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Research Note

Profile of *Salmonella* Isolates in Bullfrog Production: PFGE, MLST, and Antimicrobial Resistance

Evelyn Cristine Silva [1,*], Sthéfany da Cunha Dias [2], Priscila Cristina Costa [2], Monique Ribeiro Tiba Casas [3], Ricardo Seiti Yamatogi [4], João Pessoa Araújo Junior [1], Marcus Vinícius Coutinho Cossi [2], Fábio Sossai Possebon [1]

[1] *São Paulo State University (UNESP), Institute for Biotechnology, Tecomarias Avenue, Botucatu, SP 18607-440, Brazil*
[2] *Uberlândia Federal University (UFU), Faculty of Veterinary Medicine, João Naves de Ávila Avenue, 2121, Uberlândia, MG 38408-100, Brazil*
[3] *Bacteriology Division, Adolfo Lutz Institute (IAL), Doutor Arnaldo Avenue, São Paulo, SP 01246 000, Brazil*
[4] *Viçosa Federal University (UFV), Faculty of Veterinary Medicine, Peter Henry Rolfs Avenue, Viçosa, MG 36570-900, Brazil*

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ABSTRACT

The continued development of bullfrog farming holds great potential for expansion. However, these animals serve as reservoirs for various pathogens, including *Salmonella*, posing a risk to food safety and public health. Therefore, the primary objective of this study was to identify potential sources of *Salmonella* contamination in a bullfrog production facility in Minas Gerais, Brazil, by characterizing isolates through Pulsed-Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), and antimicrobial resistance profiling. A total of eight *Salmonella* isolates were analyzed, comprising six isolates from bullfrog carcasses and two from breeding tanks. PFGE characterization revealed four distinct profiles, with a clonal relationship among isolates belonging to the same serovar, except for *S.* Newport, where the profile of the breeding tank isolate differed from that of the carcass isolates. MLST analysis identified three sequence types (STs): ST 32, ST 614, and ST 6855, corresponding to *S.* Infantis, *S.* Newport, and *S.* 6,8:i:-, respectively. Regarding antimicrobial resistance, one isolate was resistant to azithromycin, two were resistant to neomycin, and three were resistant to ciprofloxacin. All isolates were susceptible to cephalexin, meropenem, cefotaxime, ceftriaxone, ampicillin, tobramycin, and cephalothin. The presence of distinct PFGE profiles among isolates of the same serovar suggests multiple sources of pathogen contamination within the production chain, raising significant sanitary concerns. The identified sequence types (STs) are of public health relevance, highlighting the pathogenic potential of these isolates. While most isolates were susceptible to the tested antimicrobials, the detection of azithromycin resistance is particularly concerning. The combined PFGE and MLST data indicate potential cross-contamination within the production chain, emphasizing the need for stringent control measures.

Frog farming is expanding to meet growing global demand (Cribb et al., 2013). However, during the production of American bullfrogs (Lithobates catesbeianus), the animals remain vulnerable to diseases that can reduce yields and elevate food-safety risks (Dias et al., 2009; Oliveira et al., 2020). Frog farming is susceptible to microbial contamination because rearing water harbors and disseminates pathogens. Therefore, investing in robust biosecurity measures, disease control, and effective management practices is essential to ensuring animal health and product quality (Costa et al., 2021; Oliveira et al., 2020; Pahor-Filho et al., 2019).

The farming of bullfrogs in stagnant water—a hallmark of raniculture—facilitates the accumulation of microbial contaminants in animals destined for human consumption, thereby potentially elevating the risk of foodborne illness and zoonotic transmission of pathogens (Najiah et al., 2009). Bullfrogs often harbor pathogens widespread in other animal production systems—most notably *Salmonella*—which poses serious foodborne–disease and public-health risks (Costa et al., 2021; Ribas & Poonlaphdecha, 2017). For example, a study in China detected *Salmonella* in approximately 30% of aquaculture bullfrog samples, identifying 217 isolates across 38 serovars. Additionally, 69.6% of the isolates exhibited resistance to at least one antimicrobial agent (Zhang et al., 2022). Another study analyzed edible Chinese frogs (*Hoplobatrachus rugulosus*) from wet markets in Hong Kong, where *Salmonella* spp. was isolated from 65% of the samples. The iden-

* Corresponding author.
E-mail address: evelyn.cristine@unesp.br (E.C. Silva).

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tified serovars included *S.* Saintpaul, *S.* Newport, *S.* Bareilly, *S.* Braenderup, *S.* Hvittingfoss, *S.* Stanley, and *S.* Wandsworth (Boss et al., 2023). To mitigate these risks, strict control measures, best production practices, and routine pathogen testing are essential (Dadié et al., 2017; Ribas & Poonlaphdecha, 2017).

In Brazil, Alfani (2007) reported *Salmonella* positivity ranging from 26.7% in farm water to 40% in bullfrog (*Rana catesbeiana*) carcasses, having sampled both carcasses and viscera at multiple points along the slaughter line. A survey of retail bullfrog meat marketed in Rio de Janeiro detected *Salmonella* in 10% of samples (Barreira et al., 2011). In an analysis of 140 bullfrogs (*Lithobates catesbeianus*) carcasses from two different slaughter facilities, *Salmonella* was detected in 7% of samples, with 3 isolates (16%) identified as *S.* Infantis, and 16 isolates (84%) as *S.* Newport (Costa et al., 2021). These findings emphasize the need to enforce strict safety protocols and best practices in frog meat production to minimize *Salmonella* contamination and protect consumer health (Diallo et al., 2020; Pissetti et al., 2022). Consequently, molecular tools such as PFGE and MLST are widely used for the epidemiological characterization of bacterial isolates and can help identify cross-contamination routes within bullfrog farming systems.

The principle PFGE is to digest chromosomal DNA with rare-cutting restriction enzymes (e.g., *Xba I*) and separate fragments ≥10 kb in an alternating electric field; the resulting macro-restriction fingerprints are highly discriminatory and enable precise outbreak reconstruction and source tracking. PFGE has been applied to differentiate multidrug-resistant *Salmonella* isolates in intensive poultry flocks (Zhang et al., 2022) and to demonstrate clonal transmission across species in mixed-production systems (Santana et al., 2020). In contrast, MLST sequences seven housekeeping loci, assigns numerical alleles, and combines them into portable Sequence Types (STs), allowing robust phylogeographic comparisons. The approach is widely used to delineate host-adapted lineages and infer global dissemination routes (Fu et al., 2022). Furthermore, they enable detailed genetic comparisons with international databases, contributing to global surveillance efforts (Zakaria et al., 2020).

In summary, concerns regarding the health of farmed frogs in Brazil are growing, particularly due to the risk of microbial contamination in production systems. This study aimed to characterize *Salmonella* strains from bullfrog (*Lithobates catesbeianus*) carcasses and their rearing environment in a production system located in Minas Gerais, Brazil. The objectives included determining the antimicrobial resistance profiles of the isolates and employing molecular epidemiological techniques, such as PFGE and MLST, to assess the genetic diversity and potential epidemiological links among the isolates.

Materials and methods

Bacterial strains. Eight *Salmonella* isolates analyzed in this study derived from the carcass-rinse survey of Costa et al. (2021), conducted between October 2017 and March 2018 in a closed-cycle bullfrog (Lithobates catesbeianus) facility in Uberlândia, Minas Gerais, Brazil (ethical approval CEUA 027/2017). That investigation examined 140 carcass samples and recovered *Salmonella* from 19 (13.6%) carcasses, yielding 16 serovar Newport (84%) and three serovar Infantis (16%).

To encompass both serovars and permit comparison with the aquatic environment, we also collected water samples aseptically—following standard water-sampling protocols—from the bullfrog production tanks; two S. Newport isolates recovered from these production-tank samples (as reported in the initial publication) were subsequently included. The isolation and serotyping of *Salmonella* were carried out following the international standards of identification according to ISO 6579 (ISO, 2002) and confirmed by qPCR targeting the *invA* gene (Barbau-Piednoir et al., 2013).

Antimicrobial susceptibility testing. The isolates were tested using the disk diffusion method, following the protocol described by Bauer et al. (1966). The antibiotics evaluated were azithromycin (AZI), tobramycin (TOB), cephalothin (CFL), ampicillin (AMP), cephalexin (CFE), neomycin (NEO), meropenem (MER), ciprofloxacin (CIP), cefotaxime (CTX), and ceftriaxone (CRO). After 24 h of incubation at 37 °C, the inhibition zones were measured and compared to CLSI, 2008 standards, classifying the isolates as resistant or susceptible to the antimicrobials. Interpretive criteria for susceptibility, intermediate, and resistance were defined in accordance with the breakpoints established by the Clinical and Laboratory Standards Institute (CLSI, 2008). Isolates exhibiting zone diameters ≤ 12 mm (resistant), 13–16 mm (intermediate), and ≥17 mm (susceptible). All zones were measured in millimeters with a calibrated caliper. Antimicrobial susceptibility was therefore classified exclusively by disk diffusion in strict accordance with CLSI (2008) criteria; MIC determinations were not performed.

DNA extraction. Genomic DNA was extracted using an in-house magnetic bead-based protocol (GE Healthcare Sera-Mag™ Magnetic SpeedBeads™ Carboxylate-Modified Dia.: 1 μm; 3 EDAC/PA5; 15 mL), adapted from Possebon et al. (2022). Briefly, the extraction protocol included the steps of cell lysis, binding to magnetic beads, two washes using alcohol, and elution in nuclease-free water. The purity of the extracted DNA was analyzed using the NanoDrop® ND-1000 spectrophotometer (Thermo Fisher), while quantification was performed with the Qubit 2.0 fluorometer (Thermo Fisher) using the Qubit® 1X dsDNA HS kit for high sensitivity.

Pulsed-field gel electrophoresis. The PFGE method was performed as previously described in the PulseNet protocol (Ribot et al., 2006). For cell lysis, the proteinase K enzyme was used, and for DNA restriction, the enzyme XbaI (Promega) was applied. Electrophoresis was conducted in the CHEF-DR III system (Bio-Rad) under the following conditions: 6 V/cm, temperature of 14 °C, initial switch time of 2.2 s, final switch time of 63.8 s, and a total runtime of 18 h. *Salmonella* Braenderup ATCC BAA-664 was used as a molecular weight marker for gel normalization. Image analysis was performed using BioNumerics 6.6.4 software (Applied Maths). PFGE clusters were compared using the unweighted pair group method with arithmetic mean (UPGMA) and a Dice coefficient, with a tolerance of 1.5% and optimization of 5%.

PCR amplification for Multilocus Sequence Typing (MLST). Genomic DNA extracted from *Salmonella* isolates was subjected to conventional PCR using specific primers for seven conserved housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA,* and *thrA*) from *S.* enterica (Table 1) (Yun et al., 2015). The reaction mixture included 12.5 μL of 1X GoTaq® G2 Green Master Mix (Promega Corporation),

Table 1
List of primers and product sizes for MLST characterization of *Salmonella* isolates from a bullfrog farm in Brazil

Primers		Oligonucleotide sequences (5′ → 3′)	Product size (bp)
aroC	F	CCTGGCACCTCGCGCTATAC	826
	R	CCACACACGGATCGTGGCG	
dnaN	F	TGAAATTTACCGTTGAACGTGA	833
	R	AATTTCTCATTCGAGAGGATTGC	
hemD	F	ATGAGTATTCTGATCACCCG	666
	R	ATCAGCGACCTTAATATCTTGCCA	
hisD	F	GAAACGTTCCATTCCGCGCAGAC	894
	R	CTGAACGGTCATCCGTTTCTG	
thrA	F	GTCACGGTGATCGATCCGGT	852
	R	CACGATATTGATATTAGCCCG	
sucA	F	AGCACCGAAGAGAAACGCTG	643
	R	GGTTGTTGATAACGATACGTAC	
purE	F	ATGTCTTCCCGCAATAATCC	510
	R	TCATAGCGTCCCCCGCGGATC	

Source: Yun et al. (2015).

1 μL (10 μM) of each primer, 3.5 μL of template DNA, and nuclease-free water to complete a final volume of 25 μL. The thermal cycling conditions were as follows: 94 °C for 2 min; 40 cycles of 94 °C for 30 s, 56.8 °C for 1 min, and 72 °C for 2 min; followed by a final extension at 72 °C for 10 min. PCR products were visualized by horizontal electrophoresis on a 1.5% agarose gel in 1X Tris/Borate/EDTA (TBE) buffer stained with SYBR Safe (Thermo Fisher), and the gel was visualized using an ultraviolet transilluminator. After performing conventional PCR on the isolates, the allelic profiles were determined. For all molecular assays, a previously serotyped *Salmonella* strain was used as a positive control, and nuclease-free water was used as a negative control. After PCR amplification of the seven housekeeping loci, amplicons were purified and sequenced bidirectionally (forward and reverse) by Sanger sequencing.

Sequencing and MLST analysis. The PCR products corresponding to the seven housekeeping genes were cleaned via an optimized magnetic-bead purification (De Angelis et al., 1995) and then submitted to bidirectional Sanger sequencing (forward and reverse reads). The purified amplicons were quantified and sequenced following the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) protocol using the ABI 3500 DNA Analyzer (Applied Biosystems, USA). For MLST, sequencing of the seven previously described gene fragments was performed. The FASTA sequences generated were submitted to the PubMLST database (https://pubmlst.org/organisms/salmonella-spp) to identify the allelic profiles of the sequences. The EnteroBase database (https://enterobase.warwick.ac.uk/) was used for comparison and serotype determination.

Results and discussion

Due to technical difficulties, antimicrobial resistance profiles could not be determined for the water samples from the breeding tanks. Among the six evaluated isolates, three were resistant to ciprofloxacin, two to neomycin, and one to azithromycin. All isolates were susceptible to cephalexin, meropenem, cefotaxime, ceftriaxone, ampicillin, tobramycin, and cephalothin. The PFGE, MLST, and antibiotic resistance patterns are shown in Figure 1.

All tested isolates were susceptible to cephalexin, meropenem, cefotaxime, ceftriaxone, ampicillin, tobramycin, and cephalothin. One frog carcass isolate was resistant to azithromycin, an antibiotic of clinical importance reserved for the treatment of human (Jin et al., 2019; Plumb et al., 2019; Wang et al., 2023). Regarding animal production, a study analyzing 309 *Salmonella* isolates from chicken meat in Brazil (2014–2017) found no resistance to azithromycin (Rau et al., 2021). Similarly, in a survey of *Salmonella* from pig carcasses in southern Brazil, all 61 tested strains were susceptible to azithromycin (Kich et al., 2020). However, in a study of *Salmonella* isolates from pork and poultry meat products sold in retail markets in São Paulo, 95% of the isolates from pigs showed resistance to azithromycin (Gomes et al., 2022).

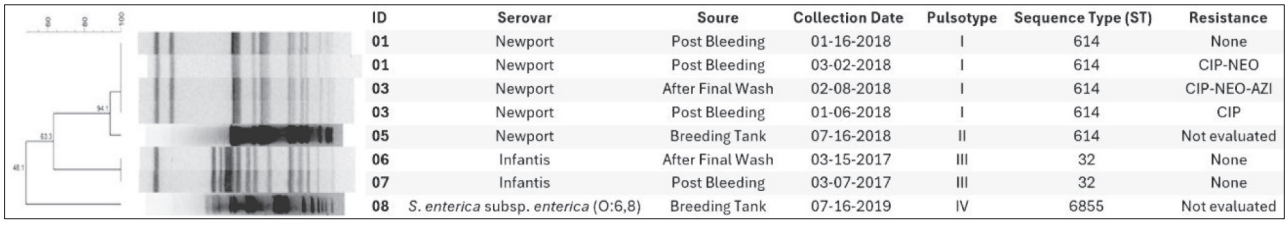
Resistance to ciprofloxacin and neomycin was also observed in this study. These two antibiotics have previously been reported as ineffective against *Salmonella* isolates from animal production and related products in other countries (Guo et al., 2023; Ripon et al., 2023). A Brazilian study revealed a significant prevalence of ciprofloxacin resistance among *Salmonella* isolates from chicken meat, with 73.78% of the 309 tested isolates exhibiting resistance to this antibiotic (Rau et al., 2021). Quinolones, the antibiotic class to which ciprofloxacin belongs, are extensively used in human clinical treatment and are considered first-line therapeutic options (Jin et al., 2019). In a study conducted in the swine production chain, all 29 *Salmonella* isolates from porcine mesenteric lymph nodes exhibited resistance to neomycin, and 79% also resisted ciprofloxacin (Azevedo et al., 2021).

By contrast, the eight isolates evaluated in the present work displayed only single-drug resistance to ciprofloxacin and/or neomycin, remaining below the multidrug-resistance (MDR; ≥3 antimicrobial classes) threshold proposed by Magiorakos et al. (2012). Nevertheless, MDR has been documented in raniculture: Boss et al. (2023) detected this profile in 21% of 67 *Salmonella* strains isolated from edible frogs in Hong Kong, mainly to tetracycline, nalidixic acid, ampicillin, and ciprofloxacin. Compared to these findings, our results indicate low levels of resistance to most tested antibiotics. In amphibian farming, the use of antimicrobials is uncommon, unlike in other livestock industries. This is mainly due to the emphasis on proper management over antibiotic use by universities and government entities in Brazil, as well as the short production cycle (~4 months), which reduces selective pressure and limits resistance. However, further research and the evaluation of a larger number of isolates are necessary to enhance our understanding of antimicrobial resistance in this sector.

Brazilian studies on antimicrobial resistance have primarily focused on products of animal origin, such as beef, pork, poultry, milk, and eggs (Mesquita et al., 2022; Rabello et al., 2020). Given this context, monitoring antimicrobial resistance in frog farming is essential to elucidate resistance dynamics and to devise targeted mitigation strategies that uphold sustainability, animal welfare, food safety, and public health.

PFGE characterization of the isolates revealed four distinct pulsotypes among the eight isolated strains. The dendrogram based on the *XbaI* PFGE patterns (Fig. 1) showed two pulsotypes for the *S.* Newport strains, exhibiting a differentiated pattern between those isolated from frog carcasses and those from breeding tank water. This distinction may indicate genetic dissimilarity between the isolates. The *S.* Infantis isolates formed highly similar band profiles, with a genetic similarity percentage close to 100%, suggesting a possible common origin or spread from the same bacterial lineage. The PFGE results indicate a clonal relationship among *Salmonella* isolates in frog farming, with a predominant finding of clonal relationships among all isolates of the same serovar. Similar patterns have been reported in other animal production chains (Perin et al., 2020; Zakaria et al., 2020).

The MLST analysis identified three distinct sequence types (STs): ST32, ST614, and ST6855, which corresponded to *Salmonella* Infantis,



ID	Serovar	Soure	Collection Date	Pulsotype	Sequence Type (ST)	Resistance
01	Newport	Post Bleeding	01-16-2018	I	614	None
01	Newport	Post Bleeding	03-02-2018	I	614	CIP-NEO
03	Newport	After Final Wash	02-08-2018	I	614	CIP-NEO-AZI
03	Newport	Post Bleeding	01-06-2018	I	614	CIP
05	Newport	Breeding Tank	07-16-2018	II	614	Not evaluated
06	Infantis	After Final Wash	03-15-2017	III	32	None
07	Infantis	Post Bleeding	03-07-2017	III	32	None
08	*S. enterica* subsp. *enterica* (O:6,8)	Breeding Tank	07-16-2019	IV	6855	Not evaluated

NEO – Neomycin, CIP- Ciprofloxacin, AZI - Azithromycin.

Figure 1. Servoar, Source, Collection Date, Pulsetype (*XbaI* enzyme), Sequence Type (ST), and Antimicrobial Resistance Profile of 8 *Salmonella* isolates from a bullfrog farm in Brazil. NEO – Neomycin, CIP – Ciprofloxacin, AZI – Azithromycin.

Salmonella Newport, and *Salmonella enterica* subsp. *enterica* serovar 6,8: i:-, respectively. ST32 and ST614 were detected in frog carcass samples, while ST614 and ST6855 were found in water samples from the rearing tank (environmental samples). Notably, ST614 was identified in both a carcass sample and a breeding tank sample, suggesting a potential link between environmental and animal isolates. ST32 is a highly conserved sequence type for serovar Infantis (Achtman et al., 2012; Vilela et al., 2022) and has been dominant in several MLST studies on *Salmonella* isolates from various sources (Papić et al., 2022; Vilela et al., 2022). In contrast, ST614 and ST6855 have been reported only once in the EnteroBase database.

Isolates from these serovars and STs have been widely reported in both human and animal production systems (Baptista et al., 2023; Jajere, 2019; Kipper et al., 2022). A study analyzing 9,014 *Salmonella enterica* isolates from human and nonhuman sources (food, animals, and the environment) identified the Infantis and Newport serovars as prevalent (Fernandes et al., 2022). Moreover, a global meta-analysis found *S.* Newport among the five most reported serovars in beef, pork, poultry, and seafood across all five continents, with an average prevalence of 5–10% in each matrix (Ferrari et al., 2019). A recent multilocus sequence-typing study of 1,842 *S.* Newport genomes showed clear host-specialised lineages—ST45 in livestock (and bearing most multidrug-resistant strains), ST5 in birds, ST118 in produce/-contaminated water, ST31 in seafood, and ST46 in reptiles—alongside two China-specific clusters from wild birds (ST808) and freshwater animals (ST2364) (Pan et al., 2019). Among the epidemiologically important serovars of nontyphoidal *Salmonella* (NTS), serovar Newport stands out due to its significant role in foodborne outbreaks worldwide (Jajere, 2019). This serovar has also been reported in the Chinese edible frog (*Hoplobatrachus rugulosus*) in China and Thailand (Boss et al., 2023; Ribas & Poonlaphdecha, 2017). Additionally, in *Hoplobatrachus occipitalis*, *S.* Newport was identified in 11.1% of muscle and intestinal isolates from fresh frog samples in Ivory Coast (Dadié et al., 2017).

Serovar Infantis has also been identified as a potential public health and food safety concern (Vilela et al., 2022). *S.* Infantis was detected in approximately 2.0% of reported human infections in Europe in 2021 (EFSA, 2022) and is present in the production chain of various animal species (Ferrari et al., 2019; Vilela et al., 2022). In a study evaluating the microbiological quality of bullfrog carcasses at different preslaughter fasting periods, 19 *Salmonella* isolates were obtained. Among them, 16% were identified as serovar Infantis and 84% as serovar Newport (Costa et al., 2021).

We also emphasize that the first four isolates of *S.* Newport were obtained from different slaughter phases and collected on separate days. This finding suggests the potential circulation and/or persistence of a single strain within the production system. Studies like this provide valuable insights into these dynamics and highlight the need for a more comprehensive analysis of the frog production chain. We underscore the critical importance of two molecular typing tools, PFGE and MLST, for epidemiological surveillance in animal production environments. However, owing to the preliminary nature of this research and the limited number of isolates ($n = 8$), the scope of our conclusions is inherently constrained; future studies employing whole-genome sequencing (WGS) are therefore warranted to afford higher-resolution insights into phylogenetic relationships, virulence determinants, and a antimicrobial susceptibility was therefore antimicrobial resistance profiles within this production system.

Conclusion

Molecular characterization of eight *Salmonella* enterica isolates recovered from bullfrog production systems revealed a predominance of serovars *S.* Newport (ST614) and *S.* Infantis (ST32), together with a single monophasic variant serovar 6,8:i:- (ST6855). Phenotypic antimicrobial susceptibility profiling demonstrated resistance to ciprofloxacin in 38% (3/8) of isolates, neomycin in 25% (2/8), and azithromycin in 12.5% (1/8). The identification of resistance to clinically important antimicrobials—namely ciprofloxacin and azithromycin, which constitute first-line therapies for human salmonellosis—high lights a critical public health threat. Considering the relatively brief production cycle and the limited application of antibiotics in raniculture, the resistance phenotypes observed could potentially stem from environmental or external sources. Likewise, the detection of multiple serovars points to a heterogeneous *Salmonella* distribution within bullfrog operations, which may contribute to cross-contamination risks along the production continuum. In conclusion, epidemiological surveys are indispensable for delineating pathogen transmission pathways and ensuring effective surveillance of *Salmonella* prevalence throughout the frog-meat production continuum.

CRediT authorship contribution statement

Evelyn Cristine da Silva: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Sthéfany da Cunha Dias:** Visualization, Methodology, Investigation, Conceptualization. **Priscila Cristina Costa:** Visualization, Methodology, Investigation, Data curation, Conceptualization. **Ricardo Seiti Yamatogi:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation. **João Pessoa Araújo Junior:** Supervision, Resources, Project administration, Funding acquisition. **Marcus Vinícius Coutinho Cossi:** Supervision, Software, Resources, Project administration, Funding acquisition, Data curation. **Fábio Sossai Possebon:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Achtman, M., Wain, J., Weill, F. X., Nair, S., Zhou, Z., Sangal, V., Krauland, M. G., Hale, J. L., Harbottle, H., Uesbeck, A., Dougan, G., Harrison, L. H., & Brisse, S. (2012). Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathogens, 8*, e1002776. https://doi.org/10.1371/journal.ppat.1002776.

Alfani, R. (2007). Ocorrência de *Salmonella* sp. em carcaças e vísceras de rãs (*Rana catesbeiana* - rã Touro): avaliação do processo de abate. *Universidade Estadual Paulista, Faculdade de Medicina Veterinária e Zootecnia*.

Azevedo, E. C., Martins, B. T. F., Tiba Casas, M. R., Possebon, F. S., Araújo Junior, J. P., Nero, L. A., & Yamatogi, R. S. (2021). Multidrug resistance and virulence profiles of *Salmonella* isolated from swine lymph nodes. *Microbial Drug Resistance, 27*, 562–570. https://doi.org/10.1089/mdr.2020.0120.

Baptista, D., Borsoi, A., Reischak, D., Nascimento, A., Montesino, L., Camillo, S., Abreu, D., & Pereira, V. (2023). *Salmonella* serovars isolated from poultry breeding flocks under the Brazilian official control programme between 2016 and 2018. *Brazilian Journal of Poultry Science, 25*, 1. https://doi.org/10.1590/1806-9061-2022-1646.

Barbau-Piednoir, E., Bertrand, S., Mahillon, J., Roosens, N. H., & Botteldoorn, N. (2013). SYBR®Green qPCR *Salmonella* detection system allowing discrimination at the genus, species and subspecies levels. *Applied Microbiology and Biotechnology, 97*, 9811–9824. https://doi.org/10.1007/s00253-013-5234-x.

Barreira, V. B., Mesquita, E. F. M., Franco, R. M., & Mello, S. C. R. P. (2011). Análise bacteriológica da carne de rã-touro comercializada no município do Rio de Janeiro, RJ. *Higiene Alimentar, 24*, 145–150.

Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*, 493–496.

Boss, S., Stephan, R., Horlbog, J. A., Magouras, I., Colon, V. A., Lugsomya, K., Stevens, M. J. A., & Nüesch-Inderbinen, M. (2023). Serotypes, antimicrobial resistance profiles, and virulence factors of *Salmonella* isolates in Chinese edible frogs (*Hoplobatrachus rugulosus*) collected from wet markets in Hong Kong. *Foods, 12*, 2245. https://doi.org/10.5167/uzh-235058.

Clinical and Laboratory Standards Institute (CLSI) (2008). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard* (3ª ed.). Clinical and Laboratory Standards Institute.

Costa, P. C., Nascimento, Y. F., Costa, L. R. M., Dias, S. C., Ventura, N. K. O., Yamatogi, R. S., Costa, F. A. A., & Cossi, M. V. C. (2021). Influence of different periods of pre-

slaughter fasting on microbiological quality of bullfrog carcasses (*Lithobates catesbeianus*). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 73*, 487–494. https://doi.org/10.1590/1678-4162-12030.

Cribb, A. Y., Afonso, A. M., & Mostério, C. M. F. (2013). *Manual técnico de ranicultura* (1ª ed.). Brasil: Embrapa.

Dadié, A., Blé, Y. C., Nadège, K., Fantodji, A., & Djè, K. M. (2017). Prevalence, serotype and presence of invasion gene in *Salmonella* isolated from frog meat obtained from western region of Côte d'Ivoire. *International Journal of Applied Microbiology and Biotechnology Research, 5*, 75–81.

De Angelis, M. M., Wang, D. G., & Hawkins, T. L. (1995). Solid-phase reversible immobilization for the isolation of PCR products. *Nucleic Acids Research, 23*, 4742–4743.

Diallo, O. O., Baron, S. A., Abat, C., Colson, P., Chaudet, H., & Rolain, J.-M. (2020). Antibiotic resistance surveillance systems: a review. *Journal of Global Antimicrobial Resistance, 23*, 430–438.

Dias, D. de C., De Stéfani, M. V., Ferreira, C. M., França, F. M., Ranzani-Paiva, M. J. T., & Santos, A. A. (2009). Haematologic and immunologic parameters of bullfrogs, *Lithobates catesbeianus*, fed probiotics. *Aquaculture Research, 41*, 1064–1071.

EFSA (European Food Safety Authority) (2022). The European Union One Health 2021 zoonoses report. *EFSA Journal, 20*.

Fernandes, S. A., Tavechio, A. T., Ghilardi, Ã. C. R., de Almeida, E. A., Silva, J. M. L., Camargo, C. H., & Tiba-Casas, M. R. (2022). Salmonella enterica serotypes from human and nonhuman sources in São Paulo State, Brazil, 2004–2020. *Revista do Instituto de Medicina Tropical de São Paulo, 64*.

Ferrari, R. G., Rosario, D. K. A., Cunha-Neto, A., Mano, S. B., Figueiredo, E. E. S., & Conte-Junior, C. A. (2019). Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. *Applied and Environmental Microbiology, 85*.

Fu, Y., M'ikanatha, N. M., & Dudley, E. G. (2022). Comparative genomic analysis of *Salmonella enterica* serovar Typhimurium isolates from passerines reveals two lineages circulating in Europe, New Zealand, and the United States. *Applied and Environmental Microbiology, 88*(9)e00205-22. https://doi.org/10.1128/aem.00205-22. PMID:35435718; PMCID:PMC9088394.

Gomes, V. T. M., Moreno, L. Z., Silva, A. P. S., Thakur, S., La Ragione, R. M., Mather, A. E., & Moreno, A. M. (2022). Characterization of *Salmonella enterica* contamination in pork and poultry meat from São Paulo/Brazil: serotypes, genotypes and antimicrobial resistance profiles. *Pathogens, 11*, 358.

Guo, L., Xiao, T., Wu, L., Li, Y., Duan, X., Liu, W., Liu, K., Jin, W., Ren, H., Sun, J., Liu, Y., Liao, X., & Zhao, Y. (2023). Comprehensive profiling of serotypes, antimicrobial resistance and virulence of *Salmonella* isolates from food animals in China, 2015–2021. *Frontiers in Microbiology, 14*.

International Organization for Standardization (2002). *ISO 6579:2002 – Microbiology of food and animal feeding stuffs — horizontal method for the detection of Salmonella spp.*. ISO.

Jajere, S. M. (2019). A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Veterinary World, 12*, 504–521.

Jin, C., Gibani, M. M., Pennington, S. H., Liu, X., Ardrey, A., Aljayyoussi, G., Moore, M., Angus, B., Parry, C. M., Biagini, G. A., Feasey, N. A., & Pollard, A. J. (2019). Treatment responses to azithromycin and ciprofloxacin in uncomplicated *Salmonella typhi* infection: A comparison of clinical and microbiological data from a controlled human infection model. *PLOS Neglected Tropical Diseases, 13*, e0007955.

Kich, J. D., Souza, A. I. A., Montes, J., Meneguzzi, M., Costa, E. F., Coldebella, A., Corbellini, L. G., & Cardoso, M. (2020). Investigation of *Listeria monocytogenes*, *Salmonella enterica*, and *Yersinia enterocolitica* in pig carcasses in southern Brazil. *Pesquisa Veterinária Brasileira, 40*, 781–790.

Kipper, D., Mascitti, A. K., De Carli, S., Carneiro, A. M., Streck, A. F., Fonseca, A. S. K., Ikuta, N., & Lunge, V. R. (2022). Emergence, dissemination and antimicrobial resistance of the main poultry-associated *Salmonella* serovars in Brazil. *Veterinary Sciences, 9*, 405.

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Mesquita Souza Saraiva, M., Lim, K., do Monte, D. F. M., Givisiez, P. E. N., Alves, L. B. R., de Freitas Neto, O. C., Kariuki, S., Júnior, A. B., de Oliveira, C. J. B., & Gebreyes, W. A. (2022). Antimicrobial resistance in the globalized food chain: a one health perspective applied to the poultry industry. *Brazilian Journal of Microbiology, 53*, 465–486.

Najiah, M., Lee, S., Wendy, W., Tee, L., Nadirah, M., & Faizah, S. (2009). Antibiotic resistance and heavy metals tolerance in gram-negative bacteria from diseased American bullfrog (*Rana catesbeiana*) cultured in Malaysia. *Agricultural Sciences in China, 8*(10), 1270–1275. https://doi.org/10.1016/s1671-2927(08)60338-7.

Oliveira, C. R., Alfaia, S. R., Ikari, F. L., Tavares, L. S., Sousa, R. L. M., Harakava, R., & Ferreira, C. M. (2020). Detection and molecular characterization of frog virus 3 in bullfrogs from frog farms in Brazil. *Aquaculture, 516*, 734575. https://doi.org/10.1016/j.aquaculture.2019.734575.

Pahor-Filho, E., Mansano, C. F. M., Pereira, M. M., & De Stéfani, M. V. (2019). The most frequently bullfrog productive systems used in Brazilian aquaculture: a review. *Aquacultural Engineering, 87*, 102023. https://doi.org/10.1016/j.aquaeng.2019.102023.

Pan, H., Zhou, X., Chai, W., Paudyal, N., Li, S., Zhou, X., Zhou, K., Wu, Q., Wu, B., Li, G., Rajkovic, A., Fang, W., Rankin, S. C., Li, Y., Xu, X., Schifferli, D. M., & Yue, M. (2019). Diversified sources for human infections by *Salmonella enterica* serovar Newport. *Transboundary and Emerging Diseases, 66*(2), 1044–1048. https://doi.org/10.1111/tbed.13099. PMID:30548172; PMCID:PMC6634944.

Papić, B., Kušar, D., Mićunović, J., Pirš, M., Ocepek, M., & Avberšek, J. (2022). Clonal spread of pESI-positive multidrug-resistant ST32 *Salmonella enterica* serovar Infantis isolates among broilers and humans in Slovenia. *Microbiology Spectrum, 10*. https://doi.org/10.1128/spectrum.02481-22.

Perin, A. P., Martins, B. T. F., Barreiros, M. A. B., Yamatogi, R. S., Nero, L. A., & dos Santos Bersot, L. (2020). Occurrence, quantification, pulse types, and antimicrobial susceptibility of *Salmonella* sp. isolated from chicken meat in the state of Paraná, Brazil. *Brazilian Journal of Microbiology, 51*, 335–345. https://doi.org/10.1007/s42770-019-00188-x.

Pissetti, C., de Freitas Costa, E., Zenato, K. S., & Cardoso, M. R. (2022). Critically important antimicrobial resistance trends in *Salmonella Derby* and *Salmonella Typhimurium* isolated from the pork production chain in Brazil: A 16-year period. *Pathogens, 11*, 905. https://doi.org/10.3390/pathogens11080905.

Plumb, I. D., Schwensohn, C. A., Gieraltowski, L., Tecle, S., Schneider, Z. D., Freiman, J., Cote, A., Noveroske, D., Kolsin, J., Brandenburg, J., Chen, J. C., Tagg, K. A., White, P. B., Shah, H. J., Francois Watkins, L. K., Wise, M. E., & Friedman, C. R. (2019). Outbreak of *Salmonella Newport* infections with decreased susceptibility to azithromycin linked to beef obtained in the United States and soft cheese obtained in Mexico — United States, 2018–2019. *MMWR, 68*, 713–717. https://doi.org/10.15585/mmwr.mm6833a1.

Possebon, F. S., Ullmann, L. S., Malossi, C. D., Miodutzki, G. T., da Silva, E. C., Machado, E. F., Braga, I. S., Pelaquim, I. F., & Araujo, J. P. Jr., (2022). A fast and cheap in-house magnetic bead RNA extraction method for COVID-19 diagnosis. *Journal of Virological Methods, 300*, 114414. https://doi.org/10.1016/j.jviromet.2021.114414.

Rabello, R. F., Bonelli, R. R., Penna, B. A., Albuquerque, J. P., Souza, R. M., & Cerqueira, A. M. F. (2020). Antimicrobial resistance in farm animals in Brazil: An update overview. *Animals, 10*, 552. https://doi.org/10.3390/ani10040552.

Rau, R. B., Ribeiro, A. R., dos Santos, A., & Barth, A. L. (2021). Antimicrobial resistance of *Salmonella* from poultry meat in Brazil: results of a nationwide survey. *Epidemiology and Infection, 149*, e228.

Ribas, A., & Poonlaphdecha, S. (2017). Wild-caught and farm-reared amphibians are important reservoirs of *Salmonella*: a study in North-East Thailand. *Zoonoses and Public Health, 64*, 106–110. https://doi.org/10.1111/zph.12286.

Ribot, E. M., Fair, M. A., Gautom, R., Cameron, D. N., Hunter, S. B., Swaminathan, B., & Barrett, T. J. (2006). Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathogens and Disease, 3*, 59–67. https://doi.org/10.1089/fpd.2006.3.59.

Ripon, R. K., Motahara, U., Ahmed, A., Devnath, N., Mahua, F. A., Hashem, R. B., Ishadi, K. S., Alam, A., Sujan, M. S. H., & Sarker, M. S. (2023). Exploring the prevalence of antibiotic resistance patterns and drivers of antibiotics resistance of *Salmonella* in livestock and poultry-derived foods: a systematic review and meta-analysis in Bangladesh from 2000 to 2022. *JAC Antimicrobial Resistance, 5*. https://doi.org/10.1093/jacamr/dlad059.

Santana, A. M., da Silva, D. G., Maluta, R. P., Pizauro, L. J. L., Simplício, K. M. M. G., Santana, C. H., Rodrigues, S. A. D., Rodrigues, D. D. P., & Fagliari, J. J. (2020). Comparative analysis using pulsed-field gel electrophoresis highlights a potential transmission of *Salmonella* between asymptomatic buffaloes and pigs in a single farm. *Frontiers in Veterinary Science, 10*(7)552413. https://doi.org/10.3389/fvets.2020.552413. PMID:33240945; PMCID:PMC7683720.

Vilela, F. P., Rodrigues, D. dos P., Allard, M. W., & Falcão, J. P. (2022). Genomic characterization and antimicrobial resistance profiles of *Salmonella enterica* serovar Infantis isolated from food, humans and veterinary-related sources in Brazil. *Journal of Applied Microbiology, 132*, 3327–3342. https://doi.org/10.1111/jam.15430.

Wang, H., Cheng, H., Huang, B., Hu, X., Chen, Y., Zheng, L., Yang, L., Deng, J., & Wang, Q. (2023). Characterization of resistance genes and plasmids from sick children caused by *Salmonella enterica* resistance to azithromycin in Shenzhen, China. *Frontiers in Cellular and Infection Microbiology, 13*. https://doi.org/10.3389/fcimb.2023.1116172.

Yun, Y.-S., Chae, S.-J., Na, H.-Y., Chung, G. T., Yoo, C.-K., & Lee, D.-Y. (2015). Modified method of multilocus sequence typing (MLST) for serotyping in *Salmonella* species. *Journal of Bacteriology and Virology, 45*, 314–318. https://doi.org/10.4167/jbv.2015.45.4.314.

Zakaria, Z., Hassan, L., Sharif, Z., Ahmad, N., Ali, R. M., Husin, S. A., Hazis, N. H. B. A., Sohaimi, N. F. M., Bakar, S. A., & Garba, B. (2020). Analysis of *Salmonella enterica* serovar Enteritidis isolates from chickens and chicken meat products in Malaysia using PFGE, and MLST. *BMC Veterinary Research, 16*, 1–8. https://doi.org/10.1186/s12917-020-02605-y.

Zhang, J. F., Shang, K., Park, J. Y., Lee, Y. J., Choi, Y. R., Kim, S. W., Cha, S. Y., Jang, H. K., Wei, B., & Kang, M. (2022). Antimicrobial resistance and PFGE molecular typing of *Salmonella enterica* serovar Gallinarum isolates from chickens in South Korea from 2013 to 2018. *Animals (Basel), 12*(1), 83. https://doi.org/10.3390/ani12010083.