JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Salmonella Schwarzengrund, Akuafo, and O:16 isolated from vacuum-packaged beef produced in the state of Mato Grosso, Brazil

Bárbara Muller[1], Adelino Cunha-Neto[1], Vinicius Silva Castro[2], Ricardo Cesar Tavares Carvalho[1,3], Larrayane Albuês Carvalho Teixeira[1], Dália dos Prazeres Rodrigues[4], Eduardo Eustáquio de Souza Figueiredo[1,5]

[1] Postgraduate program in Nutrition, Food and Metabolism, Faculty of Nutrition, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil
[2] Postgraduate program in Food Science, Chemistry Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil
[3] Postgraduate Program in Animal Bioscience, Faculty of Veterinary Medicine, Cuiabá University, Cuiabá, Mato Grosso, Brazil
[4] National Reference Laboratory for Diagnosis of Enteric Bacteria, Oswaldo Cruz Fundation, Rio de Janeiro, Rio de Janeiro, Brazil
[5] Postgraduate program of Animal Science, Faculty of Agronomy and Zootechnics, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil

Abstract

Introduction: *Salmonella* spp. is a pathogen associated with foodborne infections, mainly in foods of animal origin. In this context, the present study investigated the occurrence of *Salmonella* serotypes, genotypes and the antimicrobial resistance profiles of strains in fresh beef produced in Mato Grosso, Brazil.

Methodology: A total of 107 samples from 13 different slaughterhouses in the Mato Grosso were analyzed. Suggestive *Salmonella* spp. colonies detected during the biochemical screening were submitted to DNA extraction, and *hilA* gene amplification was used for the PCR reaction. Antimicrobial resistance analyses were performed using 17 antimicrobial agents from eight different classes by the disk diffusion method. Strains exhibiting multiple drug resistances were submitted to PCR genotyping based on repetitive sequences (rep-PCR), using a commercial semiautomatic DiversiLab® system.

Results: A total of 5.6% (6/107) of the samples tested positive by the conventional method and were confirmed by PCR, namely two *S.* Akuafo, two non-typable *Salmonella* enterica strains, one *Salmonella* O:16 serovar, and one *S.* Schwarzengrund. The antimicrobial resistance profiles indicated resistance to gentamicin (30%), tetracycline, nitrofurantoin, and trimethoprim + sulfamethoxazole (16%). Genotyping indicated a 70% difference between *S.* Schwarzengrund and the non-typable *Salmonella* strains. No genetic similarities were observed between the six *Salmonella* isolates based on rep-PCR, including two *S.* Akuafo.

Conclusions: The results obtained herein corroborate that *Salmonella* serovar Schwarzengrund is commonly isolated in animal products in the state of Mato Grosso, Brazil, also highlighting the presence of two unusual *Salmonella* serovars in beef (Akuafo and O:16).

Key words: Food production; microbial contamination; foodborne illness; beef contamination; beef exports.

J Infect Dev Ctries 2021; 15(12):1876-1882. doi:10.3855/jidc.13726

(Received 19 August 2020 – Accepted 07 April 2021)

Introduction

Salmonella are a public health concern in both high- and low-income countries [1]. Typhoid fever is mainly endemic in low to middle income countries (LMICs), although non-typhoidal *Salmonella*, including invasive serovars that cause systemic diseases, are becoming a public health concern worldwide and comprise the third leading cause of death from foodborne illnesses [1,2]. This bacterium is commonly associated with meat consumption, such as beef, pork and poultry, and derived products [2]. Food chain contamination by *Salmonella* in Brazil has been noted, and *S.* Schwarzengrund has been described for both chicken and pork [3], as well as for cheese [4]. The importance of beef to the spread of *Salmonella* spp. in different Brazilian regions, including the south [5,6], northeast [7] and southeast [8] is clear, although more data are required.

Moreover, the presence of multidrug-resistant (MDR) *Salmonella* strains has been reported in animal production [9]. Antimicrobial resistance in bacteria can lead to human infections that often require prolonged

and more expensive treatments and lead to higher disability and death rates compared to sensitive strains [10]. In this regard, a comprehensive study on *Salmonella* spp. in chicken slaughterhouses conducted by our group in the state of Mato Grosso indicated the occurrence of multidrug-resistant strains [11] and a review of available genome sequences demonstrates the paucity of current data [12]. Therefore, assessments in this regard are indispensable to the elaboration of effective disease control plans, and the accurate identification of *Salmonella* spp. in animals and food products is the key to understanding the epidemiological dynamics of salmonellosis [13,14].

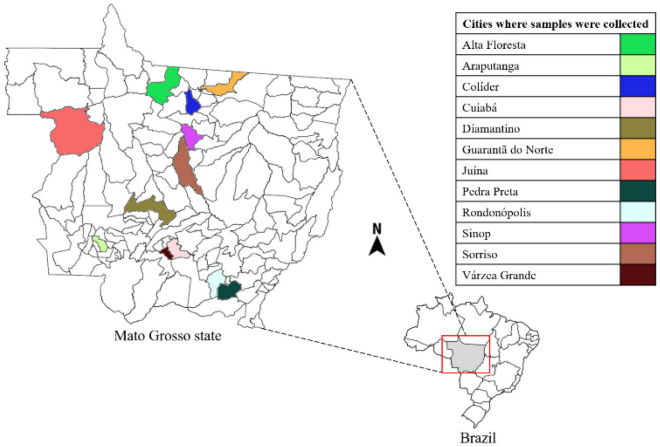
The state of Mato Grosso is located in the central-west region of Brazil and is currently the largest beef producer in the country [15]. Ninety percent of the cattle herds reared in the region are destined to beef production for both the internal and external markets [16]. This state was responsible for 15.4% (1.1 million tons) of all Brazilian exports, generating 4.3 billion dollars [15]. In this context, the present study aimed to investigate the occurrence of *Salmonella* spp. in fresh vacuum-packaged beef, through serotyping and genetic profile determinations and the identification of the presence of antimicrobial-resistant strains.

Methodology

Sample collection

A total of 107 fresh beef samples were assessed, represented by different meat cuts, with an average weight of 1.5 kg. All fresh beef samples were analyzed chilled and vacuum-packaged. The samples were collected randomly from 13 different slaughterhouses, which undergo either federal or state inspection services, located in 12 different cities in Mato Grosso (Figure 1). The selected beef cuts were: paleta (*supraspinatus*), acém (*trapezius thoracis*), picanha (*biceps femoris*), alcatra (*medium gluteos*), filet mignon (*psoas major*), lagarto (*semitendinosus*) and lombo (*longissimus dorsi*). The samples were received at the Food Molecular Microbiology Laboratory, at the Federal University of Mato Grosso (LABMMA/UFMT), chilled between 1 °C and 8 °C, from April to October 2016.

Figure 1. Geographic distribution of the cities were the beef cattle samples assessed herein were obtained, state of Mato Grosso, Brazil.



Salmonella spp. detection

The samples were submitted for *Salmonella* detection based on an adapted ISO-6579:2002 protocol [17]. Briefly, ten grams of each sample were pre-enriched in a suspension containing 90 mL of Buffered Peptone Water broth (Oxoid®, Basingstoke, United Kingdom), and incubated at 41.5 °C for 24 hours. Subsequently, 0.1 mL were added to 10 mL of Rappaport-Vassiliadis broth (Oxoid®, Basingstoke, United Kingdom) and 1 mL of the same culture in 9 mL of Muller Kauffmann Tetrathionate broth (Himedia®, Mumbai, India), incubated at 42 °C and 37 °C, respectively, for 24 hours. After incubation, one microliter of each presumptive *Salmonella* growth medium was inoculated onto Xylose Lysine Deoxycholate agar (Himedia®, Mumbai, India) and Brilliant Green Agar (Himedia®, Mumbai, India) plates and incubated at 37 °C by 24 hours. Typical colonies (red colony with a black center and red translucent halo) were picked and cultivated again on Nutrient agar incubated at 37 °C for 24 hours. Subsequently, suspected colonies were subjected to biochemical tests on Triple Sugar Iron Agar (TSI), Lysine Decarboxylase Agar (LIA) and Urea Agar. Strains with typical reactions were considered suggestive for *Salmonella* spp.

DNA extraction and quantification

Suggestive *Salmonella* spp. colonies detected in the biochemical screening were inoculated in 10 mL of Brain Heart Infusion Broth - BHI (Himedia®, Mumbai, India), and incubated at 35 °C for 24 hours. Subsequently, a 1.5 mL aliquot was centrifuged at 14,000 × *g* for 5 minutes. The bacterial pellet was then resuspended in 500 µL of ultrapure water, heated to 95 °C for 15 minutes and quickly cooled to 0 °C ± 1 °C for 20 minutes. Subsequently, an aliquot was centrifuged at 14,000 × *g* for 5 minutes, and 100 µL of the supernatant were collected and stored at -20 °C. DNA was quantified by the fluorimetry method using the QUBIT 2.0 system (Invitrogen®, St. Louis, USA), according to the manufacturer's recommendations. DNA

quantification is recommended to avoid possible false negative results associated to a low amount of target DNA in the PCR solution.

PCR assays

The *hilA* gene (*Salmonella* spp. specific) was used for the PCR reaction [18,19]. The reaction was carried out in a final volume of 25 µL, containing 1x of buffer solution, 3.5 mM MgCl2 (Fermentas®, Walthan, USA), 0.2 mM dNTPs (Fermentas®, Walthan, USA), 1.0 mM of each primer (Invitrogen®, St. Louis, USA), 1U of Taq DNA Polymerase Platinum (Invitrogen®, St. Louis, USA) and approximately 40ng/µL of bacterial DNA. The following conditions were applied: initial denaturation at 94° C for 5 minutes, followed by 35 cycles at 94 °C for 1 minute, 58 °C for 1 minute and 72 °C for 1 minute, and a final step at 72 °C for 10 minutes. The reactions were performed employing a Veriti® thermocycler (Applied Biosystems®, Massschusetts, USA), and results were observed by the electrophoresis technique using a 10 µL aliquot of the PCR product on 1.5% agarose gels with TBE buffer (45 mM Tris pH 8.3, 45 mM borate and 2 mM EDTA). A standard 100 bp label or gel ruler and 3 µL of GelRed (Biotium®, Fremont, USA) were used.

Salmonella serotyping

All the isolated strains were sent to the National Reference Laboratory on the Diagnosis of Enteric Bacteria at the Oswaldo Fiocruz Institute for serotyping. Somatic and flagellar antigens were assessed using mono and polyvalent antisera with and without flagellar-phase induction [20].

Antimicrobial Susceptibility Tests

The antimicrobial resistance analyses were performed using 17 antimicrobial agents from eight different classes by the disk diffusion method, as described by the Clinical and Laboratory Standards Institute [21]. Briefly, the isolates were spiked in 5 mL of BHI broth and incubated at 37 °C for 2 hours. Subsequently, the solutions were inoculated on Mueller Hinton 2 agar with a sterile swab. The disks containing the antibiotics (Cefar Diagnóstica®, São Paulo, Brazil) were distributed on Petri dishes (Table 1) and incubated at 37 °C for 18 hours. Subsequently, the inhibition zones were measured, and the strains were classified as either resistant, intermediate resistance or sensitive, according to the CLSI guide [22]. Strains resistant to three different classes of antimicrobials were classified as multi-resistant strains (MDR).

MDR genotyping strains

Strains exhibiting multiple drug resistances were submitted to PCR genotyping based on repetitive sequences (rep-PCR), using a commercial semiautomatic system DiversiLab® (bioMérieux, Craponne, France). The rep-PCR reaction was applied according to the manufacturer's instructions using the Diversilab® *Salmonella* kit. The rep-PCR products were separated and detected on a microfluidic chip using a Bioanalyzer 2100 (Agilent, Santa Clara, USA). All analyzed samples were compared by a Pearson's correlation analysis using the DiversiLab® software (version vr3.3.40). The distance from the matrices and the unweighted pair group were used to create a

Table 1. Antibiotic molecules evaluated by the disk-diffusion assay.

Molecule	Code	Class	Disk Content (µg)	Zone diameter breakpoints (mm) for each serotype		
				S	I	R
Ampicillin	AMP	Penicillin	10	≥ 17	14 – 16	≤ 13
Aztreonam	ATM	Monobactam	30	≥ 21	18 – 20	≤ 17
Cefepime	CPM	Cephem	30	≥ 25	19 – 24	≤ 18
Cefoxitin	FOX	Cephem	30	≥ 18	15 – 17	≤ 14
Ceftiofur	TIO	Cephem	30	≥ 21	18 – 20	≤ 17
Chloramphenicol	CL	Phenicol	30	≥ 18	13 – 17	≤ 12
Florfenicol	FFN	Phenicol	30	≥ 19	15- 18	≤ 14
Imipenem	IPM	Carbapenems	10	≥ 23	20 – 22	≤ 19
Gentamicin	GEN	Aminoglycoside	10	≥ 15	13 – 14	≤ 12
Tetracycline	TET	Tetracycline	300	≥ 15	12 – 14	≤ 11
Nalidixic acid	NAL	Quilonone	30	≥ 19	15 – 18	≤ 14
Ciprofloxacin	CIP	Fluoroquilonone	5	≥ 31	21 – 30	≤ 20
Enrofloxacin	ENR	Fluoroquilonone	5	≥ 21	17 – 20	≤ 16
Sulfamethoxazole/Trimethoprim	SXT	Folate Pathway Inhibitor	25	≥ 16	11 – 15	≤ 10
Sulfonamides	SSS	Folate Pathway Inhibitor	300	≥ 19	15 – 18	≤ 14
Trimethoprim	TMP	Folate Pathway Inhibitor	5	≥ 16	11 – 15	≤ 10
Nitrofurantoin	NIT	Nitrofuran	300	≥ 17	15 – 16	≤ 14

S: susceptible; I: Intermediate; R: Resistant; Adapted from CLSI [22].

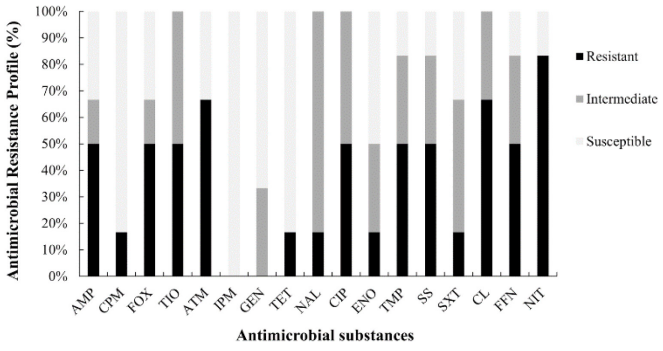
dendrogram. Samples exhibiting 95% similarity were considered clonal strains [11].

Results

Of the 107 evaluated samples, 5.6% (6/107) tested positive. The serotype results comprise two non-typable *Salmonella* enterica subspecies strains, two S. Akuafo, one *Salmonella* serovar (O:16), and one *S.* Schwarzengrund. The antimicrobial susceptibility profiles of the six isolated strains indicate that all displayed multi-drug resistance (MDR). Strains exhibited 100% resistance (0% susceptibility) or intermediary resistance to ceftiofur, nalidixic acid, ciprofloxacin, and chloramphenicol (Figure 2). Concerning antibiotic resistance, strains were sensitive to imipenem (100%), tetracycline (83.3%), cefepime (83.3%), and gentamicin (66.6%) (Figure 2).

A method based on repetitive sequences (rep-PCR) was applied to compare genetic similarities between the detected strains using the commercial semiautomatic system DiversiLab® (BioMérieux). The results indicate a difference of over 70% between *S.* Schwarzengrund (S01) and Non-Typable (NT) *Salmonella* (S02) and *Salmonella* (S04). In addition, differences are phenotypically highlighted by *S.* Schwarzengrund's sensitivity to ampicillin (AMP), cefoxitin (FOX), aztreonam (ATM), florfenicol (FFN) and resistance to (TET) tetracycline (Figure 2), which differs from the aforementioned *Salmonella* NT strains. A 92% genotypic non-clonal similarity was observed between *S.* Akuafo and *Salmonella* (O:16), respectively. It is important to note that all antibiotic results were different between the S05 and S06 strains. Further

Figure 2. Global antimicrobial resistance profile of *Salmonella* strains isolated from beef in the state of Mato Grosso, Brazil.



AMP: ampicillin; CPM: Cefepime; FOX: cefoxitin; TIO: ceftiofur; ATM: aztreonam; IPM: Imipenem; GEN: gentamicin; TET: tetracycline; NAL: nalidixic acid; CIP: ciprofloxacin; ENO: enrofloxacin; TMP: trimethoprim; SSS: sulfonamide; SXT: trimethoprim + sulfamethoxazole; CL: chloramphenicol; FFN: florfenicol; NIT: nitrofurantoin.

whole genome analyses will aid in discriminating phylogenetic relationship among strains.

Discussion

The absence of *Salmonella* spp. is a criterion established in different standard methods and country requisites for beef importation. The absence criterion is noted in the Brazilian legislation and in importing countries, such as those belonging to the European Union [23,24]. To meet this criterion, slaughterhouses apply Good Manufacturing Practices (GMP) tools and the Hazard Analysis and Critical Control Points (HACCP) plan. These control tools aim to monitor and prevent bacterial cross-contamination of beef during slaughter, processing, and commercialization. These programs are applied continuously by companies to achieve safe products, avoiding embargoes and improving the competitiveness of Brazilian beef [25].

Previous studies carried out in slaughterhouses, and beef butcher shops in Cuiabá and Barra do Garças, Mato Grosso have indicated the occurrence of *Salmonella* spp. (8.3% and 17%, respectively) in samples prior to processing [26,27]. The present study in the state of Mato Grosso demonstrated the presence of viable *Salmonella* in the final product packaged under a vacuum atmosphere. Survival of *Salmonella* in vacuum-packed products has been previously reported in the literature. For example, Djordjević *et al*. [28] estimated that the use of both vacuum and modified packaging was not sufficient to obtain a *Salmonella*-free product. These results reinforce the need to improve quality control tools to detect potential quality control program operation deficits, as vacuum packaging is the main form of beef product export and represents an actual risk to consumers.

To describe the prevalence of *Salmonella* serovars, our group has previously carried out different studies in the state of Mato Grosso in animal (fish, beef and chicken slaughterhouses) and milk derivative (cheeses) products [4,11,25,29]. It is interesting to note that the Schwarzengrund serovar was present in all analysed matrices, including those assessed in the present study. Therefore, this serovar seems to be commonly found in the state of Mato Grosso. Moreover, the presence of Schwarzengrund contamination in final products indicates an imminent risk of foodborne illness, as infections by this serovar have been previously reported in beef [30]. Another serovar isolated in the present study, S. Akuafo, has been described in environmental samples from Portugal [31], and in farmed fish from Mato Grosso, Brazil [32]. However, there is no reference to this serovar as a animal origin food

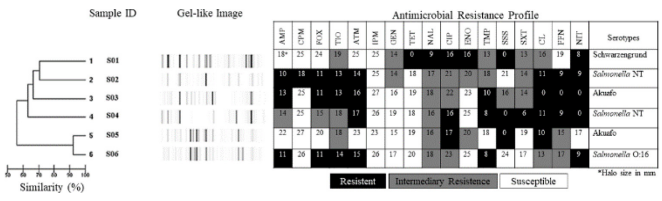
contaminant or associated to human infections. Another unusual contamination is noted herein concerning the *Salmonella* enterica subsp. enterica (O:16) serovar.

Serovars that cause enteric fever were not isolated in the present study. However, it is estimated that non-typhoid *Salmonella* is responsible for 155,000 deaths among 94 million annual infection cases worldwide [33]. The global expansion of genes that regulate the multidrug resistance (MDR) to antibiotics, mainly ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline is correlated to this increase, through horizontal transmission between *Salmonella* serovars, resulting in MDR strains [33]. A common characteristic for all the *Salmonella* spp. strains detected herein was resistance towards three or more antimicrobial classes (Figure 3), with all strains classified as multidrug-resistant (MDR). The hypothesis for this is that strains presenting a wild profile or containing several resistance genes are able to better overcome certain hurdles present both intrinsically and extrinsically in the final meat product, such as cooling temperatures and an anaerobic environment, favoring the survival of resistant strains compared to other sensitive strains [34].

This resistance may be associated with the use of antibiotics in veterinary medicine for therapeutic or preventive purposes, or their application as growth promoters in animal feed, leading to the emergence of resistant *Salmonella* strains [35]. Hong *et al*. [36] observed that *Salmonella* strains isolated from cattle from Minnesota, in the USA, displayed resistance to Sulfadimethoxine (79 - 87%), Ampicillin, Ceftiofur, Florfenicol and Oxytetracycline (30 - 70%), and Neomycin, Sulfamethoxazole/trimethoprim (15%). In the present study, resistance was lower compared to the results reported by Hong *et al*. [36], except for neomycin and sulfadimethoxine (not evaluated). Other *Salmonella* strains isolated from food produced in Mato Grosso, such as beef and chicken, also displayed resistance to nitrofurantoin, sulfonamides-generic, trimethoprim, trimethoprim/sulfamethoxazole, tetracycline, gentamicin, and chloramphenicol [11,25].

Moreover, the rep-PCR employed for the detection of *Salmonella* strains in the present study found no evidence of clonal expansion for a single strain (Figure 3), although the sample size is very small. However, it is important to note that, in contrast to the results reported by Cunha-Neto *et al*. [11], in which genetic homology was related to the horizontal transmission of resistance genes, the genetic similarities observed herein were not related to phenotypic characteristics. This suggests that the *Salmonella* present in the beef production environment may exhibit a more heterogeneous resistance profile than strains present in chicken meat production, although larger studies, including genome sequencing, are required.

Figure 3. Relationship between the *Salmonella* strains and individual AMR profiles of strains isolated from beef produced in the state of Mato Grosso, Brazil.



AMP: ampicillin; CPM: Cefepime; FOX: cefoxitin; TIO: ceftiofur; ATM: aztreonam; IPM: Imipenem; GEN: gentamicin; TET: tetracycline; NAL: nalidixic acid; CIP: ciprofloxacin; ENO: enrofloxacin; TMP: trimethoprim; SSS: sulfonamide; SXT: trimethoprim + sulfamethoxazole; CL: chloramphenicol; FFN: florfenicol; NIT: nitrofurantoin.

Conclusions

The results reported herein indicate the presence of *Salmonella* strains in 5.6% of final packaged products. Although this rate is lower compared to other studies carried in the state of Mato Grosso, all detected strains were classified as multidrug-resistant (displaying resistance to 3 or more antibiotic classes). In addition, this study highlights the presence of two rare *Salmonella* strains in beef (Akuafo and O:16) and reinforces that *Salmonella* serovar Schwarzengrund is commonly isolated in animal products in Brazil. Moreover, a genetic heterogeneity between the detected isolates was also observed, demonstrating the heterogenic presence of *Salmonella* strains in beef production. Finally, novel studies investigating the presence of *Salmonella* presence in beef and vegetal foods in the state of Mato Grosso are encouraged, to fully characterize the spread of this food pathogen, one of the leading public health problems worldwide.

Acknowledgements

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado de Mato Grosso (concession number: 222388/2015, FAPEMAT, Brasil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Financial code 001), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Doctoral Scholarship) and Process code 310462/2018-5 for financial support.

References

1. World Health Organization (WHO) (2015) WHO's first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. Available: https://www.who.int/en/news-room/detail/03-12-2015-who-s-first-ever-global-estimates-of-foodborne-diseases-find-children-under-5-account-for-almost-one-third-of-deaths. Accessed: 22 January 2020.
2. Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo SF, Conte-Junior CA (2019) Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. Appl Environ Microbiol 85: e00591-19.
3. Monte DF, Lincopan, N, Berman H, Cerdeira L, Keelara S, Thakur S, Fedorka-Cray PJ, Landgraf M (2019) Genomic features of high-priority *Salmonella enterica* Serovars circulating in the food production chain, Brazil, 2000–2016. Sci Rep 9: 11058.
4. Cunha-Neto A, Carvalho LA, Castro VS, Barcelos FG, Carvalho RCT, Rodrigues DP, Conte-Junior CA, Figueiredo EES (2019) *Salmonella* Anatum, *S.* Infantis and *S.* Schwarzengrund in brazilian cheese: occurrence and antibiotic profiles. Int J Dairy Tecnol 73: 296-300.
5. Costalunga S, Tondo EC (2002) Salmonellosis in Rio Grande do Sul, Brazil, 1997 to 1999. Braz. J Microbiol 33: 342-346.
6. Capalonga R, Ramos RC, Both JMC, Soeiro MLT, Longaray SM, Haas S, Tondo EC (2014) *Salmonella* serotypes, resistance patterns, and food vehicles of salmonellosis in southern Brazil between 2007 and 2012. J Infect Dev Ctries 8: 811-817. doi: 10.3855/jidc.3791.
7. Miranda AL, Cordeiro SM, Reis JN, Cardoso LG, Guimarães AG (2017) Phenotypic and genotypic characterization of *Salmonella* spp. isolated from foods and clinical samples in Brazil. An Acad Bras Cienc 89: 1143-1153.
8. Rowlands REG, Ristori CA, Ikuno AA, Barbosa ML, Jakabi M, Franco BDGM (2014) Prevalence of drug resistance and virulence features in *Salmonella* spp. isolated from foods associated or not with salmonellosis in Brazil. Rev Inst Med Trop Sao Paulo 56: 461-467.
9. Boonmar S, Morita Y, Pulsrikarn C, Chaichana P, Pornruagwong S, Chaunchom S, Sychanh T, Khounsy T, Sisavath D, Yamamoto S, Sato H, Ishioka T, Noda M, Kozawa K, Kimura H (2013) *Salmonella* prevalence in meat at retail markets in Pakse, Champasak Province, Laos, and antimicrobial susceptibility of isolates. J Glob Antimicrob Resist 1: 157-161.
10. Helke KL, McCrackin MA, Galloway AM, Poole AZ, Salgado CD, Marriot BP (2017) Effects of antimicrobial use in agricultural animals on drug-resistant foodborne salmonellosis in humans: a systematic literature review. Crit Rev Food Sci Nutr 57: 472-488.
11. Cunha-Neto A, Carvalho LA, Carvalho RCT, Rodrigues DP, Mano SB, Figueiredo EES, Conte-Junior CA (2018) *Salmonella* isolated from chicken carcasses from a slaughterhouse in the state of Mato Grosso, Brazil: antibiotic resistance profile, serotyping, and characterization by repetitive sequence-based PCR system. Poult Sci 97: 1373-1381.
12. Rodrigues GL, Panzenhagen P, Ferrari RG, dos Santos A, Paschoalin VMF, Conte-Junior CA (2020) Frequency of antimicrobial resistance genes in *Salmonella* from Brazil by in silico whole-genome sequencing analysis: an overview of the last four decades. Front Microbiol 11: 1864.
13. Tejada TS, Lopes NA, Silva DT, Agostinetto UM, Silva EF, Menezes DB, Timm CD (2016) DNA profiles of *Salmonella* spp. isolated from chicken products and from broiler and human feces. Rev Bras Cienc Avic 18: 693-700.
14. Fernandes AS, Camargo CH, Francisco GR, Bueno MFC, Garcia DO, Doi Y, Casas MRT. (2017) Prevalence of extended-spectrum b-lactamases CTX-M-8 and CTX-M-2-producing *Salmonella* serotypes from clinical and nonhuman isolates in Brazil. Microbial Drug Resist 23: 580-589.
15. National Confederation of Industries (2017) Brazil exports 1.4 million tons of beef and earns US$ 5.5 billion in 2016. Available:. https://www.cnabrasil.org.br/noticias/brasil-exporta-1-4-milh%C3%A3o-de-toneladas-de-carne-bovina-e-fatura-us-5-5-bilh%C3%B5es-em-2016 Accessed: 18 June 2020. [Available in Portuguese]
16. McManus C, Barcellos JO, Formenton BK, Hermuche PM, Carvalho OAJ, Guimarães R, Gianezini M, Dias EA, Lampert VN, Zago D, Neto JB (2016) Dynamics of cattle production in Brazil. PLoS One 11: e0147138.
17. International Organization for Standarization (ISO) (2012) ISO/TS 6579-2:2012. Microbiology of food and animal feed. Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 2: Enumeration by a miniaturized most probable number technique. Available: https://www.iso.org/standard/56713.html. Accessed: 17 June 2020.
18. Guo X, Chen J, Beuchat LR, Brackett RE (2000) PCR detection of *Salmonella enterica* serotype Montevideo in and on raw tomatoes using primers derived from hila. Appl Environ Microb 66: 5248–5252.
19. Voss-Rech D, Vaz CSL, Alves L, Coldebella A, Leão JA, Rodrigues DP, Back A (2015) A temporal study of *Salmonella enterica* serotypes from broiler farms in Brazil. Poult Sci 94: 433–441.
20. Bajaj V, Hwang C, Lee CA (1995) hilA is a novel ompR/toxR family member that activates the expression of *Salmonella typhimurium* invasion genes. Mol Microbiol 18: 715-727.
21. Clinical and Laboratory Standards Institute (CLSI) (2007) Performance standards for antimicrobial susceptibility testing, 17th informational supplement. CLSI document M100-S17 (ISBN 1-56238-625-5).
22. . Clinical and Laboratory Standards Institute (CLSI) (2021) Performance standards for antimicrobial susceptibility testing, 31st informational supplement. CLSI document M100-S31 (ISBN 978-1-68440-104-8).
23. Brazil (2001) Resolution-RDC Nº 12, of January 02, 2001. Technical regulation on microbiological standards for food. http://portal.anvisa.gov.br/documents/33880/2568070/RDC_12_2001.pdf/15ffddf6-3767-4527-bfac-740a0400829b. Accessed 17 June 2020. [Document in Portuguese]
24. EUR-Lex (2007) Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Available: https://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:32007R1441&from=PT. Accessed: 17 June 2020.
25. Martínez-Chávez L, Cabrera-Diaz E, Pérez-Montaño JA, Garay-Martínez LE, Varela-Hernández JJ, Castillo A, Lucia L, Ávila-Novoa MG, Cardona-López MA, Gutiérrez-González P, Martínez-Gonzáles NE (2015) Quantitative distribution of *Salmonella* spp. and *Escherichia coli* on beef carcasses and raw beef at retail establishments. Int J Food Microbiol 210: 149-155.

26. Cunha-Neto A, Castro VS, Carvalho LA, Rodrigues DP, Mano SB, Figueiredo EES, Conte-Junior CA (2017) 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. Afr J Microbiol Res 11: 1626-1631.
27. Sousa TM, Cunha-Neto A, Hernandes T, Souto PCS (2012) Pathogenic microorganisms and hygienic and sanitary conditions indicators in ground beef sold in Barra do Garças, MT. Acta Veterinaria Brasilica 6: 124-130. [Article in Portuguese]
28. Djordjević J, Bošković M, Starčević M, Ivanović J, Karabasil N, Dimitrijević M, Lazić IB, Baltić MŽ (2018). Survival of *Salmonella* spp. in minced meat packaged under vacuum and modified atmosphere. Braz J Microbiol 49: 607–613.
29. Cunha-Neto A, Panzenhagen PHN, Carvalho LA, Rodrigues DP, Conte-Junior CA, Figueiredo EES (2019) Occurrence and antimicrobial resistance profile of *Salmonella* isolated from native fish slaughtered and commercialized in Brazil. J Food Saf Food Qual 70: 94-98.
30. Aarestrup FM, Hendriksen RS, Lockett J, Gay K, Teates K, McDermott PF, White DG, Hasman H, Sørensen G, Bangtrakulnonth A, Pornreongwong S, Pulsrikarn C, Angulo FJ, Gerner-Smidt P (2007) International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. Emerg Infect Dis 13: 726-731.
31. National Institute of Health Doctor Ricardo Jorge (INSA) (2014) Epidemiological bulletin observations special issue environmental health. Available: http://repositorio.insa.pt/bitstream/10400.18/2539/3/observacoesNEspecia4-2014.pdf Accessed 16 June 2020. [Article in Portuguese]
32. Fernandes DVGS, Carvalho RCT, Castro VS, Cunha-Neto A, Muller B, Carvalho FT, Dos Prazeres Rodrigues D, Vieira BS, de Souza Figueiredo EE (2021) *Salmonella* in the processing line of farmed Tambatinga (*Colossoma macropomum* x *Piaractus brachypomus*) in Mato Grosso, Brazil: serotypes of occurrence and antimicrobial profile. Trop Anim Health Prod 53: 146.
33. World Health Organization (WHO) (2014) Antimicrobial resistance: global report on surveillance. Available: https://www.who.int/drugresistance/documents/surveillancereport/en/. Accessed: 18 June 2020.
34. Castro VS, Rosario DKA, Mutz YS, Paletta ACC, Figueiredo EES, Conte-Junior CA (2019) Modelling inactivation of wild-type and clinical Escherichia coli O26 strains using UV-C and thermal treatment and subsequent persistence in simulated gastric fluid. J Appl Microbiol 127: 1564-1575.
35. Su L-H, Chiu C-H, Chu C, Ou JT (2004) Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clin Infect Dis 39: 546–551.
36. Hong S, Rovira A, Davies P, Ahlstrom C, Muellner P, Rendahl A, Olsen K, Bender JB, Wells S, Perez A, Alvarez J (2016) Serotypes and antimicrobial resistance in *Salmonella enterica* recovered from clinical samples from cattle and swine in Minnesota, 2006 to 2015. PLoS One 11: e0168016.

Corresponding author

Professor Eduardo Eustáquio de Souza Figueiredo, PhD.
Fernando Correa da Costa. Avenue, Boa Esperança, Federal University of Mato Grosso – Campus Cuiabá, Mato Grosso, 78060-900, Brazil
Phone: +55-65-3615-8589
Fax: +55-65-3615-8852
Email: figueiredoeduardo@ufmt.br

Conflict of interests: No conflict of interests is declared.