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Original Article

Antimicrobial Resistance in Salmonella Serovars Isolated From an Egg-Producing Region in Brazil

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ABSTRACT

Fowl paratyphoid infections are caused by different *Salmonella* serovars that can affect a wide range of hosts. Due to its complex epidemiology, *Salmonella* serovar identification is crucial for the development and implementation of monitoring and control programs in poultry farms. Moreover, the characterization of the antimicrobial resistance profiles of *Salmonella* strains isolated from livestock is relevant to public health because they are a common causative agent of foodborne diseases. The objective of this study was to investigate the presence of *Salmonella* spp. and to identify the antimicrobial resistance profiles of strains isolated in the midwestern region of São Paulo state, which accounts for the highest production of table eggs in Brazil. For this purpose, 2008 fecal samples were collected on 151 commercial layer farms and submitted to microbiological analyses. Twenty-two serovars were isolated from 80 (52.9%) farms, among which *S.* Mbandaka and *S.* Braenderup were the most prevalent. All isolates expressed resistance to at least one of the 23 antimicrobials tested, and the highest resistance rates were determined against streptomycin (93.5%) and sulfonamide (84.6%). Moreover, multidrug resistance was observed in 41% of the isolates and the maximum drug resistance profile was against ten different antimicrobials. Therefore, the identification of *Salmonella* serovars in poultry production provides epidemiological knowledge to develop prevention and control measures in order to ensure poultry health and to prevent human infection by multiresistant strains.

INTRODUCTION

Commercial table eggs are an important source of protein for Brazilian consumers. In 2018, Brazil produced 44.2 billion eggs, and had a per capita consumption of 212 eggs, representing a 10.4% increase relative to 2017 (APBA, 2019). This growth emphasizes the need to improve live production and egg processing management in order to achieve higher productivity, and to develop methods to allow layer farms and egg-processing companies to comply with the standards and guidelines of importing countries, in particular those relative to product quality and pathogen control (Donato *et al.*, 2009).

Bacteria of the genus *Salmonella* are widely distributed in nature and may infect both humans and animals, and more than 2659 *Salmonella* serovars have been identified (Issenhuth-Jeanjean *et al.*, 2014). Several of these serovars cause paratyphoid infection (Salles *et al.*, 2008; Kottwitz *et al.* 2008; Freitas Neto *et al.*, 2014; Perdoncini *et al.*, 2014; Moraes *et al.*, 2016). In addition, non-typhoidal *Salmonella* serovars may persist in the digestive tract of infected chickens without causing disease and are considered the main source of foodborne infections in humans, mainly due to the consumption of poultry products (Shah *et al.*, 2017).

Due to the wide variety of infection sources and its capacity to spread between hosts and vectors, it is very difficult, and often impossible, to eradicate *Salmonella* spp. in poultry farms, because poultry are susceptible hosts (Andino & Hanning, 2015). Wild birds, rodents, and insects also play a central role in its dissemination among farms and persistence in poultry facilities (Ma *et al.*, 2018). Therefore, the knowledge on *Salmonella* spp. prevalence in poultry flocks is required to design adequate biosecurity measures to reduce infection (Freitas Neto *et al.*, 2014).

Antimicrobials are used for the treatment of bacterial diseases in both human and veterinary medicine; however, their misuse (active principles, doses, or targets), have contributed to the emergence of resistant bacterial strains (Wright, 2010). In Brazilian animal production, antibiotics are used not only for therapeutic purposes, but also for prophylaxis or as performance enhancers (Antunes *et al.*, 2016). The most frequently used antimicrobial classes are β-lactams, tetracyclines, aminoglycosides, amphenicols, quinolones, fluoroquinolones, and sulfonamides (Moreno-Bondi *et al.*, 2009). However, Brazilian studies (Antunes *et al.*, 2016; Celis-Estupiñan *et al.*, 2017) have reported ineffective antimicrobial therapy against *Salmonella* spp. infection in poultry, which may contribute to the emergence of resistance. These results emphasize the need to determine the antimicrobial susceptibility profile of *Salmonella* spp. isolates for the design of programs to aid the rational use of antimicrobials in poultry production aiming at minimizing antimicrobial resistance in both poultry and humans (Allen *et al.*, 2013).

In this context, the objectives of this study were to identify *Salmonella* spp. isolate in layer farms in the largest egg producing region of Brazil, and to characterize their antimicrobial resistance profile.

MATERIAL AND METHODS

Ethics statement

All experimental procedures were approved by the Committee of Ethics on Animal Use of the School of Agriculture and Veterinarian Sciences, São Paulo State University (Unesp), Brazil, under protocol n. 07058/19.

Sample collection

Fecal samples were collected in 151 commercial layer farms located in 11 municipalities of the midwestern region of São Paulo state, between 2016 and 2017. Fecal samples (n=2008) of 300 grams each were collected in sterile flasks and refrigerated (4-8°C) until analyses (Brasil, 2017).

Identification of *Salmonella* serovars

The isolation and identification of *Salmonella* spp. were performed at the Avian Pathology Laboratory, Department of Veterinary Pathology, School of Agricultural and Veterinary Science (FCAV), São Paulo State University (Unesp), Jaboticabal, SP, Brazil.

Salmonella spp. microbiological assays followed the protocol (Figure 1) recommended by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) (Brasil, 1995). Those isolates that had a suggestive biochemical profile for *Salmonella* were streaked on lysogen agar and then tested with somatic and flagellar antisera. All microbiological media inoculated with testing samples were incubated at 37 °C for 24 hours.

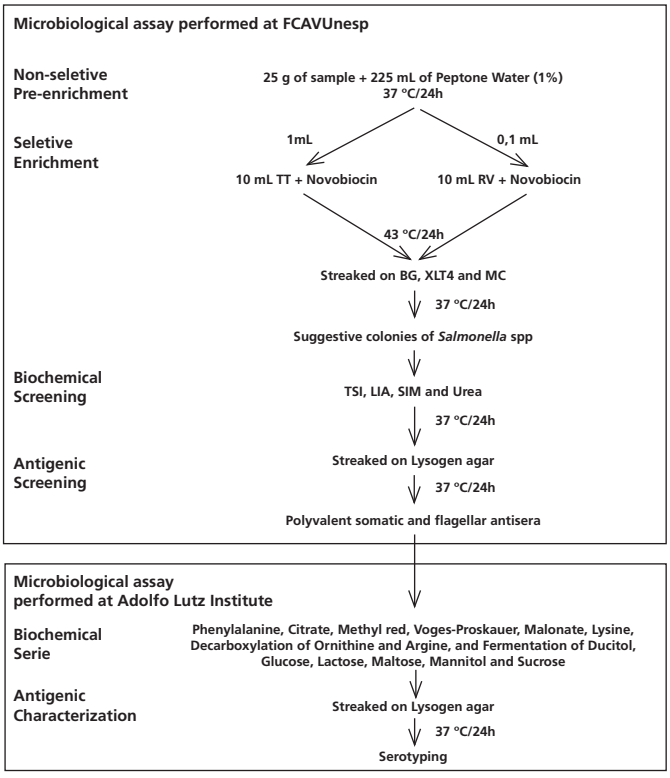

Figure 1 – Flowchart of the microbiological assays used for the identification of *Salmonella* serotypes in fecal samples of commercial layers reared in the midwestern region of the state of São Paulo, SP, Brazil, between 2016 and 2017.

TT: Tetrathionate broth; RV: Rappaport-Vassiliadis broth; BG: Brilliant Green agar; XLT4: Xylose lysine tergitol 4 agar; MC: MacConkey agar.

Presumed *Salmonella* colonies were submitted to the Polymerase Chain Reaction (PCR) targeting the *invA* gene to confirm the genus, as described by Fratamico & Strobaugh (1998). This virulence gene can only be found in *Salmonella* spp. and it is conserved among serovars (Oliveira *et al*., 2002). Positive samples in both microbiological and molecular assays were submitted

to the Enterobacteria Section of Adolfo Lutz Institute, São Paulo, SP, Brazil, for serovar identification (Brasil, 1995).

Antimicrobial sensitivity test

The antimicrobial sensitivity of *Salmonella* strains was evaluated by Kirby-Bauer disk diffusion (Bauer *et al.*, 1966) and Minimal Inhibitory Concentration (MIC) (Thamlikitkul & Tiengrim, 2014) tests. *Escherichia coli* strain ATCC® 25922™ (CLSI, 2017) was used for quality control. Twenty-three antimicrobials belonging to 8 classes were evaluated (Table 1). The MIC test was performed only for polymyxin E, and strain resistance was assumed when antimicrobial concentration was higher than 2 µg/mL (CLSI, 2017). The results were compared with the standards of the Clinical and Laboratory Standards Institute reports (CLSI, 2013; CLSI, 2017). Intermediate profiles were assumed as resistant on disk diffusion test as an interpretation criterion (Firoozeh *et al*., 2011). Serovars resistant to three or more antimicrobials from different classes were considered multiresistant (Schwarz *et al.*, 2010).

RESULTS AND DISCUSSION

The implementation of biosecurity measures directly influences the impact of foodborne salmonellosis from poultry products (Gama *et al.*, 2003; Kottwitz *et al.*, 2008). Human salmonellosis outbreaks in many countries have been associated with the consumption of table eggs (Nor Faiza *et al.*, 2013; Long *et al.*, 2017), which may be contaminated during their formation in the oviduct or their passage through the cloaca (contact with feces). *Salmonella* spp. survey on layers farms is essential for understanding the epidemiology of infection, which is influenced by farm, layer strain, production system, and technologies applied (Gama *et al.*, 2003; Kottwitz *et al.*, 2008). Although the Brazilian legislation on *Salmonella* control in poultry farms has become increasingly stringent, requiring structural changes of facilities, vaccination, and microbiological assays, *Salmonella* spp. serotyping is not mandatory, except for *S*. Gallinarum, *S*. Pullorum, *S*. Enteritidis, and *S*. Typhimurium (Brasil, 2003; Brasil, 2013; Brasil 2017).

In this study, fecal samples were collected in layer farms located in 11 municipalities in the midwestern region of the state of São Paulo, which is the largest table egg-producing region in Brazil, with 187 layer farms (ABPA, 2019). All strains suggestive of *Salmonella* in the microbiological assays were tested positive by PCR. Out of the 151 farms evaluated, 80 farms (52.9%) were positive for *Salmonella* spp. Previous reports detected *Salmonella* spp. in 3.98% cloacal

Table 1 – Antimicrobials used in sensitivity tests.

Class	Antimicrobial	Concentration (µg/mL)	Manufacturer*
Quinolones and Fluoroquinolones	Nalidixic Acid	30	Sensifar®
	Enrofloxacin	5	Sensifar®
	Norfloxacin	10	Oxoid ®
	Ciprofloxacin	5	Oxoid ®
Phenicols	Chloramphenicol	30	Sensifar®
Aminoglycosides	Kanamycin	30	Sensibiodisc®
	Streptomycin	10	Sensibiodisc®
	Gentamicin	10	Oxoid ®
	Amikacin	30	Oxoid ®
Sulfonamides	Sulfonamide	300	Sensibiodisc®
	Trimethoprim/Sulfamethoxazole	1,25/23,75	Oxoid ®
β-lactams	Cefotaxime	30	Sensifar®
	Cefepime	30	Sensifar®
	Ceftiofur	30	Sensifar®
	Ampicillin	10	Sensibiodisc®
	Amoxicillin	10	Sensifar®
	Amoxicillin/Clavulanic Acid	20/10	Oxoid ®
	Imipenem	10	Oxoid ®
	Aztreonam	30	Sensifar®
Tetracyclines	Tetracycline	30	Sensifar®
	Oxytetracycline	30	Sensi-disc®
Phosphomycins	Phosphomycin	200	Sensifar®
Polymyxins	Polymyxin E	0,125-32	Sigma-Aldrich®

*Oxoid®, Basingstoke, Hampshire, UK; Sigma-Aldrich®, St. Louis, Missouri, USA; Sensi-disc®, Franklin Lakes, Nova Jersey, EUA; Sensibiodisc®, São Paulo, BR; Sensifar®, São Paulo, BR.

swabs (Andrade *et al.*, 1995), 6.25% fecal samples (Salles *et al.*, 2008), and 25% mature flocks (Freitas Neto *et al.*, 2014) in Brazilian layer farms, suggesting that biosecurity failures may allow the dissemination of this pathogen among farms (Iwabuchi *et al.*, 2010; Moraes *et al.*, 2016).

Twenty-two serovars and two rough strains were identified in 80 isolates (Table 2). The rough samples were not subjected to antimicrobial susceptibility test. The highest prevalence was observed for serovars *S.* Mbandaka (37.2%) and *S.* Braenderup (12.8%), followed by *S*. Senftenberg and *S*. Tennessee (6.4%), *S*. Saintpaul (5.1%), *S*. Rissen (3.8%), *S*. Sandiego, *S*. Javiana, *S*. Meleagridis, *S*. Panama, *S*. I.O7:eh:- and *S*. Cerro (2.6%) and, *S*. Oranienburg, *S*. Schwarzengrund, *S*. Ouakam, *S*. Muenster, *S*. Livingstone, *S*. Agona, *S*. Yoruba, *S*. Enteritidis, *S*. Minnesota and *S*. Derby, isolated in only one sample (1.3%). Previous studies have reported the simultaneous presence of several serovars on a same layer farms, such as *S.* Agona, *S.* O: 4,5, *S.* Schwarzengrund, *S.* Cerro, *S.* Anatum, *S.* Enteritidis, *S.* Johannesburg, *S*. Corvallis (Moraes *et al.*, 2016; Du *et al*, 2017), *S.* subesp. *enterica* 4,12: r: -, *S*. Mbandaka, *S.* subesp. *enterica* 6,7: z10: -, *S*. Havana, *S.* Braenderup (Freitas Neto *et al.*, 2014), *S*. Typhimurium, and *S.* subesp. *enterica* 4,12: d: - (Snow *et al.*, 2007; Chemaly *et al.*, 2009).

The most prevalent serovar isolated in the present study (*S*. Mbandaka) was also previously reported in layer farms in Argentina and Brazil, with prevalence rates of 11% and 7.5%, respectively (Kottwitz *et al.*, 2008; Soria *et al.*, 2017). Iwabuchi *et al.* (2010) detected *S*. Mbandaka in the dust of the poultry house air circulation system, which suggests that usual poultry house disinfection procedures are not able to eliminate this serovar, contributing to its persistence on infected farms.

S. Braenderup, the second most prevalent serovar isolated in this study (12.8%), is also responsible for human infections (EFSA, 2017; CDC, 2019), and it is associated with the consumption of eggs (Nor Faiza *et al.*, 2013; Long *et al.*, 2017) and of vegetables, such as tomatoes (Micallef *et al.*, 2012) and lettuce (Gajraj *et al.*, 2012). The third most prevalent serovar isolated, *S*. Senftenberg (6.4%), has been the leading cause of foodborne disease during the last decade (Pezzoli *et al.*, 2008). In Brazil, most cases of human salmonellosis are not reported, and therefore, we were not able to find epidemiological data on the serovars causing foodborne infections (SVS, 2019).

The low prevalence of some serovars determined in the present study may be attributed to their preference for other animal or plant infection targets. For instance, *S*. Enteritidis and *S*. Schwarzengrund serovars were the most prevalent in eggs samples (Moraes *et al.,* 2016), and *S*. Agona was associated with the consumption of contaminated fishmeal by layers (Berchieri Junior *et al.*, 1985; Clark *et al.*, 1973).

In Brazil, 58.8% of human foodborne disease agents are not identified; however, when diagnosed, *Salmonella* spp. is the most prevalent (SVS, 2015). The most prevalent serovars reported in foodborne outbreaks in the USA, European Union, and Brazil are *S*. Enteritidis and *S*. Typhimurium (EFSA, 2017; EU, 2018; CDC, 2019), and are typically associated with the consumption of poultry products (Jackson *et al.*, 2013; SVS, 2019). In the present study, *S.* Typhimurium was not found, and *S.* Enteritidis was isolated only in one farm. This result was expected, as the Brazilian government established a national plan for the control of *Salmonella* in breeder flocks in 1994 (Brasil, 2003). This plan requires the mandatory destruction of breeder flocks infected by *S.* Gallinarum biovars Gallinarum and Pullorum, and eggs from flocks infected with *S.*

Table 2 – *Salmonella* serovars identified in fecal samples of commercial layers reared in the midwestern region of São Paulo state, SP, Brazil, between 2016 and 2017.

Serovar	Number of positive samples	%	Serovar	Number of positive samples	%
S. Mbandaka	29	37.2	*S.* Cerro	2	2.6
S. Braenderup	10	12.8	*S.* Oranienburg	1	1.3
S. Senftenberg	5	6.4	*S.* Schwarzengrund	1	1.3
S. Tennessee	5	6.4	*S.* Ouakam	1	1.3
S. Saintpaul	4	5.1	*S.* Muenster	1	1.3
S. Rissen	3	3.8	*S.* Livingstone	1	1.3
S. Sandiego	2	2.6	*S.* Agona	1	1.3
S. Javiana	2	2.6	*S.* Yoruba	1	1.3
S. Meleagridis	2	2.6	*S.* Enteritidis	1	1.3
S. Panama	2	2.6	*S.* Minnesota	1	1.3
S. I.O7:eh:-	2	2.6	*S.* Derby	1	1.3

Enteritidis and *S.* Typhimurium serovars cannot be incubated.

In order to minimize the occurrence of bacterial diseases, antimicrobials have been routinely used as therapeutics both in human and veterinary medicine. However, in animal production, antimicrobials have also been applied for prophylaxis and performance enhancement for many years (Rodríguez *et al.*, 2012; Chen *et al.*, 2013; Burke *et al.*, 2014; Sapkota *et al.*, 2014; Cosby *et al.*, 2015; Pande *et al.*, 2015; Antunes *et al.*, 2016; Grant *et al.*, 2016; Du *et al.*, 2017), particularly β-lactams, aminoglycosides, amphenicols, quinolones, fluoroquinolones, tetracyclines and sulfonamides (Moreno-Bondi *et al.*, 2009).

The antimicrobial sensitivity test result of the present study showed that 100% of the isolates were resistant to at least one of the antimicrobials tested, and resistance to streptomycin was the most frequent, found in 93.5% of the isolates tested (Table 3). The resistance of *Salmonella* spp. to critically important antimicrobials, such as fluoroquinolones and extended-spectrum cephalosporins, is a significant public health concern, threatening the efficiency of antimicrobial agents (Campos *et al*., 2018; Hassena *et al*., 2019). High rates of *Salmonella* spp. non-susceptible to fluoroquinolones and cephalosporins were observed in humans (Andoh *et al.,* 2017). In the present study, *Salmonella* spp. isolates showed resistance to streptomycin (93.5%), sulfonamide (84.6%) and ciprofloxacin (41%), followed by nalidixic acid and enrofloxacin (20.5%), tetracycline and oxytetracycline (17.9%), sulfamethoxazole/trimethoprim (14.1%) and amoxicillin and amoxicillin/clavulanic acid (9%). Isolates were sensitive to norfloxacin, amikacin, gentamicin, aztreonam, cefotaxime, ceftiofur, cefepime, imipenem, chloramphenicol, and phosphomycin, which should be used with caution in order to prevent the emergence of resistance, where their use is allowed (Wright, 2010).

Considering that norfloxacin and ciprofloxacin belong to the same drug class, equal resistance profiles against both antimicrobials were expected. However, in the present study, isolates were shown to be sensitive to norfloxacin and presented intermediate resistance to ciprofloxacin. This may be explained by the fact that isolates with intermediate-resistance profile were classified as resistant due to the interpretation criterion

Table 3 – Antimicrobial susceptibility of the *Salmonella* strains isolated in commercial layer farms located in different municipalities of the Midwestern region of São Paulo state, SP, Brazil, between 2016 and 2017, expressed in number of resistant strains.

Serovars (number of isolates)	Antimicrobials																				
	NAL	CIP	ENO	NOR	AMC	AMO	AMI	GEN	STR	KAN	ATM	CTX	CTF	CPM	IPM	SUL	SXT	CLO	TET	OXI	FOS
S. Mbandaka (29)	4	11	4	-	4	4	-	-	28	-	-	-	-	-	-	26	8	-	8	8	-
S. Braenderup (10)	4	6	4	-	-	-	-	-	9	1	-	-	-	-	-	9	1	-	1	1	-
S. Senftenberg (5)	3	4	3	-	-	-	-	-	5	-	-	-	-	-	-	5	1	-	2	2	-
S. Tennessee (5)	-	2	-	-	-	-	-	-	5	-	-	-	-	-	-	4	-	-	-	-	-
S. Saintpaul (4)	1	1	1	-	2	2	-	-	4	-	-	-	-	-	-	2	-	-	2	2	-
S. Rissen (3)	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	3	-	-	-	-	-
S. Sandiego(2)	-	1	-	-	-	-	-	-	2	-	-	-	-	-	-	2	-	-	-	-	-
S. Javiana (2)	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	-	-	-	-	-
S. Meleagridis (2)	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
S. Panama (2)	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	-	-	-	-	-
S. I.O7:eh:- (2)	2	2	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
S. Cerro (2)	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	-	-	-	-	-
S. Oranienburg (1)	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
S. Schwarzengrund (1)	-	1	1	-	1	1	-	-	1	-	-	-	-	-	-	1	1	-	1	1	-
S. Ouakam (1)	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-
S. Muenster (1)	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-
S. Livingstone (1)	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-
S. Agona (1)	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-
S. Yoruba(1)	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-
S. Enteritidis(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
S. Minnesota (1)	1	1	1	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-
S. Derby(1)	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-
Total	16	32	16	0	7	7	0	0	73	1	0	0	0	0	0	66	11	0	14	14	0

-: Negative; NAL: Nalidixic acid; CIP: Ciprofloxacin; ENO: Enrofloxacin; NOR: Norfloxacin; AMC: Amoxicillin/Clavulanic acid; AMO: Amoxicillin; AMI: Amikacin; GEN: Gentamycin; STR: Streptomycin; KAN: Kanamycin; ATM: Aztreonam; CTX: Cefotaxime; CTF: Ceftiofur; CPM: Cefepime; IPM: Imipenem; SUL: Sulfonamide; SXT: Trimethoprim/Sulfamethoxazole; CLO: Chloramphenicol; TET: Tetracycline; OXI: Oxitetracycline; FOS: Fosfomycin.

adopted. Nevertheless, this discrepancy between sensitive and intermediate profiles remains unclear and further studies are needed to elucidate it. It should be emphasized that the emergence and dissemination of strains resistant to fluoroquinolones (ciprofloxacin) may lead to treatment failure of serious infections in humans (Chen *et al.*, 2013). The resistance profile of the isolates in the present study may be attributed to the presence of PMQR (plasmid-mediated quinolone resistance) genes, such as *qcnr*, *aac (6')-Ib-cr*, *qepA*, and *oqxAB*. According to Campbell *et al*. (2018), isolates harboring PMQR genes may show reduced susceptibility against ciprofloxacin and may not be resistant to nalidixic acid. In the study of Hopkins *et al*. (2007), all *Salmonella* serovars expressing both reduced susceptibility against ciprofloxacin and higher susceptibility to nalidixic acid harbored qnr genes. Resistance may also be affected by the number of *qnr* genes copies and by their transcription levels (Rodríguez-Martínez *et al*., 2006; Xu *et al*., 2007).

Polymyxin-E MIC results ranged between 0.5 and 4 µg/mL, and only two isolates were considered resistant, using 2 µg/mL as a cutoff point, according to CLSI (2017). Although most strains were characterized as sensitive to this antimicrobial, it should be emphasized that it must be cautiously used. Although the use of polymyxin A was discontinued in the 1970s due to its toxicity, it was reintroduced in the 1990s due to the emergence of multiresistant bacteria (Storm *et al.*, 1977; Poirel *et al.*, 2017). In 2016, the use of colistin sulfate as a performance enhancer was banned in Brazil (Brasil, 2016) in order to prevent cross-resistance of pathogens of public health concern (Hu *et al.*, 2017). Therefore, the identification of polymyxin E-resistant zoonotic *Salmonella* strains in layer farms in the present study is a matter of concern, as this drug is one of the last treatment choices for human salmonellosis, and resistance against broad-spectrum drugs may result in high mortality rates (Costa *et al.*, 2013; Shang *et al.*, 2018).

As described in Table 4, 18 resistance profiles were observed: two isolates were resistant to only one antimicrobial, while 16 showed multiple resistance (ranging from two to 10 antimicrobials). Out of the 78 isolates evaluated, 10 showed single resistance profile to sulfonamide (3/78) or to streptomycin (7/78). Multiresistant profile (resistant to three or more antimicrobials of different classes) were observed in 32 isolates, which were grouped in 11 different profiles. *S*. Schwarzengrund serovar presented the maximum resistance profile, being resistant to 10 of the antimicrobials tested.

Table 4 – Antimicrobial resistance profiles of *Salmonella* strains isolated in commercial layer farms located in different municipalities of the Midwestern region of São Paulo state, SP, Brazil, between 2016 and 2017.

Antimicrobial resistance profile	Number of strains	Serovar (Number of strains)
SUL	3	*S.* Rissen (1), *S.* Braenderup (1), *S.* Mbandaka (1)
STR	7	*S.* Oranienburg (1), *S.* Mbandaka (2), *S.* Saintpaul (1), *S.* Tennessee (1), *S.* Meleagridis (2)
SUL/STR	31	*S.* Cerro (2), *S.* Braenderup (2), *S.* Mbandaka (12), *S.* Saintpaul (1), *S.* Panama (2), *S.* Tennessee (2), *S.* Senftenberg (1), *S.* Sandiego (1), *S.* Yoruba (1), *S.* Javiana (2), *S.* Rissen (2), *S.* Agona (1), *S.* Livingstone (1), *S.* Ouakam (1)
SUL/STR/CIP*	13	*S.* Tennessee (2), *S.* Mbandaka (5), *S.* Braenderup (2), *S.* Derby (1), *S.* Senftenberg (1), *S.* Sandiego (1), *S.* Muenster (1)
SUL/NAL/CIP	1	*S.* Enteritidis (1)
NAL/ENO/CIP	1	*S.* I. O7:eh:- (1)
NAL/ENO/STR/CIP	2	*S.* Mbandaka (1), *S.* I. O7:eh:- (1)
SUL/NAL/ENO/STR/CIP*	5	*S.* Braenderup (3), *S.* Senftenberg (1), *S.* Minnesota (1)
KAN/NAL/ENO/STR/CIP	1	*S.* Braenderup (1)
SUL/SXT/TET/OXI/STR*	1	*S.* Braenderup (1)
SUL/SXT/TET/OXI/STR/CIP*	1	*S.* Mbandaka (1)
SUL/NAL/TET/OXI/ENO/STR/CIP*	1	*S.* Senftenberg (1)
SUL/AMC/AMO/AMP/TET/OXI/STR*	1	*S.* Saintpaul (1)
SUL/SXT/NAL/TET/OXI/ENO/STR/CIP*	4	*S.* Senftenberg (1), *S.* Mbandaka (3)
SUL/SXT/AMC/AMO/AMP/TET/OXI/STR*	2	*S.* Mbandaka (2)
AMC/AMO/AMP/NAL/TET/OXI/ENO/STR/CIP*	1	*S.* Saintpaul (1)
SUL/SXT/AMC/AMO/AMP/TET/OXI/STR/CIP*	2	*S.* Mbandaka (2)
SUL/SXT/AMC/AMO/AMP/TET/OXI/ENO/STR/CIP*	1	*S.* Schwarzengrund (1)

*Serovars resistant to three or more antimicrobial classes were classified as multiresistant (Schwarz *et al.*, 2010).

NAL: Nalidixic Acid; ENO: Enrofloxacin; AMC: Amoxicillin/Clavulanic Acid; AMP: Ampicillin; AMO: Amoxicillin; STR: Streptomycin; SUL: Sulfonamide; SXT: Trimethoprim/Sulfamethoxazole; TET: Tetracycline; OXI: Oxytetracycline; KAN: Kanamycin.

The incidence of antimicrobial resistance has increased over the years due to their frequent use in human and veterinary medicine, as well as to the adaptation and genetic reorganization of bacteria, including *Salmonella* spp. (Chen *et al.*, 2013; Burke *et al.*, 2014; Sapkota *et al.*, 2014; Cosby *et al.*, 2015; Pande *et al.*, 2015; Antunes *et al.*, 2016; Grant *et al.*, 2016). The association of these factors favors the emergence of multiresistant bacteria, which is often related to human mortality rates in hospitals or intensive care units (Rodríguez *et al.*, 2012; Du *et al.*, 2017). For instance, the use of ampicillin in livestock may lead to the emergence of bacterial strains resistant to this drug, and which may also be co-resistant to third-generation cephalosporins used to treat animal infections (Jensen *et al.*, 2018).

The antimicrobial incidence rate found in the present study is higher than those previously reported for *Salmonella* in broilers, of maximum phenotype resistance profile against eight antimicrobials in Brazil (Mion *et al.*, 2014) and seven in Mexico (Arslan & Eyi, 2010). However, it is lower compared with the findings of Yan *et al.* (2010), who reported *Salmonella* spp. isolates resistant to up to 20 antimicrobials in China. Although studies evaluated different antimicrobials, they demonstrate the emergence of multiresistant bacterial strains.

Antunes *et al.* (2003) reported the 39% *Salmonella* serovars isolated from poultry products were resistant to streptomycin, while 93.5% streptomycin-resistant strains were characterized in the present study. This result may be attributed to the use of subtherapeutic doses of this antimicrobial in poultry production, which annually reduces its therapeutic effectiveness (Manie *et al.*, 1998; Liljebjelke *et al.*, 2017). Sulfonamides were the second antimicrobial class showing the second highest resistant prevalence (84.6% of the isolates); however, when associated with trimethoprim, significantly lower resistance prevalence was determined, of only 14.1% of the isolates. Castagna *et al.* (2001) and Galdino *et al.* (2013) reported higher efficacy of that association when evaluating *Salmonella* spp. sensitivity in animal isolates. Due to its effectiveness, sulfonamide-trimethoprim association is widely used for the treatment of human infections; however, a 30% annual increase in *Salmonella* strains resistant to this association has been observed (Barlow *et al.*, 2014; Tagg *et al.*, 2019).

As the emergence of *Salmonella* isolates resistant to ampicillin and sulfonamide/trimethoprim has increased over the years, broad-spectrum antimicrobials, such as ciprofloxacin and cephalosporins, have been applied for the treatment of human salmonellosis (Chen *et al.*, 2013; Antunes *et al.*, 2016). Moreover, since the use of fluoroquinolones was allowed in animal production, *Salmonella* spp. strains resistant to ciprofloxacin have been isolated in both animal products and humans (Cosby *et al.*, 2015). The 41% rate of ciprofloxacin-resistant strains determined in the present study is in agreement with previous studies, which also found high incidence rates of resistant *Salmonella* spp., of 25.0% in Ghana (Andoh *et al.*, 2017) and of 35.1% in Shanghai (Wei *et al.*, 2019). These results are a matter of concern, as the emergence of such strains may lead to failure of the treatment of severe bacterial infections in human medicine (Costa *et al.*, 2013; Shang *et al.*, 2018).

The occurrence of multi-drug resistant strains in humans may also be related to antimicrobial residues in food sources. The 2017 annual report published by the Brazilian Ministry of Agriculture on non-conformities in animal products showed that drug residues were found in 1.21% (12/3,894) of poultry products samples, with 1.03% (6/584) in eggs and 0.18% (6/3,310) in broiler products, respectively. The main drugs detected were enrofloxacin (1.81%), sulfaquinoxaline (0.17%), sulfamethazine (0.36%), doxycycline (0.08%), nicarbazin (0.85%), and arsenic (0.51%) (Brasil, 2018). This report emphasizes the public health concerns with the consumption of antibiotics in foods of animal origin, as only one of the drugs found was not derived from animal products, and resistance to two of the antibiotics reported were found in the present study, which may contribute to increase the populations of resistant and multiresistant bacteria.

The intense selective pressure imposed by the indiscriminate administration of antimicrobials has led to the emergence of bacterial strains resistant to these compounds (Wright, 2010). The emergence and dissemination of resistant *Salmonella* spp. isolated from food of animal origin has important consequences for public health, as it limits treatment efficiency and increases mortality rates in humans (Costa *et al.*, 2013; Shang *et al.*, 2018).

The present study showed that 52.9% (80/151) of the evaluated farms were positive for *Salmonella* spp., with 22 serovars isolated, and that 100% of the isolates were resistant to at least one of the tested antimicrobials, with 41% presenting multiple-drug resistance. The results emphasize the need to promote policies for the rational use of antimicrobials and for the effective implementation of biosecurity

measures. In addition, the serotyping of *Salmonella* spp. isolated from poultry farms is required to provide epidemiological knowledge for the development of prevention and control measures of this pathogen aiming at ensuring animal health and product quality certification.

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