A temporal study of *Salmonella enterica* serotypes from broiler farms in Brazil

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ABSTRACT The present study analyzes the characteristics of *Salmonella* spp. from broiler chicken farms in Brazil. In total, 82 *Salmonella* spp. strains were characterized by serotyping, determining susceptibility to antimicrobials, and using pulsed-field gel electrophoresis (PFGE). Fifteen *Salmonella* serotypes were identified, among which Minnesota (40.24%), Infantis (14.63%), Heidelberg (7.31%), Senftenberg (6.09%), and Mbandaka (6.09%) were the most frequent. *Salmonella* Minnesota occurred mostly in the state of Mato Grosso do Sul and in one of the broiler companies surveyed. Approximately 60% of the strains were resistant to at least one of the antimicrobials tested. From these isolates, 17.07% were resistant to only one antimicrobial (tetracycline or streptomycin), and 9.75% were resistant to 3 or more antimicrobial classes. Thirteen resistance profiles were characterized, the most frequent of which were the resistance to tetracycline (15.85%); to the combination of trimethroprim with sulfamethoxazole, and tetracycline (10.97%); and to the combination of streptomycin and tetracycline (9.75%). Multiple correspondence analysis revealed that susceptibility or resistance of the analyzed strains and also particular *Salmonella* serotypes were associated with broiler-producing companies where the samples were collected. Strains presented high intraserotype genetic variability, as shown by the 64 PFGE profiles, suggesting the existence of several contamination sources in the surveyed farms.

Key words: *Salmonella*, poultry, antimicrobial resistance, PFGE

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INTRODUCTION

Salmonellosis is one of the most complex zoonoses affecting worldwide public health. The over 2,600 known serotypes are found in a wide variety of animal reservoirs, and they are especially resilient, surviving in diverse environments, which explains their high potential to spread (EFSA, 2014). Broiler flocks are among the main *Salmonella* reservoirs and are an important means of transmission of the bacteria (Verge et al., 2005; Foley et al., 2008; FAO-WHO, 2009; Tabo et al., 2013). Therefore, the consumption of chicken is considered a risk factor for *Salmonella* infections in humans (FAO-WHO, 2009). Another important aspect surrounding the transmission of pathogens by animal-source foods is the likely dispersion of antimicrobial-resistant strains. The use of antimicrobials in both human and veterinary medicine promotes selective pressure, favoring the emergence of resistant strains and narrowing the choice of therapeutically efficacious drugs that are available (Verge et al., 2005; Muhammad et al., 2010; Barrow et al., 2012; Hur et al., 2012; Lai et al., 2014). The most common problems linked with antimicrobial resistance include morbidity, mortality, and the costs associated with diseases (Muhammad et al., 2010).

Due to the existence of several potential contamination sources, such as environment, transportation, equipment, litter, vectors, water and feed, the control of *Salmonella* on broiler farms is a difficult task (Foley et al., 2008; FAO-WHO, 2009). In an attempt to ensure flock health and the innocuousness of poultry products in Brazil, the Ministry of Agriculture, Livestock and Food Supply (**MAPA**) has implemented the Poultry Health National Plan (**PNSA**), which includes *Salmonella* control initiatives in the country's poultry industry. The procedures designed for breeder farms aim to control the transmission of *S.* Pullorum, *S.* Gallinarum, *S.* Enteritidis, and *S.* Typhimurium based on measures that include vaccination, sanitary culling, and termination of the incubation of eggs laid by birds of infected breeder flocks, depending on the category of bird involved (Brasil, 2003). MAPA has also implemented regular flock surveillance to detect *S.* Enteritidis, *S.* Typhimurium, and *Salmonella* spp.

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in broilers before slaughter (Brasil, 2009). However, *Salmonella* epidemiology is quite complex; therefore, other serotypes that are also able to colonize the intestinal tract of broilers and contaminate carcasses during slaughter and processing may circulate in the broiler production chain, posing an extra challenge to minimizing the risks to the end consumers.

Studies to survey and track the pathogen may be useful in *Salmonella* prevention and control programs, which require scientific knowledge of the phenotypic and genotypic diversity of the serotypes detected on farms. In recent decades, traditional *Salmonella* typing methods, such as serotyping and phage typing, have been complemented by molecular assays that afford higher discrimination and reproducibility (Tenover et al., 1995; Verge et al., 2005). Among these, pulsed-field gel electrophoresis (**PFGE**) is considered the gold standard for the molecular subtyping of *Salmonella*, with a proven contribution to investigations on the origin and spread of the pathogen (Ribot et al., 2006; Favier et al., 2013; Yang et al., 2013).

In Brazil, there are few studies published about nontyphoidal *Salmonella* serotypes on broiler farms, and those we do have are from several years ago (Tavechio et al., 2002; Kanashiro et al., 2005). Other studies have foucused only on *S.* Enteritidis due to public health issues (Oliveira et al., 2005; Ribeiro et al., 2007; Vaz et al., 2010). Therefore, additional information is needed to better understand the current distribution of *Salmonella* serotypes in broiler farms. More information is also needed to evaluate the impact of the official control program on the Brazilian poultry industry. The objectives of this study were to 1) determine the serotype and genotype diversity of *Salmonella* spp. isolates collected from broiler farms managed by different large-scale broiler-producing companies in Brazil, 2) analyze the antimicrobial resistance profile of these strains, and 3) test the relationship between the serotypes and antimicrobial resistance profiles and the broiler-producing companies where the isolates were detected.

MATERIALS AND METHODS

Sampling

This study comprised a total of 1,543 drag swabs received at the laboratory between 2009 and 2010 for salmonellosis diagnosis on commercial broiler farms. Samples were taken from broiler farms from 10 companies with integrated production systems in the states of Santa Catarina, Paraná, and Mato Grosso do Sul as part of the *Salmonella* surveillance program in flocks. These states accounted for 50.82% of the broiler production in Brazil in 2013 (UBABEF, 2014). A single broiler house was sampled at each farm. Each sample consisted of a pool of 2 drag swabs collected with sterile disposable socks moistened with 1% buffered peptone water (**BPW**) and worn over the boots. Then the collector walked along the broiler house, from wall to wall. Next, the socks were placed in a sterile plastic bag that was transported to the laboratory on ice and processed within 48 h from sampling. All surveyed broiler flocks were grown from breeder flocks vaccinated against *S.* Enteritidis.

Salmonella Isolation, Identification, and Serotyping

The MAPA-recommended isolation protocol was used (Brasil, 1995). Drag swabs were pre-enriched in brain heart infusion broth (**BHI**; Difco, Detroit, MI) at 37°C for 18 to 24 h. Aliquots of this cultivation medium were individually inoculated in tetrathionate broth (Difco) and Rappaport-Vassiliadis broth (Difco), incubated at 42°C for 18 to 24 h, streaked on brilliant green agar (Difco) and Rambach agar (Becton Dickinson, Franklin Lakes, NJ), and incubated at 37°C for 24 h. Suspected colonies were confirmed by biochemical and seroagglutination assays in polyvalent antigen on a slide. The isolates were preserved at –70°C until use.

Complete antigenic characterization and serotype identification were carried out in the National Reference Laboratory for Cholera and Enteric Diseases, Oswaldo Cruz Institute (FIOCRUZ, Rio de Janeiro, RJ, Brazil) with the rapid slide agglutination test using the somatic and flagellar antisera produced by the laboratory.

Determination of Antimicrobial Susceptibility

Antimicrobial susceptibility was determined by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2008). The measurement and interpretation of inhibition halos was performed using the criteria defined in the approved standards M31-A3 (CLSI, 2008) and M100-S21 (CLSI, 2011), when appropriate. The *Escherichia coli* ATCC 35922 strain was used to ensure the validity of the testing. The following antibiotic disks (DME, Niterói, Rio de Janeiro, Brazil) were used: amoxicillin with clavulanic acid, 20/10 μg (**Amc**); ceftiofur, 30 μg (**Cef**); ciprofloxacin, 5 μg (**Cip**); colistin, 10 μg (**Ct**); enrofloxacin, 5 μg (**Enr**); streptomycin, 10 μg (**Str**); fosfomycin, 200 μg (**Fos**); gentamicin, 10 μg (**Gen**); norfloxacin, 10 μg (**Nor**); trimethoprim with sulfamethoxazole, 23.75/1.25 μg (**Ts**); and tetracycline, 30 μg (**Tet**). Strains that presented resistance to 3 or more classes of antimicrobials were considered multiresistant (Schwarz et al., 2010).

Pulsed-field Gel Electrophoresis

Isolates were genotyped by DNA macrorestriction analysis using 40 U of the enzyme *Xba*I (New England Biolabs, Beverly, MA) followed by PFGE, as

previously described (Ribot et al., 2006). *S.* Typhimurium LT2 was used as a size standard. Restriction fragments were electrophoresed in certified 1.2% PFGE agarose gels (Bio-Rad, Hercules, CA) in tris-borate buffer (**TBE**; Tris-borate 0.045 M, EDTA 0.001 M) at 14°C using the CHEF Mapper XA system (Bio-Rad), with an initial switch time of 2.2 s and a final switch time of 63.8 s at 6 V/s for 18 h. Gels were stained in ethidium bromide (1 μg/mL) and visualized under UV light. Images were captured using a digital camera, and macrorestriction patterns were compared using Bionumerics 4.0 software (version 6.1) (Applied Maths, Sint-Martens-Latem, Belgium). The similarity was calculated by the Dice coefficient with a 1.7% tolerance. A dendrogram was generated by cluster analysis using the unweighted pair group method with arithmetic averages (**UPGMA**). Strains sharing the same number and position of DNA macrorestriction fragments were considered to belong to the same genotype.

Multiple Correspondence Analysis

Descriptive analyses were carried out to reveal the origin and phenotypic characteristics (serogroups and antimicrobial resistance) of the strains. These analyses were followed by a multiple correspondence analysis to evaluate how broiler-producing companies, *Salmonella* serogroups, and resistance to each chosen antimicrobial drug were related. The illustrative variables, *Salmonella* serogroups and number of antimicrobials to which resistance were observed, were plotted against the generated profile map. The multiple correspondence analysis was carried out using Système Pour Analyse de Données (**SPAD**) software, PC version (Centre International de Statistique et d'Informatique Appliqués, Saint-Mandé, France), taking into account only the companies where five or more *Salmonella* strains were isolated.

RESULTS

Salmonella Isolates and Serotyping

In total, 82 *Salmonella* spp. strains were isolated out of 1,543 analyzed samples. A total of 15 serotypes were identified, of which Minnesota, Infantis, Heidelberg, Senftenberg, and Mbandaka were the most common, in this order. Five strains were identified as the *Salmonella enterica* subspecies *enterica* and presented the following antigen formulae: O:4,5:-:1,2 (2/82); O:13,23:i:- (1/82); O:4,5 (1/82); and O:9,12 (1/82).

S. Minnesota was the most frequent serotype in the samples from the state of Mato Grosso do Sul (33/38, 86.8%). Four other serotypes were detected in the same state. In Paraná, 13 serotypes were identified, among which *S.* Infantis was the most prevalent (10/29, 34.5%). In the state of Santa Catarina, 10 different serotypes were detected, and *S.* Senftenberg was the most common (5/15, 33.3%).

Antimicrobial Susceptibility of Strains

Of the analyzed strains, 49 isolates (59.75%) presented resistance to 1 or more antimicrobials (Table 1). Of these, 14 (17.07%) were resistant to 1 drug, and 24 (29.26%) were resistant to 2 drugs. Multiresistance was observed in 8 isolates, 7 isolates (8.53%) were resistant to 3 antimicrobial classes, and 1 isolate (1.22%) was resistant to 4 antimicrobial classes. All of the characterized strains were susceptible to fosfomycin,

Table 1. Frequency of *Salmonella* serotypes and distribution according to antimicrobial resistance in broilers farms in Brazil.

Serotypes	O group	Isolates (%)	Resistance pattern[1]					Resistant isolates (%)
			0	1	2	3	4	
Minnesota	O:21 (L)	33 (40.24)	1	12	14	6		32 (39.02)
Infantis	O:7 (C_1)	12 (14.63)	8		4			4 (4.87)
Heidelberg	O:4 (B)	6 (7.31)	4	1	1			2 (2.44)
Senftenberg	O:1,3,19 (E_4)	5 (6.09)			4	1		5 (6.09)
Mbandaka	O:7 (C_1)	5 (6.09)	4	1				1 (1.22)
Schwarzengrund	O:4 (B)	4 (4.87)	3		1			1 (1.22)
Bredeney	O:4 (B)	2 (2.44)	1	1				1 (1.22)
Cerro	O:18 (K)	2 (2.44)	1		1			1 (1.22)
Anatum	O:3,10 (E_1)	2 (2.44)	2					0
O:4,5:-:1,2[2]	O:4 (B)	2 (2.44)	2					0
Orion	O:3,10 (E_1)	1 (1.22)	1					0
Livingstone	O:7 (C_1)	1 (1.22)					1	1 (1.22)
Saintpaul	O:4 (B)	1 (1.22)			1			1 (1.22)
Agona	O:4 (B)	1 (1.22)	1					0
O:13,23:i:-[2]	O:13 (G)	1 (1.22)	1					0
O:4,5[2]	O:4 (B)	1 (1.22)	1					0
O:9,12[2]	O:9 (D_1)	1 (1.22)	1					0
London	O:3,10 (E_1)	1 (1.22)	1					0
Ohio	O:7 (C_1)	1 (1.22)	1					0
Total		82	33	15	26	7	1	49 (59.75)

[1]Number of strains that were sensitive (0) or resistant to up to 4 antimicrobial classes.

[2]Incomplete antigenic formula.

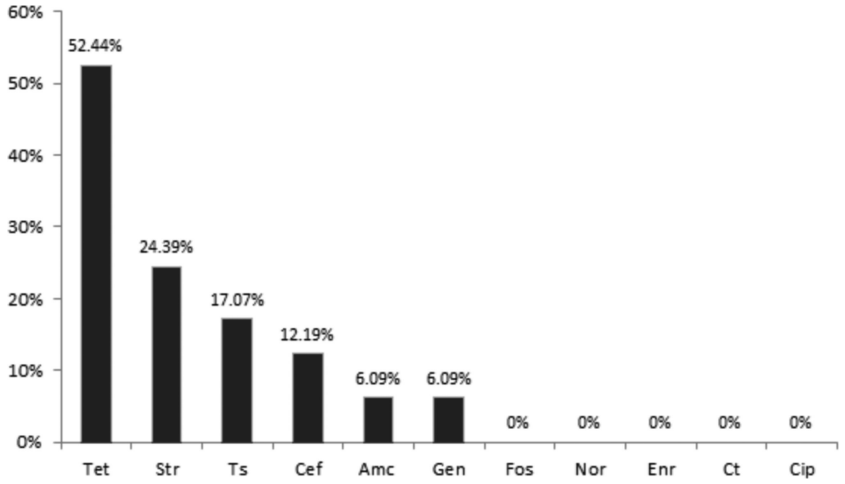

Figure 1. Frequency (%) of antimicrobial resistance in *Salmonella* isolates from broiler farms in Brazil. **Tet**: tetracycline; **Str**: streptomycin; **Ts**: trimethoprim with sulfamethoxazole; **Cef**: ceftiofur; **Amc**: amoxicillin with clavulanic acid; **Gen**: gentamicin; **Fos**: fosfomycin; **Nor**: norfloxacin; **Enr**: enrofloxacin; **Ct**: colistin; **Cip**: ciprofloxacin.

norfloxacin, enrofloxacin, colistin, and ciprofloxacin (Figure 1).

Thirteen different resistance profiles were identified (Figure 2), the most frequent of which were resistance to tetracycline only (15.85%), to the combination tetracycline and trimethoprim with sulfamethoxazole (10.97%), and to the combination streptomycin and tetracycline (9.75%).

The Relationship between Phenotypic Patterns and the Origin of Strains

Figure 3 shows the profile map generated by the multiple correspondence analysis. On the bottom right, broiler-producing companies 1, 5, and 6 were associated with antimicrobial susceptibility and with serogroups E_1, C_1, D_1, and G, respectively. Susceptibility to trimethoprim with sulfamethoxazole and to streptomycin was associated with serogroups B and K, respectively. On the bottom left, *Salmonella* strains of serogroup L (*S.* Minnesota) were closely associated with company 3, just as serogroup E_4 (*S.* Senftenberg) was linked to company 4. In this group, companies 2, 3, and 4 were associated with resistance to tetracycline, trimethoprim with sulfamethoxazole, and streptomycin, respectively. Because only one strain was resistant to 5 antimicrobials (4 classes) (Table 1), there was no direct association between this isolate and the company plotted on the map.

Salmonella Genotype Diversity

PFGE differentiated the strains analyzed into 64 genotypes (Figure 2), of which 30 were identified in the 33 *S.* Minnesota strains, 8 in the 12 *S.* Infantis strains, 3 in the 6 *S.* Heidelberg strains, and 2 in the 5 *S.* Senftenberg strains. The samples of the other serotypes were of a single genotype. All of the genotyped *Salmonella* serotypes presented PFGE profiles distinct from the other serotypes. Most strains of *S.* Minnesota, *S.* Infantis, *S.* Heidelberg, *S.* Senftenberg, and *S.* Mbandaka formed specific clusters. *S.* Minnesota presented the highest intraserotype genetic diversity, shown by the number of obtained PFGE profiles, and only 3 of these genotypes were shared by more than one strain. In spite of this variability, most *S.* Minnesota strains formed one main cluster, with similarity levels over 70% (Figure 2). Six strains (7.31%) of the serotypes Ohio (1), Saintpaul (1), Cerro (2), Schwarzengrund (1), and *Salmonella enterica* subspecies *enterica* (O:9,12) could not be genotyped using the protocol adopted in the present study. The samples that were genotyped presented between 9 and 18 bands, whose sizes varied between 35 and 821 kb (data not shown).

DISCUSSION

The present study identified several nontyphoidal *Salmonella* strains isolated from drag swabs collected from broiler farms in 3 Brazilian states. In total, 15 serotypes were identified and found to be distributed unevenly in the surveyed region. *S.* Minnesota was the most commonly isolated strain (40.24%), followed by *S.* Infantis (14.63%), *S.* Heidelberg (7.31%), *S.* Senftenberg (6.09%), and *S.* Mbandaka (6.09%) (Table 1). However, most *S.* Minnesota strains were from a single broiler-producing company (company 3) in the state of Mato Grosso do Sul, suggesting the regional predominance and distribution of this serotype. The variability of serotypes by geographical area has also been reported by Yang et al. (2013).

S. Minnesota has been considered one of the most prevalent serotypes in laying hens in Chad (Tabo et al., 2013) and also in broiler farms in Belgium (CODA-CERVA, 2014). However, it is rarely responsible for human salmonellosis outbreaks worldwide (CDC, 2013a,b; EFSA, 2014). In contrast, *S.* Infantis is one of the

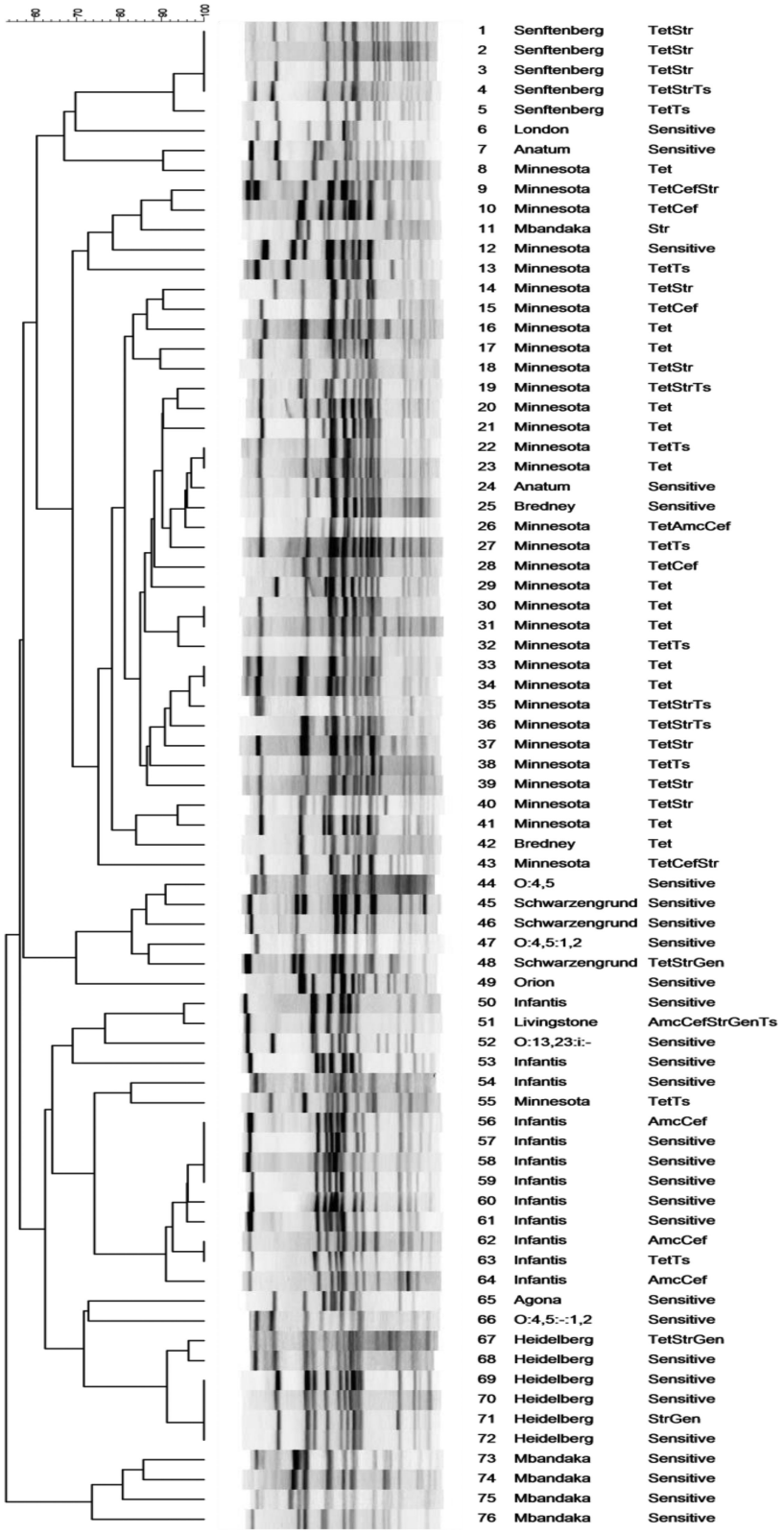

Figure 2. *Xba*I PFGE and antimicrobial resistance patterns of *Salmonella enterica* isolates from broiler farms in Brazil. **Tet**: tetracycline; **Str**: streptomycin; **Ts**: trimethoprim with sulfamethoxazole; **Cef**: ceftiofur; **Amc**: amoxicillin with clavulanic acid; **Gen**: gentamicin; **Fos**: fosfomycin; **Nor**: norfloxacin; **Enr**: enrofloxacin; **Ct**: colistin; **Cip**: ciprofloxacin.

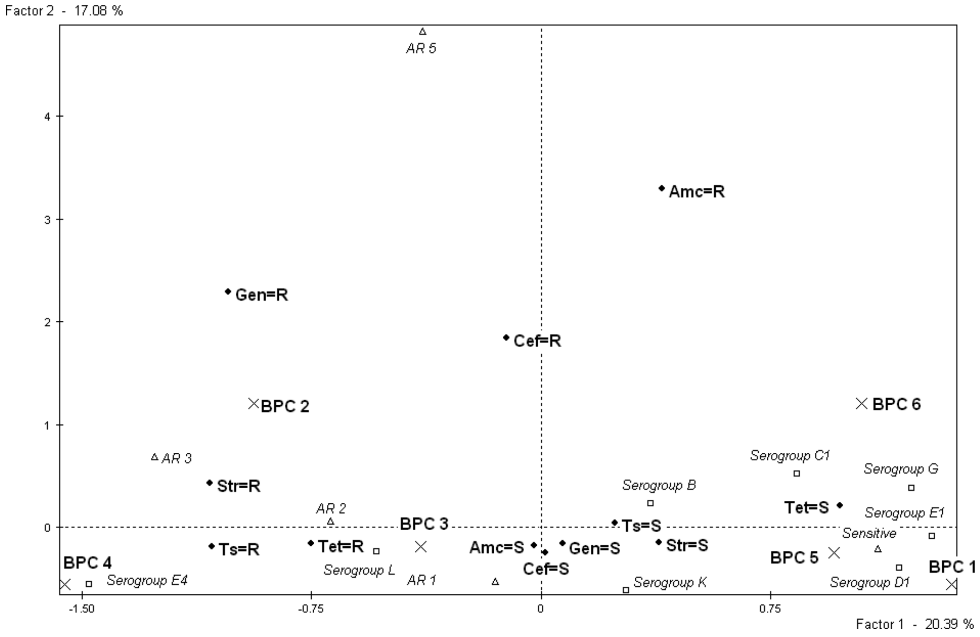

Figure 3. Map of the multiple correspondence analysis describing associations among *Salmonella* serogroups, antimicrobial resistance patterns, and broiler-producing companies sampled in Brazil. **BPC**: broiler-producing company; **AR**: number of antimicrobials to which resistance was observed; **S**: sensitive; **R**: resistant; **Amc**: amoxicillin with clavulanic acid; **Cef**: ceftiofur; **Str**: streptomycin; **Gen**: gentamicin; **Ts**: trimethoprim with sulfamethoxazole; **Tet**: tetracycline.

serotypes that is regularly surveyed in breeder flocks in the European Union because it has been involved in salmonellosis outbreaks in member countries (CODA-CERVA, 2014; EFSA, 2014). In turn, *S.* Heidelberg was listed among the 4 most commonly isolated serotypes in broiler chickens and humans in the United States from 1970 to 2009 (CDC, 2013a). In Brazil, *S.* Heidelberg, *S.* Senftenberg, and *S.* Mbandaka have been identified in broiler chickens for years (Tavechio et al., 2002; Kanashiro et al., 2005; Duarte et al., 2009), but there are no data concerning their occurrence in outbreaks of salmonellosis in humans because the causal serotype has not been characterized in most of these events. Due to the multifactorial nature of *Salmonella* transmission in the food chain, it is often not possible to establish a correlation between the most prevalent serotypes in poultry and the most common ones implicated in officially reported human salmonellosis.

In the past two decades, *S.* Enteritidis was the predominant serotype in poultry flocks, broiler meat, and table eggs in Brazil (Tavechio et al., 2002; Kanashiro et al., 2005; Ribeiro et al., 2007; Duarte et al., 2009; Vaz et al., 2010); however, it was not found in this study. In fact, the gradual reduction in *S.* Enteritidis frequency, in comparison to other nontyphoidal serotypes isolated on broiler farms, has become apparent in our laboratory during this period. This reduction may be a consequence of *Salmonella* control programs implemented by the Brazilian poultry industry. In addition to the strict biosecurity measures adopted, the immune prophylaxis of breeder flocks with inactivated vaccines against *S.* Enteritidis began in 2003 under official supervision (Brasil, 2003), which may have helped reduce its prevalence in broilers. The immunity given by vaccination also protects against antigenically similar serotypes (Foley et al., 2011) and may have led to a drop in the prevalence of related *Salmonella* serotypes. Actually, in the present study, we identified only one isolate from serogroup D (Table 1) that was antigenically related to *S.* Enteritidis. It is estimated that when the prevalence of one serotype falls, other serotypes emerge and take over its ecological niche (Verge et al., 2005; Foley et al., 2008; Barrow et al., 2012). In Belgium, after the implementation of vaccination as part of an integrated control strategy for *S.* Enteritidis, several *Salmonella* serotypes have emerged, taking over its former ecological niche and subsequently disappearing (Jasson & Butaye, 2012). Notably, a rise in the occurrence of *S.* Minnesota has been seen in Belgium, from 2% in 2009 to 17.6% in 2012, when it became the second most common serotype in poultry (CODA-CERVA, 2014).

Regarding antimicrobial resistance, 59.75% of the evaluated strains were resistant to one or more chosen antimicrobials (Table 1). Multiresistance to 3 or 4 classes of antimicrobials was identified in 9.75% of the strains. The highest levels of resistance were found for tetracycline, streptomycin, and trimethroprim/sulfamethoxazole (Figure 1). Different antimicrobial resistance levels have been identified in *Salmonella* spp. isolated from poultry worldwide. In Brazil, these levels have been shown to vary between 56% and 100% (Oliveira et al., 2005; Cardoso et al., 2006; Duarte et al., 2009; Vaz et al., 2010). In the United States, 74.1% of strains isolated from chicken meat have been found resistant to antimicrobials and, as in the present study, the highest resistance frequencies were observed for tetracycline (65.8%) (USDA, 2012). *Salmonella* strains isolated from chicken have presented resistance

frequencies of 70.4% and 99.1% in China (Yang et al., 2013; Lai et al., 2014) and 100% in Argentina (Favier et al., 2013) and Spain (Álvarez-Fernadéz et al., 2012). However, these results should be compared with care due to the effect of variation in factors, such as methodology, antimicrobials tested, and origin of the strains (Schwarz et al., 2010).

The high frequency of resistance to tetracycline is expected because this antimicrobial was one of the first adopted for use in animal production (Muhammad et al., 2010). For instance, high levels of resistance to tetracycline have been observed in *Salmonella* spp. isolated from chicken meat sampled at processing plants in Brazil, ranging from 80% (Ribeiro et al., 2007) to 100% (Cardoso et al., 2006). Although tetracyclines were banned as additives in animal feed in Brazil in 1998, they are still used therapeutically and therefore exert selective pressure on microorganisms. In addition, the emergence of bacteria resistant to cephalosporins and fluoroquinolones raises concerns. Both classes of antimicrobials are used to treat severe human infections, and resistance to these drugs may cause grave complications in terms of successful treatment (Verge et al., 2005; Hur et al., 2012; Kilonzo-Nthenge et al., 2013; Lai et al., 2014). In the present study, 12.19% of the strains were resistant to ceftiofur (Figure 1), a third-generation cephalosporin that has been approved for veterinary use. Although resistance to this antimicrobial was low in the analyzed strains, these findings may also indicate a decrease in the susceptibility to other cephalosporins (Zhao et al., 2008; Lai et al., 2014). In Brazilian hatcheries, ceftiofur has been used in chicks during their first day of life and may represent a critical factor in the selection of resistant strains. On the other hand, all *Salmonella* strains were susceptible to fluoroquinolones tested in this study: enrofloxacin, norfloxacin, and ciprofloxacin. Although enrofloxacin is used exclusively in veterinary medicine, the resistance to this antimicrobial may indicate increasing resistance or a drop in susceptibility to other fluoroquinolones of medical importance (Schwarz et al., 2010). For this reason, the therapeutic use of enrofloxacin in production animals has been questioned due to the possibility that resistant strains might be transmitted to humans through the food chain (Jones-Dias et al., 2013).

The multiple correspondence analysis linked the antimicrobial susceptibility of *Salmonella* to broiler-producing companies 1, 5, and 6, while antimicrobial resistance was associated with companies 2, 3, and 4 (Figure 3). In Brazil, although antimicrobial classes licensed for therapeutic use in food-producing animals and their respective judicious uses are defined in specific guidelines, the actual choice of the specific drugs to be used is defined based on criteria that vary from company to company. How these differences in choice of the respective antimicrobial drugs actually influence the susceptibility or resistance phenotypes of microbial populations is unclear, and the data presented here did not allow us to reach a conclusion on this subject. At any rate, careful administration of antimicrobials and continuous surveillance are important initiatives that help define the best treatment and prevent the selection and spreading of resistant strains across flocks.

As a subtyping technique, PFGE has been widely used because of its high discriminatory power (Ribot et al., 2006; Favier et al., 2013; Yang et al., 2013). In the present study, the analyzed *Salmonella* isolates presented high genotypic variability (Figure 2) in the number of identified serotypes and the diversity of strains belonging to the same serotype. Most analyzed *S.* Minnesota strains came from farms that were located in the same geographic region and that operated in an integrated system of broiler production that was managed by the same company. For this reason, some *S.* Minnesota strains were closely related, showing similarities of over 95% (Figure 2). Strains that differ by two or three distinctive bands typically result from one single mutation, e.g., a DNA insertion or deletion, which generates the subtypes of an ancestral strain. This type of change has been observed in strains subcultured several times or reisolated from the same patient (Tenover et al., 1995). This suggests that strains that grouped into clusters of high genetic similarity may actually be subtypes of the same sample that have spread over space and time. On the other hand, the total genetic variability between clusters (Figure 2) suggests the inexistence of a clonal relationship, indicating that *S.* Minnesota transmission on the surveyed farms was predominantly horizontal and through different contamination sources. Actually, the epidemiology of *Salmonella* in poultry farming is quite complex, and the bacteria may be introduced on farms through different sources, such as feed, water, insects, rodents, and even the boots worn by the workers (Foley et al., 2008).

Among the other serotypes, most *S.* Infantis strains presented a similarity of over 90%, a value that was also observed for all *S.* Heidelberg and *S.* Senftenberg strains, which reveals little intraserotype variability. Four *S.* Infantis and 4 *S.* Heidelberg strains isolated from farms controlled by different broiler-producing companies presented one same genotype in each serotype, although the absence of epidemiological information about these samples does not allow us to infer their origin. Among the 5 analyzed *S.* Senftenberg strains, 4 shared the same PFGE profile, which was closely related to the alternative genotype that was also identified in this serotype (Figure 2). Although a small number of *S.* Senftenberg isolates was analyzed, the close relationship between these strains also suggests a common origin, an assumption supported by the association of serogroup E_4, to which they belong, with broiler company 4 (Figure 3). As a rule, PFGE revealed high genetic variability between the isolated *Salmonella* strains, suggesting that the bacteria entered farms through a variety of contamination sources.

In conclusion, *S.* Minnesota was the most frequent serotype in the sampled broiler flocks and was concentrated in one geographical area (the state of Mato

Grosso do Sul). Other nontyphoidal *Salmonella*, such as *S.* Infantis, *S.* Heidelberg, *S.* Senftenberg, and *S.* Mbandaka, were found in the states of Paraná and Santa Catarina. In contrast to previously published results, *S.* Enteritidis was not isolated in the present study, which suggests a profile change of the most frequent nontyphoidal serotypes in the Brazilian broiler industry. Approximately 60% of the strains were resistant to at least one of the evaluated antimicrobials, mainly tetracycline. All isolates were susceptible to fosfomycin, norfloxacin, enrofloxacin, colistin, and ciprofloxacin. The susceptibility or resistance of the analyzed strains was associated with the broiler-producing companies where the samples were collected, while *Salmonella* serogroups L and E4 showed a strong correlation with particular broiler-producing companies sampled. Additionally, the genetic diversity of the *Salmonella* strains suggests the involvement of different transmission sources on the surveyed farms. These results reveal the need, due to public health concerns, for continuous surveillance and molecular typing and subtyping analyses as ancillary tools in strategies to control *Salmonella* on broiler farms.

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