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Serotypes and antimicrobial resistance profiles of *Salmonella* isolated from fresh beef processing and chilled fresh beef samples produced and marketed in the metropolitan region of Cuiabá, in the State of Mato Grosso, Brazil

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***Salmonella* spp. in food products of animal origin can cause foodborne infections, and when antimicrobial-resistant strains are present, can evolve to severe disease. This study aims to determine the occurrence of *Salmonella* and identify the serotypes present in the beef production chain of the metropolitan region of Cuiabá, State of Mato Grosso, Brazil, and evaluate their antimicrobial resistance profile. The presence of *Salmonella* was determined by the ISO-6579:2002 method and evaluation of resistance to 17 antibiotics was performed by the CSLI 2014 method. The following samples were analyzed: (1) environmental samples: corral swab, bleeding, skinning and deboning knives, ribbon saw, boning bench; (2) animal samples: anal swab, internal and external carcass sponge, organ pool, boning shavings and by-products in the slaughterhouse; and (3) ground meat samples from retail sales from 10 butcher shops and permanent fair stalls. *Salmonella* was present in ten of the 182 samples (5.5%), five of which were *Salmonella* Panama detected in the corral, boning tables, meat from the butcher's shop and permanent market. These strains were sensitive to all antibiotics characteristics and similar to *Salmonella enterica* rough, and being isolated from the ribbon saw used in the production chain. However, *Salmonella* Anatum and *Salmonella* Infantis isolated from carcasses, bone meal and viscera presented resistance to sulfonamides, trimethoprim, sulfametazol/ trimethoprim and nitrofurantoin in relation to the use of antibiotics in animal production and the selective pressure of multiresistant *Salmonella* spp. in products derived from these animals.**

Key words: Serotyping, bovine meat, slaughterhouse, butcher, antibiotic resistance.

INTRODUCTION

Salmonella is the most frequently isolated bacteria responsible for diagnosed cases of foodborne diseases worldwide (Puig Peña et al., 2011). *Salmonella* presents more than 2600 serotypes among which *Salmonella* Typhi is pathogenic to humans (Grimont and Weill, 2007; Issenhuth-Jeanjean et al., 2014).

However, some nontyphoidal serotypes considered as pathogenic to humans also exist which are frequently isolated from food infection cases as well as from systemic infections, such as meningitis and bacteremia (Zaidi et al., 2006). These include *Salmonella* Enteritidis and *Salmonella* Typhimurium, although *Salmonella* Infantis, *Salmonella* Anatum, and *Salmonella* Panama have also been cited as infectious agents and/or have been detected in food products involved in cases of food or systemic infections (Hendriksen et al., 2011; Huang et al., 2013; Capalonga et al., 2014).

Serotypes *S.* Infantis, *S.* Panama, and *S.* Anatum are among the 15 main *Salmonella* serotypes considered agents of human infection and food contaminants occurring in Brazil, in both the southeastern (São Paulo) and southern (Rio Grande do Sul) regions in the 1990s and between 2007 and 2012, respectively (Tavechio et al., 2002; Huang et al., 2013). Globally, the distribution of these serotypes is diverse, with *S.* Infantis found in Africa, North America and Latin, Asia, Europe and Oceania; *S.* Anatum reported in Africa, Latin America and Oceania and, *S.* Panama present only in Asia and Latin America. These serotypes frequently range from the fifth to the fourteenth position as agents of human infection (Hendriksen et al., 2011).

Worldwide, meats, their derivatives, and foods produced from mechanically deboned meat are more frequently involved in food infection cases caused by *Salmonella* (Puig Peña et al., 2011). In Brazil, the food products involved in these cases have been reported, mainly, as eggs, chickens and their by-products. However, reports of cases, where red meat and its by-products are implicated, are on the rise, especially regarding *S.* Infantis and *S.* Panama (Capalonga et al., 2014).

The emergence of Multi-Resistant Drug (MDR) *Salmonella* is a worldwide concern. This is a consequence of the extensive use of antibiotics in humans and animals, being a risk condition that increases the severity of the disease and hospitalization rates, as well as the possibility of death (Ribeiro et al., 2007; Huang et al., 2013). The antimicrobial resistance of *Salmonella* currently, represents a worldwide problem for both the veterinary and public health sectors. An increase in the incidence of antibiotic-resistant *Salmonella* strains in production animals and their by-products is observed, especially in Latin America, particularly in cattle, although studies in this regard are still scarce (Perez-Montaño et al., 2012; Capalonga et al., 2014). Therefore, more information is needed on the occurrence of MDR *Salmonella* in this matrix to assist in the control of the indiscriminate use of antibiotics.

Brazil is one of the largest beef producers in the world, with a production of 9.56 million tons in 2015. Of this total, 19.63% was exported, and 81% was consumed in the local market (ABIEC, 2016). In this context, the State of Mato Grosso leads the slaughter capacity among all Brazilian States, slaughtering 35.466 cattle a day (ABIEC, 2016). Hence, the importance of *Salmonella* prevalence investigation in this state contributes significantly to the Brazilian food safety and public health surveillance. The present study aimed to investigate the occurrence of *Salmonella* and identify the serotypes and their antimicrobial resistance profiles, by evaluating the beef production chain, from the production environment to the wholesale market in the slaughterhouse, as well as retail sales from butcher shops and municipal market stalls, in the metropolitan region of Cuiabá, Mato Grosso, Brazil.

MATERIALS AND METHODS

Sampling

A total of 182 samples were evaluated; 156 slaughterhouse samples and 26 butcher shop samples. The slaughterhouse samples were collected during the processing of 13 batches of animals from different cities and farms. Environmental samples were obtained from the corral and boning tables (I, II and III), utensils (bleeding, skinning and boning knives) and equipment (ribbon saw). Animal samples were obtained from anal swabs, internal and external carcass sponges, organ pool (esophagus, diaphragm, and masseter muscle), boning shavings and bone and viscera meal.

All environmental and anal region samples from the animals were harvested with 3M ™ Sampler swabs containing Buffered Peptone Water (BPW). 3M™ Sponge-Sticks containing buffered peptide water were used for sample collection from the neck, thorax, abdomen and hindquarter regions, using a sterilized field boundary bracket (10 cm by 10 cm, or 10 0 cm^2). The tissue fragments were packed in sterile plastic bags. Subsequently, for convenience, another 26 samples consisting of portions of 500 g of chopped meat (*quadriceps femoris*) were obtained from five butcher's shop in the city districts, and five-meat stall at a permanent fair, 12 and 14 samples, respectively. The samples from both the slaughterhouses and retailers were collected under refrigeration and immediately taken to the laboratory and analyzed, from April to July 2015, in the Cuiabá metropolitan region, latitude: 15°35'46 "S; Longitude: 56°05'40 "W, the State of Mato Grosso, Brazil.

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Isolation and identification of *Salmonella* species

The isolation method was based on the protocol recommended by the International Standardization Organization (ISO-6579:2002). Briefly, 25 g of the sample were inoculated in Buffered Peptone Water (Himedia®, Mumbai, India), incubated at 37°C for 24 h, enriched in Rappaport-Vassiliadis Broth (Oxoid®, United Kingdom), incubated at 42°C for 24 h and then in Muller Kauffmann Novobiocin Tetrathionate Broth (Himedia®, Mumbai, India) at 37°C for 24 h, with subsequent plating on Xylose Lysine Deoxycholate Agar (Himedia®, Mumbai, India) and Rambach Agar (Merck, Darmstadt, Germany), incubated at 37°C for 24 h.

The typical colonies were selected, purified on Nutrient Agar and subsequently inoculated on API 20E (BioMérieux®, Lyon, France). The strains that showed a typical *Salmonella* reaction were subjected to serum-agglutination by the anti-salmonella polyvalent O serum.

DNA extraction

The strains biochemically identified as *Salmonella* were inoculated in 10 mL of BHI Broth (Brain Heart Infusion) and incubated at 35°C for 24 h. A 1.5 mL aliquot was then centrifuged at 14,000 x *g* for 5 min; the pellet was dissolved in 500 µL Mili-Q water, and heated at 100°C for 10 min on a heating plate (BioGPRO, Brazil), then cooled at 4°C for 10 min. The lysate was then centrifuged at 14,000 x *g* for 5 min, and 200 µL of the supernatant was removed, maintained in a freezer and subsequently subjected to multiplex PCR.

Multiplex-PCR

The reaction was performed in a total volume of 25 µL containing 1U Taq Polymerase (Invitrogen®), 1x Taq buffer (5 mM KCl Tris-HCl, pH 8.5) 1.5 mM MgCl 2, 0.1 mM dNTP's (Promega®), 0.9 MM primer Inv-A, and 0.4 µM of IE-1 and Flic-C primers (Invitrogen®). The conditions were based on the study performed by Paião et al. (2013).

The m-PCR assay was performed with an initial denaturation for 5 min at 95°C, followed by 30 cycles one min at 95°C, 1 min at 58°C, and 30 sec at 75°C, with a final extension step at 72°C for 7 min. The PCR product was analyzed by electrophoresis on 1.5% agarose gels, TBE buffer (45 mmol L^{-1} Tris pH 8.3, 45 mmol L^{-1} borate, and 2 mmol L^{-1} EDTA) as the running buffer. The gels were then stained with Gel Red (Invitrogen®) and photo documented (MiniBis-Pro DNT, Bio-Imaging Systems®).

Antimicrobial susceptibility test

The isolates were submitted to antimicrobial susceptibility tests by the disc diffusion method employing 17 antimicrobials: ampicillin, 10 mcg; aztreonam, 30 mcg; cephalothin, 30 mcg; cefoxitin, 30 mcg; ceftiofur, 30 mcg; chloramphenicol, 30 mcg; florfenicol, 30 mcg; streptomycin, 300 mcg; gentamicin, 10 mcg; nalidixic acid, 30 mcg; ciprofloxacin, 5 mcg; enrofloxacin, 5 mcg; tetracycline, 300 mcg; sulfamethoxazole trimethoprim. 25 mcg; sulfonamide, 300 mcg; trimethoprim, 5 mcg and nitrofurantoin, 300 mcg (Cecon®, Brazil). The discs were distributed equidistantly in plates which were then incubated at 35°C for 16 to 20 h (CLSI, 2014).

Inhibition halos were measured, and the results were compared to standards contained in the Clinical and Laboratory Standards Institute (Patel and CLSI, 2016), and classified as sensitive, intermediate or resistant. Screening of strains suggestive of Extended-Spectrum Beta-lactamase (ESBL) producers was also carried out, using the β-Lactams breakpoint criterion for *Enterobacteriaceae* that detects all resistance mechanisms, including ESBL and plasmid-mediated AmpC. According to Cavalieri et al. (2005) strains with halos ≤22 mm for ceftazidime (30 mcg), and ≤27 mm for aztreonam (30 mcg) are potential β-lactamase-producing strains suggestive of ESBL.

***Salmonella* spp. serotyping**

Salmonella serotype identification was carried out at the National Reference Laboratory Diagnosis of Enteric Bacteria, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), by detection of somatic and flagellar antigens, using polyvalent and monovalent antisera, with or without flagellar phase induction (Voss-Rech et al., 2015).

RESULTS AND DISCUSSION

Among the 182 bovine samples (from the environment, carcass, and byproducts), 5.5% (10/182) were positive for *Salmonella* in the biochemical, serological and molecular tests (multiplex PCR).

The presence of this bacterium in the 5.1% (8/156) of the slaughterhouse samples, 8.3% (1/12) of the butcher shop samples and 7.1% (1/14) of the permanent fair samples (Figure 1), is in disagreement with the Brazilian legislation, which advocates its absence in bovine meat (BRASIL, 2001). Studies carried out in Mato Grosso, in the city of Barra do Garças, in ground butchered meat indicated the occurrence of 17% *Salmonella* (Sousa et al., 2012). This microorganism was also detected in 12.5% of fresh meat samples evaluated before and after deboning in Cuiabá butcher shops (Sigarini et al., 2006). These percentages are higher than those detected in the present study, although the strain serotypes were not determined in the studies above. This high detection may be because only butcher shops, not slaughterhouses, were included in these evaluations.

Salmonella was found in environmental samples in the evaluated slaughterhouse: serotype *S.* Panama was detected in the corral (1 strain) and at deboning (2 strains), while *Salmonella enterica* subspecies *enterica* rough was observed in the ribbon saw strains isolated from four different lots (2, 4, 6 and 11) during different weeks. Serotypes *S.* Anatum and *S.* Infantis were isolated from carcasses and bone and viscera meal in slaughterhouse samples (Table 1). Two strains of *S*. Panama were isolated from samples of chopped meat (*quadriceps femoris*) collected one at the neighborhood butcher's shop and another at the meat stall of a permanent fair in Cuiabá (Table 1).

Usually, contamination by these bacteria occurs due to inadequate hygienic and handling conditions in the slaughterhouse. In this context, microbiological evaluations performed on utensils, environment, and carcasses of cattle, pigs, and sheep in the slaughterhouse of Australia (Bakhtiary et al., 2016), in Mato Grosso (Santos et al., 2017) and Rio de Janeiro (Cabral et al., 2014), Brazil have been reported. In these studies, the occurrence of microorganisms was observed

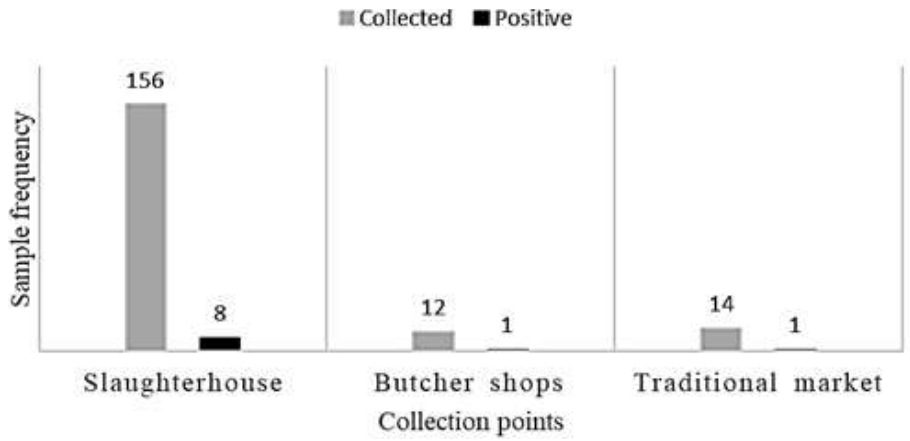

Figure 1. Distribution of the evaluated samples by collection points, and their positivity frequency for *Salmonella* in beef from Mato Grosso, Brazil.

Table 1. Frequency of the *Salmonella* serovars and antibiotic resistance profile of 10 strains isolated from a bovine slaughterhouse and butcher shops in the State of Mato Grosso, Brazil.

Serotype *Salmonella*	Number of strains	Samples	Antimicrobial resistance
S. Anatum	2	Carcass sponge	Tri, Sul
		Bone meal	Sut, Tri, Sul
S. Infantis	2	Carcass sponge	Nit, Sut, Tri, Sul
		Viscera meal	Sut, Tri, Sul
S. *enterica* rough	1	Ribbon saw swab	-
S. Panama	5	Corral Swab (1)	-
		Boning stand (2)	-
		Chopped meat (2)	-

Nit, nitrofurantoin (300 mcg); Tri, trimethoprim (5 mcg); Sut, sulfamethoxazole/trimethoprim (25 mcg); Sul, sulfonamide 300 mcg.

which are indicators of fecal contamination, belonging to the *Enterobacteriaceae* family, species such as *Escherichia coli*, and *Salmonella enterica* in utensils, environment, and carcasses indicating hygiene problems in the slaughter process.

S. Anatum and *S.* Infantis were observed both in the bovine carcasses and by-products (bone meal and viscera). *S.* Anatum is commonly found in cattle and has frequently been detected in feces, skin, lymph nodes, meat fluids and bovine carcasses of both dairy and beef in the southern United States (Kunze et al., 2008), as well as in beef from Mexico (Varela-Guerrero et al., 2013), Namibia (Shilangale et al., 2015) and South Africa (Madoroba et al., 2016). Serotypes *S*. Infantis and *S*. Panama were also reported in carcasses and processed meat from four slaughterhouses in the State of Jalisco, Mexico (Perez-Montaño et al., 2012). *S.* Anatum *S*. Panama and *S.* Infantis have also been detected in ground beef from three cities in the same Mexican State (Cabrera-Diaz et al., 2013).

In Brazil, only *S.* Panama and *Salmonella enterica* subspecies *enterica* rough were found in a salami production line in the State of Paraná, with *S.* Panama being the most frequently detected (Ribeiro et al., 2007). In the present study, these serotypes were detected in the slaughterhouse as environmental contaminants present in the corral, deboning tables and ribbon saw. *S*. Panama was present in the ground meat samples (*quadriceps femoris*) from the retail trade, butcher shops and the permanent fair stalls in Cuiabá. This data is similar to reported for South Korea, where *S*. Panama was detected in beef, although those samples were marketed wholesale (Hyeon et al., 2011). Serotypes *S.* Panama and *S.* Infantis were also detected in beef and other animals (poultry and pork) in the retail trade in São Paulo, Brazil (Jakabi et al., 2004). In Italy, serotype *S.* Panama was found in pork, while *S.* Infantis occurred in pork and poultry and *S.* Anatum was detected in pork and beef (Busani et al., 2005).

The presence of serotypes *S.* Anatum and *S.* Infantis in

carcasses from the slaughterhouse and *S.* Panama in samples from the butcher and permanent fair samples are concern from public health point of view. These serotypes have also been detected in processed beef implicated in cases of food poisoning in southern Brazil (Capalonga et al., 2014). In Yucatan, Mexico, a study on *Salmonella* as an agent of food infections observed that serotypes *S.* Anatum, *S.* Infantis, and *S.* Panama were isolated from both patients and food, including poultry, beef and pork (Gutiérrez-Cogco et al., 2000). Epidemiological studies have shown that serotypes *S.* Anatum, *S.* Infantis, and *S.* Panama are frequently isolated from contaminated foods and human infections in southeastern Brazil since the 1990s (Tavechio et al., 2002), and in southern Brazil from 2007 to 2012 (Capalonga et al., 2014). In Mexico, the *S.* Anatum serotype was isolated more frequently from products of non-human origin, whereas *S.* Infantis and *S.* Panama serotypes were isolated from humans (Gutiérrez-Cogco et al., 2000). The distribution of *Salmonella* serotypes isolated from humans, animals, and food worldwide indicates that serotypes *S.* Anatum and *S.* Infantis are frequently isolated in certain countries in Africa and Oceania, while *S.* Anatum, *S.* Infantis, and *S*. Panama have been reported in Asia, Latin America and Europe and *S.* Infantis is among the most frequently isolated serotypes in North America (Hendriksen et al., 2011).

In the present study, *S.* Anatum and *S.* Infantis displayed resistance to antibiotics belonging to the antifolate (sulfonamide, trimethoprim, and sulfamethoxazole/trimethoprim) and nitrofuran classes, while *S.* Panama and *Salmonella enterica* subspecies *enterica* rough strains were sensitive to all tested antimicrobials (Table 1). *S.* Infantis displaying resistance to trimethoprim/sulfamethoxazole has been observed in carcasses and *in natura* bovine meat from Mexico, in strains isolated from slaughterhouses (Cabrera-Diaz et al., 2013), as well as *S.* Anatum from retail markets (Perez-Montaño et al., 2012). *S.* Panama sensitive to all tested antibiotics have also been detected in slaughtered carcasses (Cabrera-Diaz et al., 2013; Varela-Guerrero et al., 2013), corroborating the present findings. *S.* Panama strains resistant to ampicillin, chloramphenicol, streptomycin, gentamicin, tetracycline, and sulfamethoxazole/trimethoprim have also been observed in ground beef samples from the State of Jalisco, Mexico (Cabrera-Diaz et al., 2013). In the present study, no difference in the antimicrobial susceptibility profile was observed among the *S. Panama* strains from the slaughterhouse and in the retail trade.

Non-typhoid *Salmonella* strains, including serotypes *S.* Anatum, *S.* Infantis, and *S.* Panama, can cause gastroenteritis, which can progress to systemic infections (bacteremias and meningitis) (Huang et al., 2013; Rowlands et al., 2014). These strains may display an invasive capacity, a characteristic of multi-resistance to antimicrobial drugs, which are genes encoded capacity that may potentiate their persistence in the organism and hence, their pathogenicity (Ribeiro et al., 2007; Huang et al., 2013).

Studies suggest that treatment in cases of contamination by antibiotic-resistant *Salmonella* Typhimurium strains can promote their permanence within the host, thus increasing the virulence, transmissibility, and spread of the disease (Diard et al., 2014). This leads to the possibility that the serotypes *S.* Anatum and *S.* Infantis, present in carcass, bones and viscera meal may display higher pathogenic potential than serotypes *S.* Panama and *S. enterica* rough, present in the slaughterhouse environment and in retail meat since they display sensitivity to all evaluated antibiotics.

Conclusions

We verified that *Salmonella* serotypes *S.* Anatum, *S. enterica* subspecies *enterica* rough, *S.* Infantis and *S.* Panama were present in the meat production chain from the metropolitan region of Cuiabá, in the State of Mato Grosso, Brazil.

For some serotypes of *Salmonella* detected in this, productive chains were mentioned in other regions of Brazil, and the world as the causal agent of foodborne diseases, which was transmitted by the meat. Isolated from carcasses and animal by-products (*S.* Anatum and *S.* Infantis) were resistant to the antifolate and nitrofuran classes. This suggests the need to use antibiotics cautiously in veterinary medicine and human to limit the resistance of these microorganisms to antimicrobials. For these, results may be related to the use of antibiotics in animal production and selection of multiresistant *Salmonella* spp. that persists in animal products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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