ABSTRACT

Salmonella spp. remain among the most important agents of foodborne diseases worldwide. The importance of Salmonella spp. in public health is linked to their wide range of antimicrobial resistance and to their pathogenicity and virulence in both human and animal hosts. The aim of this study was to determine the antimicrobial resistance patterns for Salmonella serotypes isolated from poultry sources in Brazil and to detect virulence-associated genes and verify their association with specific serotypes. A total of 163 strains of Salmonella enterica isolated from poultry sources in Southern Brazil were selected, and each belonged to one of 11 different serotypes. They were tested against ten antibiotics and examined for the presence of 26 virulence-associated genes by PCR. S. Typhimurium, S. Bredeney, S. Schwarzengrund and S. Tennessee showed the highest overall resistance rates. Approximately 18% of Salmonella strains were classified as multidrug-resistant strains. Our results indicate associations between antimicrobial resistance and specific serotypes. Most of the investigated genes presented a high frequency and a regular distribution, regardless of the serotype. Eight genes are positively or negatively associated with at least one serotype. The observed associations between antimicrobial resistance and specific serotypes are useful in developing specific control and treatment measures for each serotype. Despite the virulence genes being evenly distributed among the serotypes, some of these genes are associated with specific serotypes, and sefA, sopE and lpfA were selected as possible markers of Salmonella serotypes.

INTRODUCTION

Salmonella spp. remain one of the main pathogens responsible for foodborne disease worldwide, and salmonellosis outbreaks are commonly associated with the consumption of poultry and poultry-derived products (Centers for Disease Control, 2015; European Food Safety Authority 2017b; Brasil, 2018). In the US, Salmonella serotypes are responsible for approximately 34% of reported infections (Centers for Disease Control, 2015). In Europe, the authorities reported Salmonella as the second most important agent of foodborne diseases, with more than 94,530 salmonellosis cases (European Food Safety Authority, 2017b). In Brazil, Salmonella is responsible for more than 30% of foodborne disease, according to the Brazilian Ministry of Health (Brasil, 2018).

For human salmonellosis cases, S. Enteritidis, S. Typhimurium (including its monophasic variant), S. Infantis, S. Derby, S. Newport, S. Heidelberg, S. Schwarzengrund and S. Javiana are the main serotypes isolated in humans worldwide (Robinsom, 2013; Capalonga et al.,...
of the strain (Madigan et al., 2010). For many pathogens, virulence is conferred by a single region of the genome. However, Salmonella pathogenesis and its interaction with the host are a complex and multifactorial phenomenon that depends on several virulence factors (Wallis & Galyov, 2000; Skyberget et al., 2006). These factors are encoded by many virulence-associated genes that are distributed along its chromosome and/or in mobile genetic elements such as plasmids (Wallis & Galyov, 2000). Some virulence factors are related to the components of the bacterial structure such as fimbriae and play an important role in the virulence of the strains (Clouthier et al., 1993). Salmonella Pathogenicity Islands (SPI) are large genetic elements with pathogenic properties (Hacker & Carniel, 2001). SPI-1 encodes the components of a Type III Secretion System (TTSS), a complex protein secretion system, and other proteins required for the invasion of non-phagocytic cells and the activation of the inflammatory response (de Jong et al., 2012; Wisner et al., 2012). The islands are also involved in Salmonella recognition and multiplication within macrophages, in iron metabolism, and in endotoxin production (Álvarez, 2007).

In this context, the aim of this study was to determine the antimicrobial resistance patterns for different Salmonella serotypes isolated from poultry sources and to detect virulence-associated genes and verify their association with specific serotypes.

**MATERIALS AND METHODS**

**Bacterial strains**

For this study, 163 strains of S. enterica were isolated from poultry sources, and they belonged to 11 different serotypes in total. The following serotypes were included: S. Enteritidis (n=70), S. Heidelberg (n=49), S. Hadar (n=14), S. Typhimurium (n=8), S. Anatum (n=5), S. Bredeney (n=5), S. Agona (n=4), S. Tennessee (n=3), S. Infantis (n=2), S. Brandenburg (n=2) and S. Schwarzengrund (n=1). Strains were previously serotyped by the Oswaldo Cruz Institute Foundation (Fiocruz, Brazil). The bacterial isolates were kept frozen at -80 °C in brain heart infusion (BHI) broth (Oxoid®, United Kingdom) and were supplemented with 15% glycerin (Synth®, Brazil).

**Antimicrobial susceptibility test**

Antimicrobial susceptibility was determined by the disc diffusion method according to the Clinical and Laboratory Standards Institute (Clinical and...
Laboratorial Standards Institute, 2014a) instructions. An interpretation was performed using the criteria described in the approved standards VET01-S2 (Clinical and Laboratorial Standards Institute, 2014b) and M100-S26 (Clinical and Laboratorial Standards Institute, 2016). An *Escherichia coli* (ATCC 25922) strain was selected to ensure the validity of the test. The discs with the following antibiotics (Oxoid®, United Kingdom) were used: amoxicillin (AMX), 10 µg; ceftiofur (TIO), 30 µg; ciprofloxacin (CIP), 30 µg; chloramphenicol (CHL), 30 µg; enrofloxacin (ENR), 5 µg; gentamicin (CN), 10 µg; spectinomycin (SPT), 100 µg; sulfafurazole (SOX), 300 µg; sulfamethoxazole with trimethoprim (SXT), 1.25 µg/23.75 µg; and tetracycline (TCY), 30 µg. All strains classified as being intermediate resistant were considered non-susceptible. Strains that presented resistance to three or more classes of antimicrobials were considered multidrug resistant (MDR) (Schwarz et al., 2010). The multiple antibiotic resistance (MAR) index was calculated as previously described (Krumperman, 1983) using the following formula: \( a/b \), where \( a \) represents the number of antibiotics to which a particular isolate was resistant and \( b \) the total number of antibiotics tested.

**Detection of virulence-associated genes**

DNA extraction was carried out by heat treatment as described by Borges et al. (2017a). PCRs for the *invA* gene were carried out to confirm the presence of *Salmonella* DNA in the extracted samples. Individual or multiplex PCR protocols were conducted to detect the presence of 26 virulence-associated genes (*hilA, lpfA, lpfC, sefA, agfA, spvB, spvC, pefA, sopE, avrA, sivH, orgA, prgH, spaN, tolC, sipB, sitC, pagC, msgA, spiA, sopB, cdtB, iroN, sifA, sseL, and stn*) in *Salmonella* strains. Gene function, primer sequences, amplicon sizes, cycling conditions and reaction mixtures (25 µL) were previously described by Borges et al. (2017b). The cycling program was performed in the Esco Swift MaxPro thermal cycler (Esco, Singapore). The amplified products were separated by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide. Fragments were transilluminated with UV light. *Mannheimia haemolytica* ATCC 29694 and *Salmonella Enteritidis* ATCC 13076 were used as negative and positive controls, respectively, for all PCRs except that of the *cdtB* gene, for which a strain of *Salmonella Senftenberg* (from our laboratory stock collection) was used as a positive control. In all PCRs, a mixture of all constituents of the PCR except the extracted DNA were mixed and used as a PCR control.

**Statistical analysis**

Chi-square (\( \chi^2 \)) and Fisher’s tests were used to analyse the susceptibility of the strains to the different antimicrobials tested, to compare the resistances and to analyse the presence of virulence genes among *Salmonella* serotypes. Discriminant analysis was used to build decision tree and identify possible serotype marker genes.

**RESULTS**

**Antimicrobial susceptibility test**

The antimicrobial resistances of *Salmonella* strains regardless of the serotype are described in Figure 1. Among the 163 analysed strains, only 5 (3.1%) were susceptible to all tested antimicrobials. No antimicrobial agent was efficient in inhibiting the growth of 100% of tested strains. Amoxicillin, ceftiofur, chloramphenicol, gentamicin and sulfamethoxazole with trimethoprim inhibited the growing of more than 90% of the strains. Ciprofloxacin and sulfafurazole were the antimicrobial agents that presented the significantly (p<0.05) highest numbers of non-susceptible strains.

![Figure 1](image)

**Figure 1** – Antimicrobial susceptibility (%) of *Salmonella* strains to ten antimicrobial agents by disc diffusion tests, regardless of serotype.

There were important differences in antimicrobial resistance among *Salmonella* serotypes, as described in Table 1. *Typhimurium*, *S. Bredeney*, *S. Schwarzengrund* and *S. Tennessee* showed the highest overall resistance rates. However, this result can be influenced by the reduced number of samples of the three last serotypes. Statistical associations between each serotype and its resistance for specific antibiotics were determined considering only the serotypes *S. Enteritidis*, *S. Heidelberg*, *S. Hadar* and *S. Typhimurium*. Amoxicillin resistance was associated with *S. Heidelberg*, ciprofloxacin with *S. Enteritidis* and *S. Typhimurium*, spectinomycin with *S. Heidelberg*...
Antimicrobial Resistance and Molecular Characterization of Salmonella Enterica Serotypes Isolated from Poultry Sources in Brazil

Table 1 – Antimicrobial susceptibility and multiple antibiotic resistance (MAR) indices of Salmonella enterica serotypes isolated from poultry sources.

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Total of strains</th>
<th>Resistance (%)</th>
<th>Overall (%)</th>
<th>Average MAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TIO</td>
<td>CIP</td>
<td>SPT</td>
<td>ENR</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>70</td>
<td>6</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>S. Heidelberg</td>
<td>49</td>
<td>3</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>S. Hadar</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>S. Anatum</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S. Agona</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S. Tennessee</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S. Infantis</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. Brandenburg</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. Schwarzengrund</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Legend: amoxicillin (AMX), cepfirofur (TIO), ciprofloxacin (CIP), chloramphenicol (CHL), enrofloxacin (ENR), gentamicin (GEN), spectinomycin (SPT), sulfafurazole (SOX), sulfamethoxazole with trimethoprim (SXT) and tetracycline (TET).

and S. Typhimurium, sulfafurazole with S. Enteritidis, tetracycline with S. Hadar and S. Heidelberg, and chloramphenicol and sulfamethoxazole with trimethoprim with S. Typhimurium.

The maximum and minimum MAR indices of isolates were 0.1 and 0.6, respectively, and the average MAR was 0.2. The MAR distribution according to serotype is described in Table 1. Approximately 18% (30/163) of Salmonella strains were classified as MDR strains. The majority of MDR strains belonged to the serotypes S. Enteritidis (9/30), S. Typhimurium (7/30) and S. Bredeney (5/30).

Detection of virulence-associated genes

Most of the investigated genes presented a high frequency and a regular distribution regardless of the serotype. The frequencies for the twenty-six genes are described according to serotype in Table 2. Serotype S. Enteritidis presented the highest average (24) number of detected genes (of the 26 virulence-associated genes analysed), followed by S. Heidelberg (21), S. Typhimurium (21), S. Infantis (21), S. Hadar (20) and S. Tennessee (20).

For statistical analyses of the association between a given gene and serotypes, only S. Enteritidis, S. Heidelberg, S. Hadar and S. Typhimurium were used in the comparison because they had the highest numbers of samples. Eight genes were positively associated ($p<0.05$) with at least one serotype, and one gene was negatively associated ($p<0.05$) with the four serotypes. This negative association indicates that this gene was restricted to some groups of strains and was not usually related to one of the four analysed serotypes. Based on the distribution of virulence-associated genes in these serotypes, a decision tree was constructed (Figure 2) considering the sefA, sopE and lpfA genes.

DISCUSSION

Salmonella spp. are considered priority bacteria by the World Health Organization (WHO) and the World Organisation for Animal Health (OIE) in monitoring the emergence of resistant strains in animals due to the increase in their antimicrobial resistance over the years. Thus, in vitro tests are important not only for the choice of antimicrobial for the treatment of infections but also for the monitoring of resistance (Jorgensen & Ferraro, 2009). Unfortunately, Brazil does not have integrated programmes for monitoring the antimicrobial resistance of the main pathogens of humans and production animals, such as Salmonella spp. and Campylobacter jejuni. The analysis of the behaviour of these pathogens in these populations would allow the adoption of new measures to control and restrict the use of antimicrobials.
Table 2 – Absolute and relative frequencies of twenty-seven virulence-associated genes in *Salmonella enterica* serotypes isolated from poultry sources.

<table>
<thead>
<tr>
<th>Virulence gene</th>
<th><em>S. Enteritidis</em> (%)</th>
<th><em>S. Heidelberg</em> (%)</th>
<th><em>S. Hadar</em> (%)</th>
<th><em>S. Typhimurium</em> (%)</th>
<th><em>S. Bredeney</em> (%)</th>
<th><em>S. Anatum</em> (%)</th>
<th><em>S. Agona</em> (%)</th>
<th><em>S. Tennessee</em> (%)</th>
<th><em>S. Infants</em> (%)</th>
<th><em>S. Brandenburg</em> (%)</th>
<th><em>S. Schwarzengrund</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>invA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>hiaA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>avrA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>zefA</td>
<td>70 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>lipA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>6 (75)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>agfA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sopE</td>
<td>69 (98.6)</td>
<td>45 (91.8)</td>
<td>6 (42.9)</td>
<td>5 (62.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (100)</td>
<td>0</td>
</tr>
<tr>
<td>spvC</td>
<td>64 (91.4)</td>
<td>2 (4.1)</td>
<td>0</td>
<td>1 (12.5)</td>
<td>0</td>
<td>0</td>
<td>1 (25)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sivH</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>spvB</td>
<td>64 (91.4)</td>
<td>12 (24.5)</td>
<td>0</td>
<td>1 (12.5)</td>
<td>2 (40)</td>
<td>2 (40)</td>
<td>1 (25)</td>
<td>1 (33.3)</td>
<td>2 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>spaA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>paxC</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>cdbB</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (60)</td>
<td>2 (40)</td>
<td>0</td>
<td>2 (66.7)</td>
<td>2 (100)</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>msgA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>stdA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>orgA</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>4 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>tolC</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>iroN</td>
<td>39 (55.7)</td>
<td>45 (91.8)</td>
<td>13 (92.9)</td>
<td>8 (100)</td>
<td>4 (80)</td>
<td>5 (100)</td>
<td>2 (50)</td>
<td>2 (66.7)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>sitC</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>pefC</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>6 (75)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sifA</td>
<td>69 (98.6)</td>
<td>45 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>3 (75)</td>
<td>1 (33.3)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>sopB</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>pefA</td>
<td>63 (90)</td>
<td>1 (2)</td>
<td>0</td>
<td>1 (12.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sel</td>
<td>70 (100)</td>
<td>49 (100)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>4 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>str</td>
<td>63 (90)</td>
<td>46 (93.9)</td>
<td>14 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>3 (75)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>
Resistance to sulfonamides is common in production animals, and it has been widely described in the literature (Benacer et al., 2010; World Health Organization, 2011a; Proroga et al., 2015; European Food Safety Authority 2017a). These high rates of resistance are possibly related to the wide use of these substances, which would result in an increase in selective pressure (Grave et al., 2010; Maka et al. 2015; Food and Drug Administration, 2017). More than 70% of the strains resistant to ciprofloxacin also showed resistance to enrofloxacin. This fact can be explained by the similar structures of these antimicrobials (Marshall & Levy, 2011). Fluoroquinolones are considered the preferred antimicrobial agents for the treatment of salmonellosis in humans (World Health Organization, 2011b; European Food Safety Authority, 2017a). The WHO classifies these antimicrobials as extremely important and recommends special attention be paid to the surveillance of antimicrobial resistance in animals, as resistance may be the result of the transfer of strains from non-human sources. The WHO also supports the interruption or the reduction of their use in production animals (World Health Organization, 2011a).

Official data show that the potential for antimicrobial resistance acquisition may vary among serotypes (Canadian Integrated Program for Antimicrobial Resistance Surveillance, 2013; Centers for Disease Control, 2015; European Food Safety Authority, 2017a). Thus, the relative contribution of each serovar may also influence the overall level of resistance in the genus Salmonella (European Food Safety Authority, 2015). S. Typhimurium strains presented the highest overall resistance, and almost all strains were classified as MDR in our study. This serotype has shown high resistance rates to the most commonly used drugs, regardless of the source of isolation (Ahmed et al., 2016; Almeida et al., 2016; Lopes et al., 2016; Wang et al., 2017). Recently, S. Heidelberg strains have become more resistant to antibiotics, limiting therapeutic options (Center for Infectious Disease Research and Policy, 2017). In addition, the frequency of finding MDR S. Heidelberg has increased dramatically in the last few years (Centers for Disease Control, 2014). However, our strains did not present a higher frequency of multidrug resistance.

Although the frequency of MDR strains found in this study was lower than previously reported frequencies (Pulido-Landinez et al., 2014; Proroga et al., 2015), these results indicate that the increase in antimicrobial resistance is a matter of worldwide concern, even though there are differences between the methodologies used (Lertworapreecha et al., 2013). Almost all strains of S. Typhimurium, frequently isolated from human salmonellosis, were classified as MDR, which is of great concern to public health.

Although there is evidence that the use of antimicrobials in production animals is responsible for resistance in human to some pathogens such as Salmonella spp., control has not been effectively adopted in all sectors of the poultry production chain (World Health Organization, 2011a; World Health Organization, 2011b; Collignon, 2012). In addition, globalization and the consequent trade in animal products between countries allow MDR strains to be disseminated to different regions (World Health Organization, 2011b; European Food Safety Authority, 2015). Some factors such as foreign travel, international trade in food, the breeding of different species in the same environment and the vertical structure of some animal production systems may also influence the propagation of resistant strains (European Food Safety Authority, 2015).

The presence of the sefA gene was restricted to S. Enteritidis, since the gene had positive association (p<0.05) with this serovar and negative association (p>0.05) with the others. This gene is a marker of this serotype (Amini et al., 2010). A positive association (p<0.05) of _lpfA_ and _lpfC_ with S. Enteritidis, S. Heidelberg and S. Hadar serotypes was also observed, demonstrating that despite being considered conserved within the genus Salmonella (Bäumler & Heffron, 1995; Doran et al., 1996), the operon _lpfABCD_E_ is more frequent in some serotypes. The plasmidial genes _spvB, spvC_ and _pefA_ were positively associated (p<0.05) with S. Enteritidis. A negative association (p<0.05) between these genes and the serotypes S. Hadar and S. Heidelberg was also found. According to Rychlik et al. (2009), S. Enteritidis and S. Typhimurium tend to present plasmids, whereas other serotypes such as S. Typhi, S. Hadar and S. Infantis usually do not. The _sopE_ gene is positively associated (p<0.05) with S. Enteritidis and S. Heidelberg. The frequency variation of this gene among Salmonella serotypes may be related to its location, since it is found in a bacteriophage. Phage have predilections for certain serotypes, and they facilitate the horizontal transmission of bacterial genes (Mirol et al., 1999). The _iroN_ gene showed a positive association (p<0.05) with S. Typhimurium, S. Heidelberg and S. Hadar and a negative association (p<0.05) with S. Enteritidis. This result differs from the results published by Skyberg et al.(2006), which
indicated that the gene would be distributed equally among the different *Salmonella* serotypes.

A decision tree computes binary classifications based on univariate divisions of categorical predictors. It finds the best data partition and discards variables that do not fully explain the categories of the variable response. In this context, classification trees are useful in determining serotype marker genes. In our study, *sefA*, *sopE* and *lpfA* are potentially markers for *S. Enteritidis*, *S. Typhimurium*, *S. Heidelberg* and *S. Hadar*. Although *sopE* and *lpfA* may be present in all serotypes and *sefA* is exclusively detected in *S. Enteritidis*, simultaneous analysis of the presence or absence of these genes through the construction of decision trees can significantly predict the probable involved serotype.

The observed association between antimicrobial resistance and specific serotypes is useful in developing specific control and treatment measures for each serotype. Despite the virulence genes being evenly distributed among the serotypes, some of these genes are associated with specific serotypes. Further studies are needed to understand how the molecular patterns of each serotype influence pathogenicity and virulence *in vivo*. In addition, *sefA*, *sopE* and *lpfA* are possible markers of *Salmonella* serotypes.

**ACKNOWLEDGEMENTS**

This work was supported by the Brazilian National Council of Technological and Scientific Development - CNPq [grant number 476092/2013-2].

**REFERENCES**


Borges KA, Furian TQ, de Souza SN, Tondo EC, Streck AF, Salle CT; et al. Spread of a major clone of *Salmonella enterica* serotype Enteritidis in poultry and in salmonellosis outbreaks in Southern Brazil. Journal of Food Protection 2017a;80(1):158–163.


