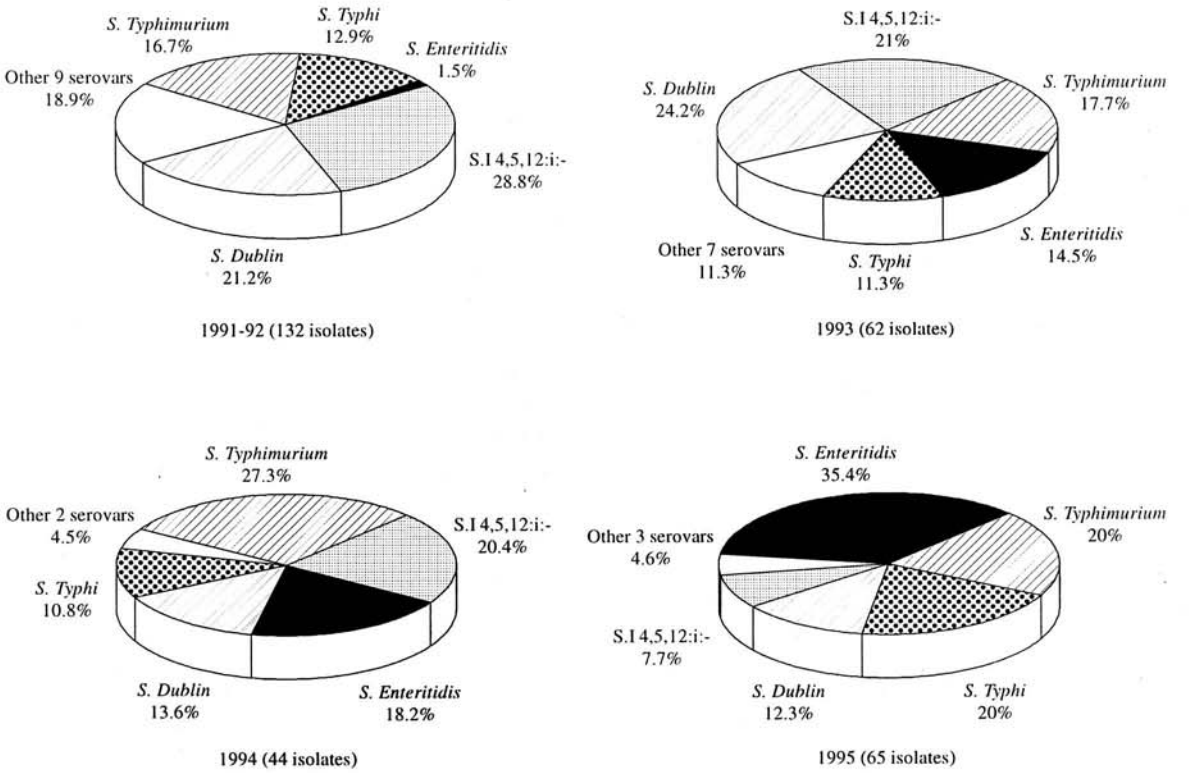


Fig. 2 – Increase in the isolation of *S.* Enteritidis from blood cultures.

Among human isolates, 13%, 6.5%, 1.1% and 4.3% were resistant, respectively to one, two, three and four or more (up to eleven) antimicrobials, while among non-human strains, 23.1% were resistant to one drug, 2.6% to two antimicrobials, and 2.6% to four drugs. Resistant strains could be detected among strains isolated since 1975.

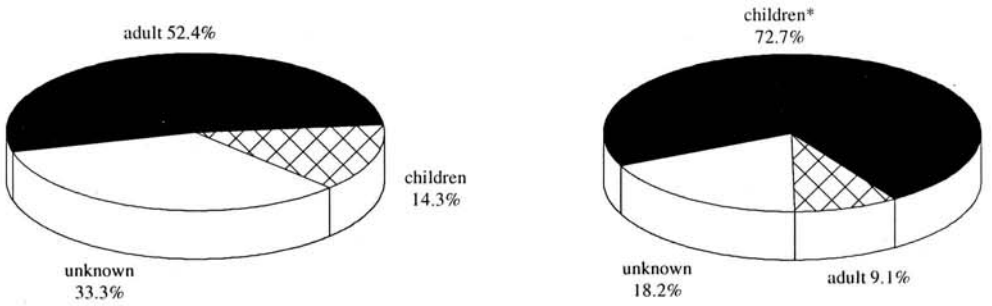
Although in the United States and in the United Kingdom[22,27] there was no substantial changing in antimicrobial resistance pattern in *S.* Enteritidis, acquisition of multiple resistance (up to 11 antimicrobial agents) is a great problem, taking into account the short period of time of its prevalence in this State.

In Europe and in the United States, *S.* Enteritidis dominated over long periods[10,12,21,24]. Although we cannot predict which pattern it will follow in our country, it is very likely that this serovar will be the prevalent type as occurred with *S.* Typhimurium.

While the prevalence, in the late 1970's, of *S.* Typhimurium in São Paulo was associated mainly with nosocomial origin[18,26], the increase of *S.* Enteritidis in the 1990's is connected with food borne outbreaks. However, taking into account that most of sporadic cases of gastroenteritis were related with children, *S.* Enteritidis can become a major concern in nosocomial infections, particulary in paediatric units.

In the late 70's, São Paulo State hospitals witnessed a dramatic problem of these infections caused by *S.* Typhimurium, especially among infants. Difficulties of control of these long lasting outbreaks could be deduced by the rapid dissemination of multiple resistance strains as shown by PESSOA et al.[18] and FERNANDES et al.[8].

The rise of *S.* Enteritidis, in 1993, of almost 13 fold over one year period, ranking first among human and non-human isolates in 1994 and 1995, probably, should be an evidence of its domination over long period. In this way, efforts should be concentrated to elaborate efficent strategies in order to control the expansion of this serovar, preventing substantially high social and economical loss.



blood isolates (42 strains)

C.S.F. isolates (11 strains)

* less than 5 years

Fig. 3 – *S.* Enteritidis from blood and C.S.F. cultures according to the age group of patients – 1991-95.

RESUMO

Mudança na prevalência dos sorotipos de *Salmonella*: aumento da *Salmonella* Enteritidis no Estado de São Paulo, Brasil

Foram avaliados os sorotipos de 5.490 cepas de *Salmonella* isolados no período, 1991-95, de infecções humanas (2.254 cepas) e de materiais de origem não humana (3.236 cepas) bem como o perfil de sensibilidade aos agentes antimicrobianos de 131 cepas de *S.* Enteritidis (92 de origem humana e 39 de origem não humana). No período estudado, foram determinados 81 diferentes sorotipos. *S.* Enteritidis correspondeu a 1,2% em 1991, 2% em 1992, 10,1% em 1993, 43,3% em 1994 e 64,9% em 1995. Um aumento significativo no isolamento de *S.* Enteritidis foi verificado em 1993 associado à ocorrência de surtos de enfermidades transmitidas por alimentos. É relatado também o aumento deste sorotipo a partir de hemoculturas, principalmente daquelas oriundas de pacientes com síndrome de imunodeficiência. *S.* Enteritidis foi também o sorotipo prevalente em materiais de origem não humana, particularmente em ovos, aves (matrizes) e em amostras do meio ambiente. Ressalta-se a importância da contaminação, das matérias primas, componentes de ração de aves, pela *S.* Enteritidis, o que representa um preocupante problema para a avicultura brasileira. Assinala-se a ocorrência de cepas multiresistentes aos agentes antimicrobianos em cepas de *S.* Enteritidis de origem humana e não humana.

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