

## ***Salmonella enterica* serotypes from human and nonhuman sources in Sao Paulo State, Brazil, 2004-2020**

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### ABSTRACT

Salmonellosis ranks among the most frequently reported zoonosis worldwide and is often associated with foodborne outbreaks. Since the 1950s, the distribution of *Salmonella* serotypes in Sao Paulo State, Brazil, has been documented and periodically reported. In this study, we updated the data on the distribution of *Salmonella* serotypes received in our reference laboratory, isolated from human infections and nonhuman sources, from 2004 to 2020. In that period, a total of 9,014 *Salmonella* isolates were analyzed, of which 3,553 (39.4%) were recovered from human samples, mainly of stool (65%) and blood (25.6%), and 5,461 (60.6%) were isolated from nonhuman origins, such as animals (47.2%), food (27.7%) and animal environments (18.6%). In human isolates, a total of 104 serotypes were identified and the most frequent ones were Enteritidis, Typhimurium, S. I. 4,[5],12:i:-, Dublin and Typhi. A consistent reduction of the Enteritidis proportion was observed over the years. Among the 156 serotypes identified in isolates with nonhuman origins, Enteritidis, Mbandaka, Typhimurium, Agona and Anatum were ranked as the top five *Salmonella* serotypes; in more recent years, S. Heidelberg has increased in frequency. Although with different proportions, the top 10 prevalent serotypes were identified in both human and nonhuman origins, underscoring the role of animals, food products and environment as reservoirs of *Salmonella* with potential to cause human salmonellosis.

**KEYWORDS:** Salmonellosis. *Salmonella*. Human. Nonhuman. Serotype. Serotyping.

### INTRODUCTION

*Salmonella enterica* is commonly acquired from contaminated food and non-typhoidal *Salmonella* (NTS) is an important worldwide cause of human foodborne infections, such as gastroenteritis and bacteremia<sup>1,2</sup>. Outbreaks of foodborne disease due to *Salmonella* are continually reported in many parts of the world<sup>3,4</sup>. Currently, more than 2,500 serotypes of *S. enterica* have been identified; however, most human infections are caused by a limited number of serotypes<sup>5</sup>.

Changes in the prevalence of specific strain types and serotypes in human and animal populations may follow the introduction of the strain through international travel, human migration, food, animal feed, and livestock trade, underscoring the role of One Health in this pathogen<sup>1</sup>. The *Salmonella* serotypes may differ in their natural reservoirs, their geographic and seasonal distributions, and their ability to cause human infection<sup>5</sup>. Numerous reservoirs and the capacity for environmental persistence give *Salmonella* multiple entry points into the human population. A wide variety of animals, particularly animals for human nutrition, have been identified as reservoirs for non-typhoidal *Salmonella* (NTS) serotypes<sup>1,6</sup>.

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Most of the information regarding the prevalence of *Salmonella* has been provided by laboratorial surveillance, especially serotyping data that allow broad comparisons and identify trends, reservoirs and routes of transmission of *Salmonella* serotypes<sup>5,7</sup>. In Sao Paulo State, Brazil, the identification of *Salmonella* serotypes has been conducted by the Laboratory of Enteric Pathogens in Adolfo Lutz Institute since 1950, and the obtained data have been showing the significant changes in the epidemiology of salmonellosis in our region over the past years<sup>7-9</sup>.

In this study, we evaluated the trends on distribution and prevalence of *Salmonella* serotypes isolated from clinical human and nonhuman sources in Sao Paulo State, Brazil, updating the data until 2020.

**MATERIALS AND METHODS**

**Bacterial isolates**

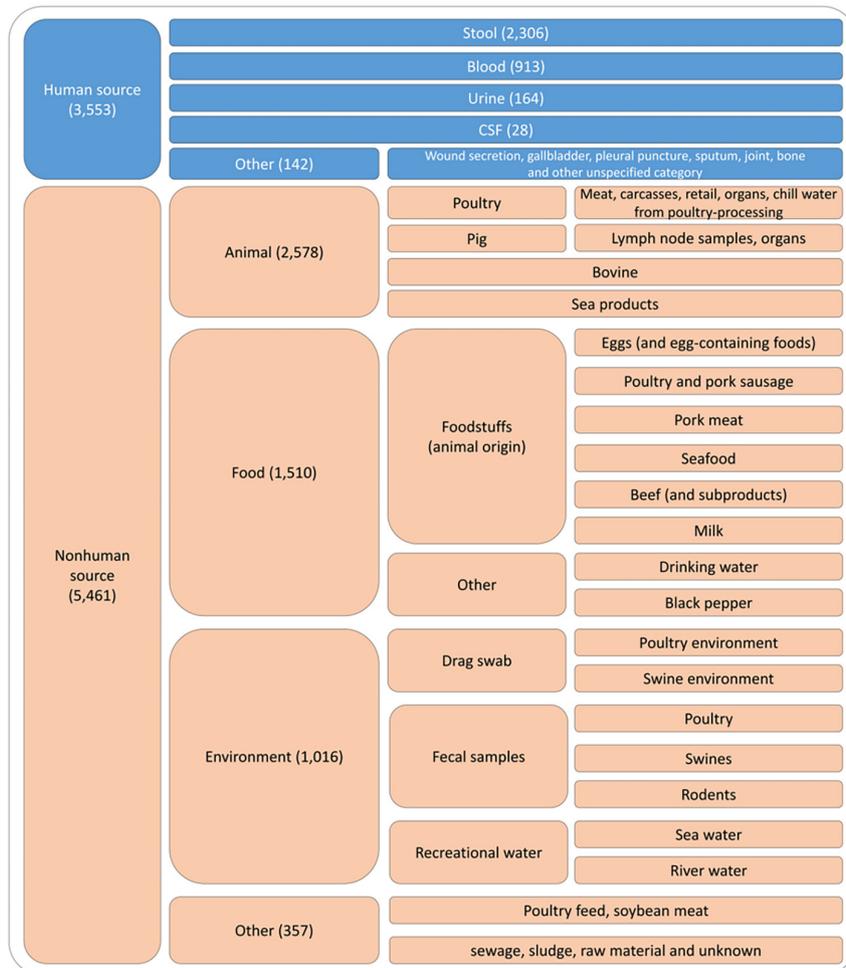
In this study, we analyzed 9,014 *Salmonella* isolates from human infections and nonhuman sources, received

from 2004 to 2020 for serotyping at Institute Adolfo Lutz, a reference laboratory for public health in Brazil. These isolates had been isolated and presumptively identified in private and public hospitals, local public health, animal pathology and food microbiology laboratories from different geographic locations of the Sao Paulo State.

The 3,553 clinical non-duplicate *Salmonella* isolates were mainly from: stool (2,306), blood (913), cerebrospinal fluid (CSF) (28), urine (164) and other body fluids or unspecified origins (142). The other 5,461 *Salmonella* isolates were representative of the nonhuman sources, isolated from animals (2,578), mostly from poultry, pigs and bovine; food (1,510), including food-producing animals and other foodstuffs; environment (1,016), mainly poultry and pig environments; and other unspecified sources (357). The distribution of the human and nonhuman sources of *Salmonella* isolates are detailed in [Figure 1](#).

**Biochemical identification and serotyping**

First, all isolates were confirmed to be of the genus



**Figure 1** - Distribution of human and nonhuman sources of *Salmonella* isolates, 2004-2020 (n=9,014).

*Salmonella* based on conventional biochemical tests<sup>10</sup> and the subspecies level determination was based on biochemical characteristics of addition<sup>11</sup>. The *Salmonella* serotyping was performed according to the 9<sup>th</sup> edition of the White-Kauffmann-Le Minor scheme<sup>11-13</sup>, on the basis of somatic O and H flagellar antigens by agglutination tests with antisera (prepared in the Laboratory of Enteric Pathogens, Adolfo Lutz Institute, Sao Paulo).

**Data analysis**

The results are presented in frequency and, for statistical analysis, three time periods were created: P1, comprising isolates from 2004-2008; P2, with isolates from 2009-2014; and P3, with isolates from 2015-2020. The chi-squared test was used to compare frequencies with the OpenEpi webserver, and a P value < 0.05 was considered significant.

**RESULTS**

***Salmonella* serotypes from human sources**

During the last 17-year laboratorial surveillance period, from 2004 to 2020, a total of 104 different serotypes were identified among 3,512 *Salmonella* isolates from human infection, another 41 “rough” strains were not serotyped (Supplementary Table S1).

The top ten identified serotypes: *S. Enteritidis*, *S. Typhimurium*, variant monophasic *S. Typhimurium* (*S. I. 4,[5],12:i:-*), *S. Dublin*, *S. Typhi*, *S. Newport*, *S. Saintpaul*,

*S. Infantis*, *S. Panama* and *S. Javiana* accounted for 2,464 (77.0%) of the strains.

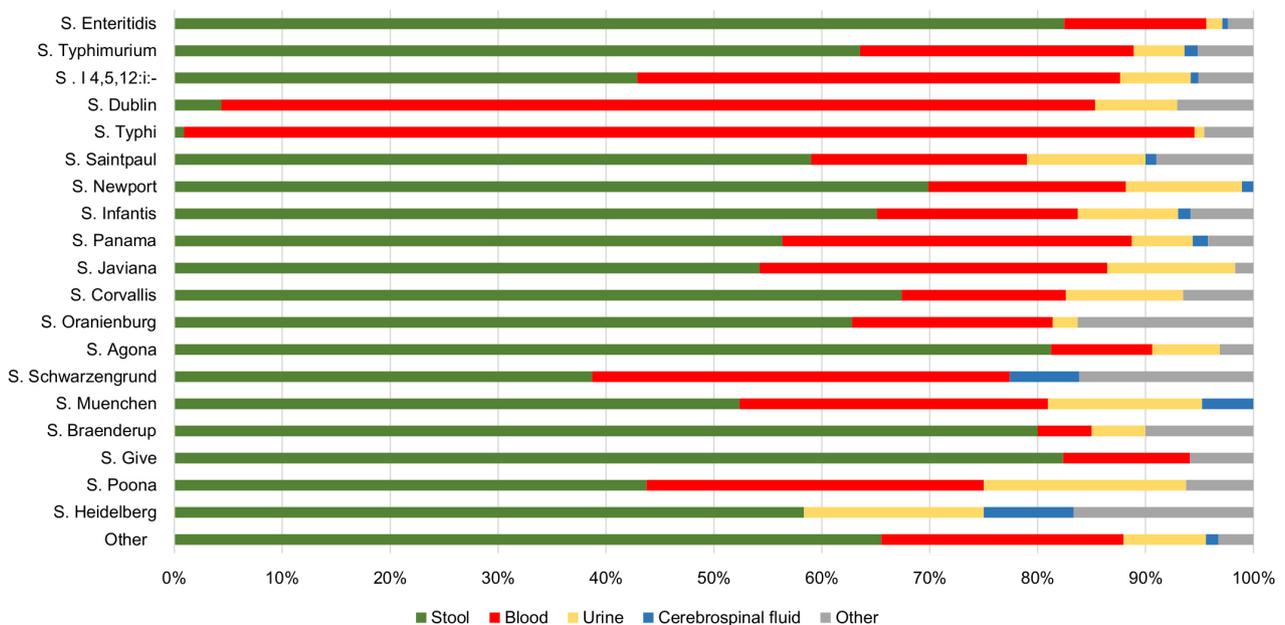
These serotypes — except *S. Typhi* and *S. Dublin*, which were mostly detected in blood samples (93% and 81%, respectively) — were the most frequent serotypes identified from stool, blood, urine and CFS samples. The distribution of serotypes associated with human source isolation is presented in Figure 2.

*S. Enteritidis* was the most frequently reported *Salmonella* serotype in human infections, accounting for 41.9% (1,489) of all isolates, followed by *S. Typhimurium* with 11.4% (406), and *S. I. 4,[5],12:i:-* with 7.7% (275). By comparing the frequencies of main serotypes over the analyzed periods, a strong and significant decline was observed for *S. Enteritidis* ( $p < 0.05$ ), while other serotypes presented a consistent growth in their frequencies (notably *S. Typhimurium*, *S. Dublin*, and *S. Schwarzengrund* – Figure 3, Supplementary Table S2).

***Salmonella* serotypes from nonhuman sources**

A total of 156 different serotypes were identified among the 5,461 *Salmonella* isolates from nonhuman origins, excluding 107 “rough” *Salmonella* strains (Supplementary Table S3).

The isolation source and annual distribution of the most prevalent serotypes are presented in Figures 4 and 5. Despite the high diversity of serotypes detected, the majority of isolates were limited to just a few serotypes. The top 10 most prevalent *Salmonella* serotypes (*Enteritidis*, *Mbandaka*, *Typhimurium*, *Agona*, *Anatum*, *Senftenberg*, *Heidelberg*,



**Figure 2** - Distribution of the most frequent *Salmonella* serotypes according to human sources.

*S. I. 4,[5],12:i:-*, Infantis and Saintpaul) accounted for 47.5% (2,592) of the total isolates recovered from food, animals, and the environment.

A strong reduction in the isolation ratio of *S. Enteritidis* was observed between the periods P1 and P2 ( $p < 0.05$ ), and remained consistently low in the most recent years (P3). The

Human serotypes (n)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total (%)
<i>S. Enteritidis</i> (1489)	58	68	74	59	53	40	39	35	40	34	20	9	17	18	12	10	18	42
<i>S. Typhimurium</i> (406)	10	8	8	8	11	14	13	14	10	12	13	23	12	11	13	16	7	11
<i>S. I 4,[5],12:i:-</i> (275)	5	5	1	7	6	6	7	14	11	13	12	8	12	8	6	8	11	8
<i>S. Dublin</i> (184)	2	0	2	2	5	5	7	3	4	10	16	10	5	9	11	6	7	5
<i>S. Typhi</i> (110)	2	2	2	5	3	1	1	1	1	1	1	3	0	14	9	8	8	3
<i>S. Saintpaul</i> (99)	3	1	0	2	3	5	2	3	2	2	5	3	6	7	3	3	2	3
<i>S. Newport</i> (93)	3	2	1	2	3	2	2	1	4	2	8	5	5	1	3	4	3	3
<i>S. Infantis</i> (86)	0	1	1	3	0	2	2	2	2	2	3	3	8	3	6	6	3	2
<i>S. Panama</i> (71)	1	1	1	0	3	1	2	3	2	2	0	3	4	2	3	6	8	2
<i>S. Javiana</i> (59)	1	1	1	1	1	1	1	0	1	1	3	3	3	1	3	6	6	2
<i>S. Corvallis</i> (46)	3	1	0	1	1	2	2	2	0	2	0	1	1	0	1	2	1	1
<i>S. Oranienburg</i> (43)	0	1	1	0	1	0	1	2	1	3	4	0	1	2	3	1	1	1
<i>S. Agona</i> (32)	0	2	0	1	2	0	0	2	1	0	1	1	2	0	2	2	0	1
<i>S. Schwarzengrund</i> (31)	0	0	0	0	0	1	2	1	1	0	0	2	1	2	4	3	1	1
<i>S. Muenchen</i> (21)	0	0	0	0	0	0	0	1	1	2	3	2	1	0	0	1	1	1
<i>S. Mbandaka</i> (14)	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0	1	1	0
<i>S. Heidelberg</i> (12)	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1	1	1	0
<i>S. Anatum</i> (10)	0	0	0	0	0	0	0	0	0	0	1	2	1	0	1	0	1	0
Other (472)	10	6	6	8	8	19	20	16	17	12	6	21	19	23	18	17	21	13
Total (3553) (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Figure 3 - Heatmap showing the annual frequency of the most prevalent human *Salmonella* serotypes.

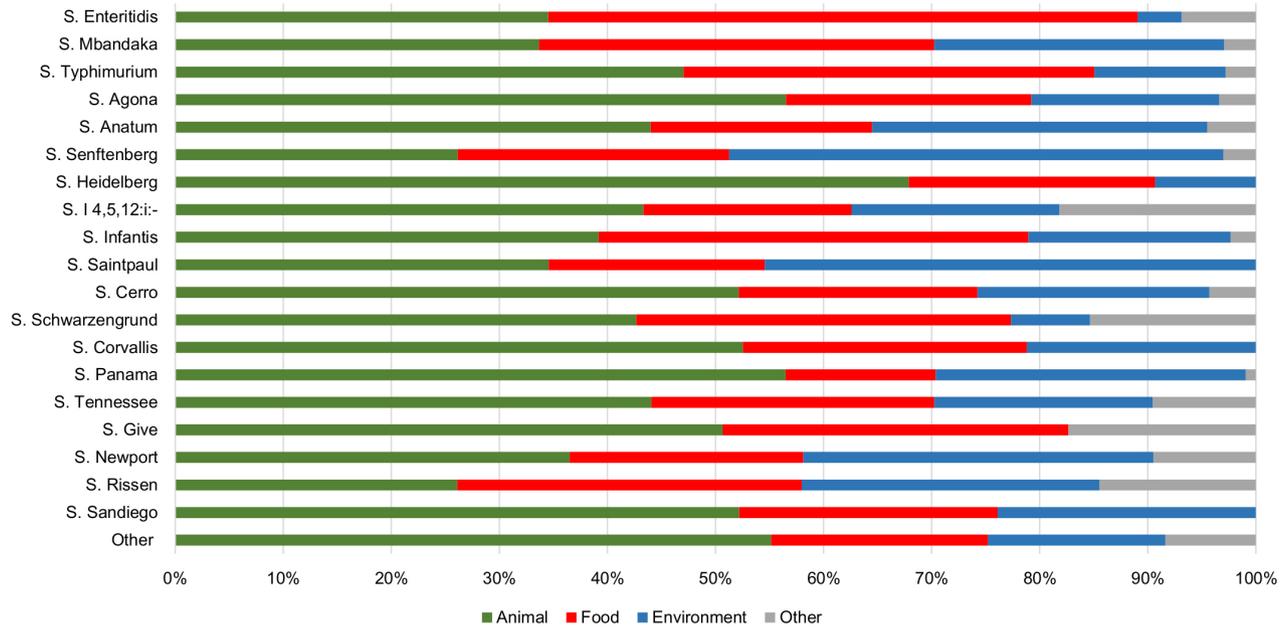


Figure 4 - Distribution of the most frequent *Salmonella* serotypes according to nonhuman sources.

Non-human serotypes (n)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total (%)
<i>S. Enteritidis</i> (539)	24	12	13	15	11	6	6	1	8	4	3	1	20	7	2	0	3	10
<i>S. Mbandaka</i> (410)	10	17	5	8	8	12	6	6	5	3	2	1	2	1	9	18	16	8
<i>S. Typhimurium</i> (321)	2	8	5	8	7	8	6	4	3	4	9	15	5	3	5	4	1	6
<i>S. Agona</i> (207)	6	2	0	1	1	1	4	12	4	4	0	15	1	0	1	2	0	4
<i>S. Anatum</i> (200)	5	3	1	3	4	2	6	5	4	4	10	5	1	0	0	2	0	4
<i>S. Senftenberg</i> (199)	3	3	3	6	8	9	4	2	3	3	1	3	1	1	2	0	1	4
<i>S. Heidelberg</i> (193)	1	1	1	0	2	1	1	0	1	0	8	3	5	24	30	5	5	4
<i>S. I 4,[5],12:i:-</i> (187)	1	2	1	2	4	5	1	3	4	4	4	6	17	5	1	1	12	3
<i>S. Infantis</i> (171)	2	4	2	4	3	4	3	5	4	3	0	6	4	2	1	1	1	3
<i>S. Saintpaul</i> (165)	7	3	1	1	1	1	2	13	1	3	0	0	0	4	1	7	1	3
<i>S. Cerro</i> (163)	2	1	3	2	2	2	4	4	4	5	5	0	4	0	5	3	6	3
<i>S. Schwarzengrund</i> (150)	3	2	1	1	1	2	11	1	2	8	0	3	1	0	4	0	5	3
<i>S. Corvallis</i> (118)	0	3	1	2	2	4	0	6	2	5	6	1	0	0	0	1	3	2
<i>S. Panama</i> (108)	1	0	0	1	1	1	0	0	0	8	15	3	0	1	1	2	3	2
<i>S. Tennessee</i> (84)	1	2	3	4	1	2	2	1	1	1	0	0	0	0	1	2	0	2
<i>S. Newport</i> (74)	1	1	1	1	2	3	1	0	2	3	1	1	0	1	0	1	5	1
<i>S. Gallinarum</i> (59)	0	2	5	0	0	0	0	0	3	2	0	1	0	8	0	2	1	1
<i>S. Muenchen</i> (39)	1	0	0	0	1	0	0	0	2	1	0	0	0	1	0	5	8	1
<i>S. Pullorum</i> (28)	0	1	4	0	0	0	0	1	0	0	0	1	0	2	0	0	0	1
Other (2046)	27	36	48	40	41	37	42	37	47	35	34	36	38	40	37	45	29	37
Total (5461) (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Figure 5 - Heatmap showing the annual frequency of the most prevalent nonhuman *Salmonella* serotypes.

same pattern was observed for *S. Mbandaka*. Conversely, the frequency of *S. Typhimurium* increased only in P3 compared to P2 and P1, as also observed for *S. Heidelberg*, the serotype with the highest increase in frequency among nonhuman sources (Figure 5, Supplementary Table S4).

**Comparison of *Salmonella* serotypes from different sources**

When we compared the results of serotype prevalence in human and nonhuman *Salmonella* isolates (Figure 6), we observed that *S. Enteritidis*, *S. Typhimurium*, *S. I. 4,[5],12:i:-*, *S. Saintpaul*, *S. Infantis*, *S. Newport* and *S. Panama* were frequently detected in both origins. On the contrary, *S. Mbandaka* was the second most commonly isolated from nonhuman origins (7.5%), but only rarely isolated from humans (0.4%).

The additional statistical data analysis showed that *S. Saintpaul*, *S. Panama*, and *S. Muenchen* were homogeneously found in human and nonhuman sources ( $p > 0.05$ ). While *S. Enteritidis*, *S. Typhimurium*, *S. I. 4,[5],12:i:-*, and *S. Newport* were more frequently found in human specimens ( $p < 0.05$ ), the other eight serotypes (*S. Mbandaka*, *S. Infantis*, *S. Agona*, *S. Anatum*, *S. Heidelberg*, *S. Schwarzengrund*, *S. Corvallis*, and *S. Give*) were mainly recovered from nonhuman sources ( $p < 0.05$ ).

**DISCUSSION**

This study integrates data of the distribution of *Salmonella enterica* serotypes identified among human

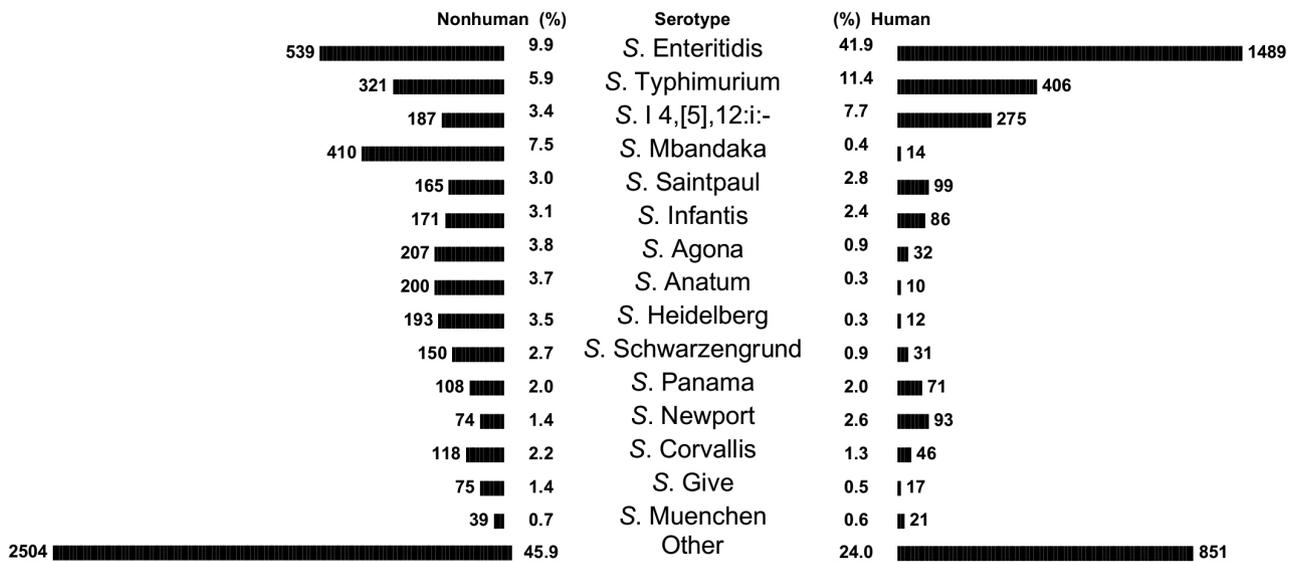
infections, animals, food products and the environment in Sao Paulo State, Brazil.

**Distribution of *Salmonella* serotypes from human infections**

Our findings indicate that no significant change was detected in the prevalence of the three main serovars isolated from humans (*S. Enteritidis*, *S. Typhimurium* and *S. I. 4,[5],12:i:-*) when compared to previous studies in the State of Sao Paulo, Brazil<sup>7,9</sup>. *S. Enteritidis* and *S. Typhimurium* are the two most important NTS serotypes transmitted from animal to human infections reported in most parts of the world<sup>3,6,14-17</sup>.

In Sao Paulo State, a remarkable increase of human *S. Enteritidis* frequency was observed in 1994, mainly associated with foodborne disease outbreaks and sporadic gastrointestinal disease<sup>7,18</sup>. *S. Enteritidis* remained the most commonly reported serotype worldwide, especially associated with foodborne outbreaks<sup>19</sup>. However, in this study, a major decrease in *S. Enteritidis* frequency was observed over the years, from 73.6% in 2006 to 19.9% in 2014. Similar patterns have been reported in the USA and Europe<sup>1,20</sup>. The decrease in the proportion of *S. Enteritidis* in humans may reflect the ongoing efforts made to control its spread through eggs by implementing quality-assurance programs.

*S. Typhimurium* and *S. I. 4,[5],12:i:-*, a variant of *S. Typhimurium*, were respectively the second and third most prevalent serotypes detected in clinical samples in this study. *S. Typhimurium* has a well-characterized ability to infect various species which can survive for a long time in



**Figure 6** - Frequency of the common serotypes identified in *Salmonella* isolates recovered from human and nonhuman sources in Sao Paulo, Brazil, 2004-2020 (n=9,014).

the environment, enhancing its ability to be one of the most common causes of salmonellosis<sup>15</sup>. *S. Typhimurium* are important foodborne pathogens in all parts of the world<sup>6,20</sup>.

*S. I. 4,[5],12:i:-* was first reported in the 1980s, with an increased prevalence since the late 1990s in humans and animals, especially in Europe and the United States<sup>21</sup>, and also in Brazil<sup>7,22</sup>. *S. I. 4,[5],12:i:-* has been responsible for an increasing number of foodborne outbreaks, attributed to pigs and pork products and frequently associated with the emergence of the multidrug-resistant phenotype<sup>21,23</sup>.

The majority of the NTS isolates evaluated in this study were recovered from gastrointestinal diseases (64.9%). However, a significant percentage (32.1%) of *Salmonella* infections was recovered from extra intestinal sources. The frequency of serotypes *S. Dublin*, *S. Typhimurium* and *S. I. 4,[5],12:i:-* were notably high in extra intestinal sources, which may require antimicrobial treatment. This fact is of public health concern, considering that over the years an increasing proportion of *Salmonella enterica* have acquired antimicrobial resistance — especially the extended-spectrum B-Lactamases (ESBL) that produces *Salmonella* serotypes from both clinical and nonhuman sources, as also reported in our region<sup>24-26</sup>, indicating a reduction in the available drug options for patient treatment.

The typhoidal *Salmonella* serotypes are known to cause different clinical manifestations in comparison to NTS infections. Here, we identified that *S. Typhi*, ranked as the fifth most prevalent serotype from human sources, is mainly isolated from septicemia infections. Although the proportion of isolates has decreased over the years (2004-2016), *S. Typhi* re-emerged in 2017, when cluster cases of typhoidal fever were reported in Sao Paulo city, Brazil, affecting 14 people, including infants<sup>26</sup>. *S. Typhi* remains endemic in developing countries typically associated with poor sanitation and a weaker healthcare system<sup>1,7,8</sup>. *S. Saintpaul* rose from tenth to sixth position; *S. Newport* and *S. Javiana* appeared among the ten most identified serotypes, while *S. Agona* and *S. Corvallis* are out of this rank when compared to those reported in the last period (1996-2003)<sup>7</sup>. A distinct difference between the isolates in this new period was the increase of the *S. Newport* and *S. Javiana* serotypes among the human isolates.

#### Distribution of *Salmonella* serotypes from nonhuman sources

Among the various 156 serotypes identified in nonhuman isolates of this study, *Salmonella* Enteritidis was the most frequent, but with a decline in its frequency in recent years. *S. Enteritidis* is the most frequent cause of

salmonellosis worldwide, which can be associated with eggs and contaminated poultry consumption, as identified in Europe and the USA<sup>14,17</sup>. However, recent studies have revealed that, in addition to eggs, foodstuffs including meat, chicken, vegetables, and dairy products played a significant role in causing SE disease outbreaks all over the USA between 1990 and 2015<sup>27</sup>.

*Salmonella* Mbandaka, the second most prevalent serotype in this study, was extensively detected in poultry meat, particularly poultry carcasses, as well as in isolates from animals environment. This finding is in agreement with previous reports that described the presence of this serotype in such sources, as well as in connection with foodborne outbreaks<sup>28,29</sup>. In our region, *S. Senftenberg* was the second most detected serotype strain in the 1990s, losing its position to *S. Mbandaka* which is currently the second most detected serotype. *Salmonella* Mbandaka has been identified by the CDC as an important outbreak-causing serotype of *Salmonella* and it has been reported as a cause of human salmonellosis in several countries, making this serotype globally important for human and animal health<sup>28,30</sup>. Studies have shown that this serotype has rarely been reported and is currently adapted for multi-host propagation<sup>30</sup>. According to outbreak investigation reports, *S. Mbandaka* can originate from both live animals and processed food. The increase of *S. Mbandaka* in this last period of 2004-2020, ranking second among animal, food and environmental strains, showed evidence that the increase of this serotype requires epidemiological monitoring. Thus, efforts should be focused on the elaboration of efficient strategies in order to control the expansion of this serovar, to substantially avoid economic loss. These results also provide a baseline for future comparisons.

The *Typhimurium* serotype – which is not only known for infecting poultry and pigs, but also acts as a potential agent for human gastroenteritis – was found to be the third most prevalent serovar during the study period. As in other studies, *S. Typhimurium* and *S. I. 4,[5],12:i:-* were also detected in both animal and food samples<sup>14</sup>, and ranked from fifth to third when comparing the present results with our previous studies on nonhuman strains.

*S. Heidelberg*, which was not frequently detected, is now among the top ten frequently identified serotypes, mainly associated with poultry samples. *Salmonella* Heidelberg ranks among the most prevalent causes of human salmonellosis in the United States<sup>16</sup> and Canada<sup>31</sup>. In the last few years, the *S. Heidelberg* serotype has also emerged in various countries such as Argentina<sup>32</sup>, Brazil<sup>33</sup> and Europe<sup>20</sup>. This serotype has been observed in other studies<sup>32,33</sup> and has also been involved in foodborne outbreaks<sup>34</sup>. The increasing isolation of *S. Heidelberg*, as well as its antimicrobial

resistance in our region<sup>35</sup>, highlights its emergence, spread and maintenance in the environment, and reinforces the need to improve measures for prevention and surveillance.

When comparing the present results with our previous studies<sup>7,9,18</sup> we see, over the years, especially more recently, an increase in the prevalence of *Salmonella* serotypes among nonhuman isolates in Sao Paulo State such as *S. Mbandaka*, *S. Anatum*, *S. Heidelberg* and *S. Saintpaul*, with the decline of *S. Senftenberg* and *S. Hadar*. These results also provide a baseline for future comparisons for *Salmonella* epidemiological surveillance.

## CONCLUSION

Regarding the results detected in both human infection and nonhuman sources, *S. Enteritidis* and *S. Typhimurium* remain the most frequent in Sao Paulo State, Brazil, which are the two most important NTS serotypes transmitted from animal to human in most parts of the world. The top five serotypes most commonly found in nonhuman sources – *S. Enteritidis*, *S. Typhimurium*, *S. I. 4,[5],12:i:-*, *S. Infantis* and *S. Saintpaul* – were also ranked as the most frequent, associated with more than 65% of human disease over the period studied. Nevertheless, other serotypes common in nonhuman sources, such as *S. Anatum*, *S. Agona*, *S. Schwarzengrund*, *S. Corvallis*, *S. Muenchen* and *S. Heidelberg*, were also detected in human infections, but in a comparative reduced frequency.

The data presented here support the established importance of animals, food products and the environment as sources of salmonellosis. The overall similarity between the types and frequencies of serotypes isolated in humans and nonhuman sources is consistent with other studies that hypothesize that animals might serve as reservoirs for salmonellosis. *Salmonella* serotyping remains an important laboratory tool that helps public health researchers to better understand and define the epidemiology of salmonellosis in a geographic area. The measurement of trends in serovars over time can provide information about emerging serotypes and about the efficacy of prevention and control measures.

## CONFLICT OF INTERESTS

The authors declare no conflict of interests.

## FUNDING

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