Genetic diversity and multidrug-resistance among Salmonella *Typhimurium isolated from swine carcasses and slaughterhouses in Rio de Janeiro, Brazil*

Claudius Cabral[1], Pedro Panzenhagen[1,2*], Karina Delgado[1], Gabriela Rodrigues[1], André Mercês[3], Dalia Rodrigues[2], Robson Franco[1] and Carlos Conte-Junior[1,2,3]

[1]*Universidade Federal Fluminense, Brazil.*
[2]*Universidade Federal do Rio de Janeiro, Brazil.*
[3]*Fundação Oswaldo Cruz, Brazil.*

[*]*Corresponding author at: Universidade Federal do Rio de Janeiro, Brazil.*
E-mail: panzenhagen@ufrj.br.

Veterinaria Italiana 2020, **56** (4), 245-250. doi: 10.12834/VetIt.1688.8954.1
Accepted: 14.02.2019 | Available on line: 31.12.2020

Keywords
Antimicrobial resistance,
PFGE,
Rio de Janeiro,
Salmonella,
Swine.

Summary
This study, conducted in the State of Rio de Janeiro, aimed to genetically distinguish 29 isolates of *S.* Typhimurium isolated from 344 samples of swine carcasses by PFGE (pulse-field gel electrophoresis) and to evaluate their antimicrobial resistance profile. Out of the 29 isolates, 26 (90%) were resistant to at least one antimicrobial. Sulfonamides (66%), nalidixic acid (66%), trimethoprim (66%) and tetracycline (52%) were the most frequent resistant drugs. Multidrug-resistance (MDR) profile was frequent (60% of isolates). The profile Eno-Na-Nxn-Fc-C-S-Gm-G-T-Te (14%), Cp-Eno-Na-Fc-C-S-G-T-Te (10%) and Na-G-T (7%) were the most frequent. Five isolates within the predominant PFGE pulsetype came from lymph nodes of distinct animals from multiple slaughterhouses indicating that this particular clone might be widespread in the Rio de Janeiro State. This paper reveals a threat to the population in the entire State and highlights the necessity of the strict control in the use of antimicrobials in swine production in the entire country.

Introduction

The foodborne pathogen *Salmonella* is responsible for human salmonellosis, an infection that has the potential to become life-threatening. In the United States, *Salmonella* is the second most prevalent foodborne pathogen, and is responsible for the highest number of hospitalizations (CDC 2018). In Brazil, this pathogen is the most frequent cause of foodborne diseases, and responsible for 39% of the notified and confirmed cases (Finger *et al.* 2019). Although chicken is considered as the main reservoir of *Salmonella* , swine is also crucial in the transmission of this pathogen and is capable of periodically shedding this bacterium through feces (Mataragas *et al.* 2011).

In Brazil, several studies already revealed contamination of pork and pork-based products with *Salmonella* (Cabral *et al.* 2014, Silva *et al.* 2009, Teixeira 2007). The Rio de Janeiro State has a pork production center with slaughterhouses producing meat that supplies the local consume within the municipality or inside the State borders. However, evaluation of the microbiological quality of pork production in the Rio de Janeiro State is out of date (Lázaro *et al.* 1997, Zebral *et al.* 1974) and new studies need to be performed. Recently, we reported 36 out of 344 (10.5%) samples from swine slaughterhouses contaminated with *Salmonella* including carcasses, lymph nodes, ham, jowl, knives and even the cleaning water (Cabral *et al.* 2017). These results evidence how high is the consumer potential exposure to *Salmonella* causing concern to public health authorities.

A serious concern about *Salmonella* strains regards their antimicrobial susceptibility profile. In severe cases of human salmonellosis, it is expected that the first-choice antimicrobial therapy will be able to control systemic infection. Because antimicrobial resistance has been increasingly

detected in *Salmonella*, public health has been continually threatened (CDC 2005). Another hazard is related to the possibility of the multidrug-resistant (MDR) strains to disseminate resistance genes to non-resistant bacteria transferring those genes between human and animal populations, mostly in the gut environment (Trobos *et al.* 2009). It is now well established that the selective pressure created by the inappropriate antimicrobial use in human and veterinary medicine is one of the main reasons for the increase of antimicrobial resistance (Tenover 2006).

Within more than 2,600 *Salmonella enterica* serovars, *Salmonella* Typhimurium is the most surveyed and frequent serovar transmitted from animals to humans worldwide (Hendriksen *et al.* 2011). In Brazil, several studies provide evidence that this serovar has been the most frequently isolated in swine and pork-based products (Bandeira *et al.* 2007, Castagna *et al.* 2004, Kich *et al.* 2011, Michael *et al.* 2002, Pissetti *et al.* 2012, Seixas *et al.* 2009, Viott *et al.* 2013). Previously, we have demonstrated that *Salmonella* Typhimurium is prevalent (55% of isolates) in the State of Rio de Janeiro (Cabral *et al.* 2017). Now, the understanding of the diversity level of these isolates is indispensable to monitoring interventions strategies if outbreaks investigations are necessary (Kich *et al.* 2011). In this context, Pulsed-field gel electrophoresis (PFGE) is eligible due to the rapid standardized protocol, parameters analysis and nomenclature, and the ability to exchange information in real-time through internet by the center of PulseNet's (Ribot *et al.* 2006). Also, it is routinely used for foodborne outbreaks investigation and studies regarding animal infections and food pathogens (Kich *et al.* 2011, Vigo *et al.* 2009).

Hence, the current study was designed to evaluate the antimicrobial susceptibility profile of 29 isolates of *Salmonella* Typhimurium, along with their molecular typing with PFGE in the purpose of epidemiologically differentiate and trace the sources of those bacteria.

Materials and methods

Bacterial isolates

We selected the twenty-nine isolates of *S.* Typhimurium previously obtained from 344 samples of swine carcasses (intestinal faeces, mesenteric and submandibular lymph nodes, jowl, ham) and from the water for cleaning the carcasses in swine slaughterhouses (S1, S2, and S3) in the Rio de Janeiro State (Cabral *et al.* 2017). Isolates were kept frozen at - 18 °C in Brain Heart Infusion Broth (BHI) (Himedia®) with 25% glycerol. These isolates were reactivated by transferring 100 µL to a sterilized BHI broth, followed by incubation at 37 °C for 20 hours. Newly grown isolates were then transferred to Mueller-Hinton Broth (MH broth) and incubated at 37 °C for 20 hours.

Antimicrobial susceptibility test

Isolates were grown in MH broth to prepare the inoculum for the Kirby-Bauer antimicrobial susceptibility test (AST), which was performed as described at the Clinical and Laboratory Standards Institute - CLSI (CLSI 2017). Eighteen antimicrobials from eight classes were tested, including the most commonly used veterinary drugs in swine production and the first-choice drugs for the treatment of human enteric infections. The diffusion disks (Oxoid, Basingstoke, UK) with the following drugs were used: amoxycillin/clavulanic acid (Amc, 30 µg), cephalothin (Cf, 30 µg), cefoxitin (Cfx, 30 µg), ceftazidime (Caz, 30 µg), ceftriaxone (Cax, 30 µg), ciprofloxacin (Cp, 5 µg), enrofloxacin (Eno, 5 µg), nalidixic acid (Na, 30 µg), norfloxacin (Nxn, 10 µg), florfenicol (Fc, 30 µg), chloramphenicol (C, 30 µg), streptomycin (S, 10 µg), gentamicin (Gm, 10 µg), tobramycin (To, 10 µg), imipenem (Imp, 10 µg), sulfonamides (G, 300 µg), trimethoprim (T, 5 µg), tetracycline (Te, 30 µg). *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. aeruginosa* 10536 were tested in parallel and served as control strains according to CLSI.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed at the National Reference Laboratory for Enteroinfections at Oswaldo Cruz Institute (FIOCRUZ) according to the Standard Operating Procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-157 (STEC), *Salmonella* serovars, *Shigella sonnei* and *Shigella flexneri*, established by the Centers for Disease Control and Prevention (CDC 2013). The DNA fingerprints were generated by macrorestriction with 40 U of enzyme XbaI (New England Biolabs, Beverly, MA, USA). Restriction fragments were separated in certificated 1.2% PFGE agarose gels (Bio-Rad, Hercules, CA, USA) in tris-borate buffer (TBE; tris-borate 0.045 M, EDTA 0.001M) at 140 °C, using the CHEF DR III system (Bio-Rad). Electrophoresis runs with an initial switch time of 2.2 s and a final switch of 63.8 s at 6V/s for 18 h. *Salmonella* Braenderup was used as standard and similarity was calculated by the Dice coefficient with 1.5 to 2.0% tolerance. The generated profile assembly and dendrogram analysis were performed with BioNumerics Software 7.5 (Biomerieux®).

Table I. *Antimicrobial susceptibility profile of 29* Salmonella *Typhimurium isolates obtained from swine carcasses and slaughterhouses in Rio de Janeiro, Brazil.*

Antimicrobial	Susceptibility profile [n (%)]		
	Sensitive	Intermediate resistance	Resistant
Streptomycin	14 (48%)	10 (35%)	5 (17%)
Gentamicin	19 (66%)	3 (10%)	7 (24%)
Tobramycin	28 (97%)	-	1 (3%)
Amoxicillin + clavulanic acid	24 (83%)	4 (14%)	1 (3%)
Cephalothin	21 (72%)	2 (7%)	6 (21%
Cefoxitin	23 (79%)	2 (7%)	4 (14%)
Ceftazidime	27 (93%)	1 (7%)	1(3%)
Ceftriaxone	28 (97%)	-	1 (3%)
Imipenem	28 (97%)	1(3%)	-
Chloramphenicol	14 (48%)	-	15 (52%)
Florfenicol	14 (48%)	-	15 (52%)
Nalidixic acid	6 (21%)	4 (14%)	19 (66%)
Ciprofloxacin	12 (41%)	5 (18%)	12 (41%)
Enrofloxacin	15 (52%)	-	14 (48%)
Norfloxacin	23 (79%)	4 (14%)	2 (7%)
Sulfonamide	9 (31%)	1 (3%)	19 (66%)
Trimethoprim	9 (34%)	-	20 (66%)
Tetracycline	14 (48%)	-	15 (52%)

Results

Twenty-six out of the 29 selected isolates (90%) exhibited resistance to at least one antimicrobial. Also, two isolates had only intermediate resistance to at least one antibiotic and two isolates were pan-susceptible. Seventeen isolates (60%) were MDR since they showed resistance to more than three different antimicrobial classes. Sixty-six percent of the isolates were resistant to sulfonamide, nalidixic acid, and trimethoprim. Also, 97% of them were susceptible to tobramycin, ceftriaxone, and imipenem (Table I).

PFGE-based sub-typing was performed to determine the diversity of the isolates selected in this study, (Figure 1). This analysis revealed a total of 11 different pulsetypes wherein the three predominant types were formed by at least six isolates each (clusters A and B). Pulsetype 1 (cluster A) harbored eight isolates whereas pulsetype 2 (cluster B1) harbored six isolates and pulsetype 3 (cluster B2) also harbored six isolates. In the cluster C, the pulsetypes eight and nine were 92% similar. The cluster D harbored two isolates with the pulsetype 11. The use of the single XbaI enzyme was able to genetically distinguish 7 out of 29 isolates (pulsetypes 4, 5, 6, 7, 8, 9 and 10) (Figure 1). However, the majority of the isolates which share identical PFGE profile (pulsetypes 1, 2, 3 and 11) showed different antimicrobial resistance profiles,

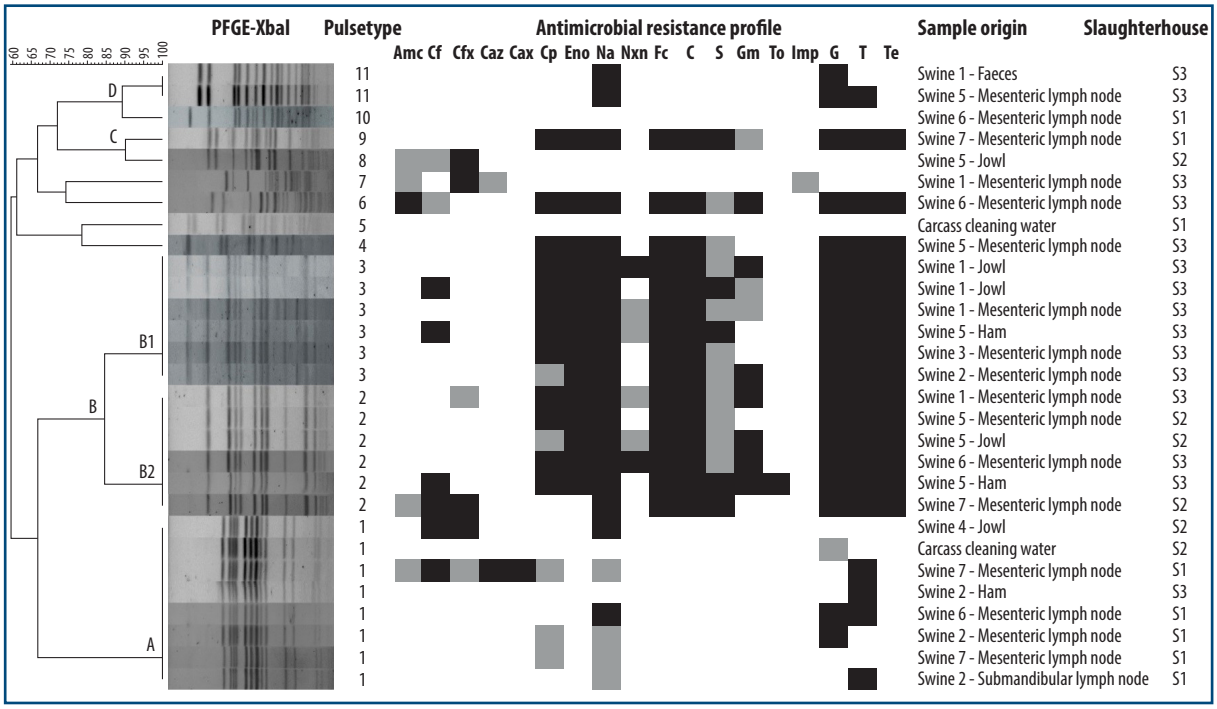

Figure 1. *Pulsed-field gel electrophoresis (PFGE) dendrogram and antimicrobial resistance profile showing the genetic and phenotypic diversity among 29 isolates of* Salmonella *Typhimurium obtained from swine slaughterhouses in the Rio de Janeiro State.* Antimicrobial resistance profile abbreviations: amoxycillin/clavulanic acid (Amc), cephalothin (Cf), cefoxitin (Cfx), ceftazidime (Caz), ceftriaxone (Cax), ciprofloxacin (Cp), enrofloxacin (Eno), nalidixic acid (Na), norfloxacin (Nxn), florfenicol (Fc), chloramphenicol (C), streptomycin (S), gentamicin (Gm), tobramycin (To), imipenem (Imp), sulfonamides (G), trimethoprim (T), tetracycline (Te). Grey boxes represent intermediate resistance.

suggesting that they might be genetically different although not distinguished by the use of the single XbaI enzyme. Overall, AMR profile was quite diverse, but four isolates (14%) shared the common profile Cp-Eno-Na-Nxn-Fc-C-S-Gm-G-T-Te, three isolates (10%) shared the profile Cp-Eno-Na-Fc-C-S-G-T-Te, and two isolates (7%) shared the profile Na-G-T. No association between the antimicrobial resistance profile and pulsetype were found. However, isolates with resistance to four or more classes were in cluster B (Figure 1).

Discussion

Slaughterhouses in the Rio de Janeiro State plays a crucial role in the economics of local municipalities as a provider of quality protein to those consumers. In 2017, we published the first study reporting that those slaughterhouses were producing *Salmonella*-contaminated pork (Cabral *et al.* 2017). Here, we selected all the 29 *S.* Typhimurium isolated from that study to profile a phenotypically and genetically characterization by accessing the antimicrobial susceptibility profile and PFGE, respectively.

In Brazil, few studies have evaluated the antimicrobial susceptibility profile of *Salmonella* isolates from swine carcasses, pork or slaughterhouse environment, although those performed reported a high prevalence of isolates resistant to at least one antimicrobial. Lopes and colleagues (Lopes *et al.* 2015) reported that 76% of *Salmonella enterica* serovars isolated from pigs and carcasses were resistant to at least one antimicrobial. Lima and colleagues (Lima *et al.* 2016) have evaluated 357 from pork and pork by-products and found 257 isolates (72%) resistant to one or more drugs. Also, Almeida and colleagues (Almeida *et al.* 2016) reported that 17 out of 27 (63%) isolates had resistance to at least one drug. Here, we reported 90% of the tested isolates showing resistance to at least one drug. Although these studies did not reveal resistance patterns specifically from Typhimurium serovar, they reported that drug resistance is wide-spreading across Brazilian foodborne *Salmonella* isolates.

In the present study, most of the *Salmonella* isolates were resistant to sulfonamides, nalidixic acid, trimethoprim, and tetracycline. Resistance to these four antibiotics is routinely reported in *Salmonella*. For instance, Bessa and colleagues (Bessa *et al.* 2007) reported high rates of *Salmonella* resistant to sulfonamide (91%), tetracycline (85%) and streptomycin (66%). This profile was also reported by Lopes and colleagues (Lopes *et al.* 2015) with 55%, 40%, 34% and 34% resistance to tetracycline, sulfonamide, streptomycin and nalidixic acid, respectively. Lima and colleagues (Lima *et al.* 2016) reported high resistance to tetracycline (44%) and nalidixic acid (25%) in *Salmonella* isolates obtained from pigs and multiple pork by-products. Interestingly, high frequency of isolates with resistance to tetracycline is not unexpected in Brazil since this antibiotic was routinely used as a growth promoter in swine breeding. The Ministry of Agriculture, Livestock and Supply has forbidden its use as growth promoter since 1998, although it is still allowed for infection therapy. Sulfonamide resistance along with gentamycin and fluoroquinolones resistance also are routinely detected among *Salmonella* isolates, and this can be explained by their widespread use in swine breeding (Silva *et al.* 2009). In our study, sulfonamide and trimethoprim resistance were separately evaluated, but nowadays they are commonly used in combination due to their synergism (cotrimoxazole). Trimethoprim resistance is also common, however resistance is lower when it is associated with sulfamethoxazole (Lima *et al.* 2016). In this study, 22/29 (76%) isolates were nalidixic acid resistant, 13/29 (44%) to ciprofloxacin and 16/29 (55%) to enrofloxacin. This last finding could be a matter of great concern to public health because, in human medicine, ciprofloxacin is the first choice drug for cases of salmonellosis, especially when dealing with septicemic strains (Souza *et al.* 2010). The implications of the indiscriminate use of antimicrobials in animal production on bacterial resistance have been continually reviewed (Landers *et al.* 2012). The uncorrect use by animal breeders and the little control by the authorities contribute to the increase of bacterial resistance.

Multidrug-resistant (MDR) strains are defined as isolates resistant to three or more different antimicrobials classes (CLSI 2017). Despite few isolates studied here, the high frequency of MDR isolates (60%) remains alarming. In Brazil, most of the studies have shown frequency of *S.* Typhimurium in swine with MDR profile below fifty percent: Almeida and colleagues (Almeida *et al.* 2016) 37%, Lopes and colleagues (Lopes *et al.* 2015) 40.4%, Kich and colleagues (Kich *et al.* 2011) 43%. MDR isolates are dangerous anywhere since they are commonly more virulent than susceptible ones, and this is a constant threat to human health (DiMarzio *et al.* 2013). A high variety of MDR profiles were found in our isolates even in that obtained from the same slaughterhouses. Although the antimicrobial use records from the swine breeding were not available, it is possible to speculate that the breeders might have adopted different protocols and these variations may have resulted in the selection and widespread of many different multi-resistant profiles within the isolates.

Pulsed-field gel electrophoresis identified 11 distinct pulsetypes among the twenty-nine samples. Pulsetype 1 (cluster A) is the biggest one

with eight isolates (Figure 1). Curiously, five clones in this cluster came from lymph nodes of different animals (swine 2, 6, 7) raised in the same breeder. Strains isolated from lymph nodes indicate that these animals are harboring *Salmonella* and can asymptomatically carry this pathogen since they are capable of periodically shed the bacteria through feces. According to Silva and colleagues (Silva *et al.* 2006), infection at the farm level mainly at the finishing step is the most common event responsible for swine infection. Samples within pulsetype 1 provide evidence that this particular clone is widespread in the same swine breeder. Because these isolates were obtained from three different slaughterhouses (S1, S2, and S3), it is possible to speculate that they are also circulating not only in the breeders but also among the slaughterhouses in the Rio de Janeiro State. Conversely, PFGE also revealed *S.* Typhimurium isolates with distinct pulsetypes isolated from the same animal. For instance, we obtained isolates from different parts in swine number five belonging to the pulsetype 2, 3, 4 and 11. This finding supports the evidence that isolates from the same serovar are worth to be typed since they may not belong to the same clone and consequently exhibit distinct epidemiological importance.

In conclusion, the results of PFGE typing along with the antimicrobial resistance profile revealed a high variety of *S.* Typhimurium isolates among swine samples from slaughterhouses in Rio de Janeiro State. The high frequency of MDR profile among these isolates indicates that pigs in that region are reservoirs with potential risk to transmit multidrug-resistant *S.* Typhimurium. This paper reveals a threat to the population public health in the entire State and highlights the necessity of strict control in the use of antimicrobials in the swine production in Brazil.

Acknowledgments

The authors are thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES for the financial funding.

References

Almeida F., Medeiros M.I.C., Kich J.D. & Falcão J.P. 2016. Virulence-associated genes, antimicrobial resistance and molecular typing of *Salmonella* Typhimurium strains isolated from swine from 2000 to 2012 in Brazil. *J Appl Microbiol*, **120**, 1677-1690.

Bandeira R., da Cruz Payão, Pellegrini D. & Cardoso M. 2007. Ocorrência de *Salmonella* sp. em cortes de pernil provenientes de lotes suínos portadores ao abate. *Acta Sci Vet*, **35**. Retrieved from http://www.redalyc.org/resumen.oa?id=289021845010.

Bessa M.C., Michael G.B., Canu N., Canal C.W., Cardoso M., Rabsch W. & Rubino S. 2007. Phenotypic and genetic characterization of *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolated from pigs in Rio Grande do Sul, Brazil. *Res Vet Sci*, **83**, 302-310.

Cabral C.C., Conte-Junior C.A., Silva J.T. & Paschoalin V.M.F. 2014. *Salmonella* spp. contamination in fresh pork and chicken sausages marketed in Niterói and Rio de Janeiro, Brazil. *J Für Verbraucherschutz Leb*, **9**, 243-249.

Cabral C.C., Panzenhagen P.H.N., Delgado K.F., Silva G.R.A., Rodrigues D. dos P., Franco R.M. & Conte-Junior C.A. 2017. Contamination of carcasses and utensils in small swine slaughterhouses by *Salmonella* in the Northwestern Region of the State of Rio de Janeiro, Brazil. *J Food Prot*, 1128-1132.

Castagna S.M.F., Schwarz P., Canal C.W. & Cardoso M. 2004. Presença de *Salmonella* sp. no trato intestinal e em tonsilas/linfonodos submandibulares de suínos ao abate. *Arq Bras Med Veterinária E Zootec*, **56**, 300-306.

CDC. 2005. National Antimicrobial Resistance Monitoring System (NARMS) Frequently Asked Questions (FAQ) about antibiotic resistance - Why is antibiotic resistance a food safety problem? Department of Health and Human Diseases - Center of Disease Control and Prevention. Retrieved from http://www.cdc.gov/narms/faq_pages/5.htm.

CDC. 2013. Centers for Disease Control and Prevention. Standard Operating Procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*. Centers for Disease Control and Prevention. Retrieved from https://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf.

CDC. 2018, April 16. Outbreaks involving *Salmonella* | CDC. Retrieved May 9, 2018, from https://www.cdc.gov/salmonella/outbreaks.html.

CLSI. 2017. Performance standards for antimicrobial susceptibility testing (27th ed.). Wayne, PA, Clinical and Laboratory Standards Institute.

DiMarzio M., Shariat N., Kariyawasam S., Barrangou R. & Dudley E.G. 2013. Antibiotic resistance in *Salmonella enterica* serovar Typhimurium associates with CRISPR sequence type. *Antimicrob Agents Chemother*, **57**, 4282-4289.

Finger F.F.A.J., Baroni V.G.S.W., Maffei F.D., Bastos M.H.D. & Pinto M.U. 2019. Overview of foodborne disease outbreaks in Brazil from 2000 to 2018. *Foods*, **8**, 1-10.

Hendriksen R.S., Vieira A.R., Karlsmose S., Lo Fo Wong

D.M.A., Jensen A.B., Wegener H.C. & Aarestrup F.M. 2011. Global monitoring of *Salmonella* serovar distribution from the World Health Organization global foodborne infections network country data bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog Dis*, **8**, 887-900.

Kich J.D., Coldebella A., Morés N., Nogueira M.G., Cardoso M., Fratamico P.M., Fedorka-Cray P. & Luchansky J.B. 2011. Prevalence, distribution, and molecular characterization of *Salmonella* recovered from swine finishing herds and a slaughter facility in Santa Catarina, Brazil. *Int J Food Microbiol*, **151**, 307-313.

Landers T.F., Cohen B., Wittum T.E. & Larson E.L. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep*, **127**, 4-22.

Lázaro N.S., Tibana A. & Hofer E. 1997. *Salmonella* spp. in healthy swine and in abattoir environments in Brazil. *J Food Prot*, **60**, 1029-1033.

Lima A.L., Rodrigues D.P., Araújo M.S., Reis E.M.F., Festivo M.L., Rodrigues E.C.P. & Lázaro N.S. 2016. Serovars and antimicrobial susceptibility of *Salmonella* spp. product isolated from swine. *Arq Bras Med Veterinária E Zootec*, **68**, 39-47.

Lopes G.V., Pissetti C., da Cruz Payão Pellegrini D., da Silva L.E. & Cardoso M. 2015. Resistance phenotypes and genotypes of *Salmonella enterica* subsp. *enterica* isolates from feed, pigs, and carcasses in Brazil. *J Food Prot*, **78**, 407-413.

Mataragas M., Dimitriou V. ,Skandamis P.N. & Drosinos E.H. 2011. Quantifying the spoilage and shelf-life of yoghurt with fruits. *Food Microbiol*, **28**, 611-616.

Michael G.B., Simoneti R., Cardoso M.R. de I. & Costa M. da. 2002. Sorotipos de *Salmonella* isolados em uma propriedade de suínos de terminação no Sul do Brasil. *Ciênc Rural*, **32**, 525-527.

Pissetti C., Werlang G.O., Biesus L.L., Kich J.D. & Cardoso M.R. de I. 2012. Detecção de *Salmonella enterica* e *Listeria monocytogenes* em carcaças suínas na etapa de pré-resfriamento. *Acta Sci Vet*, **40**, 1-8.

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 Pathog Dis*, **3**, 59-67.

Seixas F.N., Tochetto R. & Ferraz S.M. 2009. Presença de *Salmonella* sp. em carcaças suínas amostradas em diferentes pontos da linha de processamento. *Ciênc Anim Bras*, **10**, 634-640.

Silva M.C., Faria G.S., Paula D.A.J., Martins R.P., Junior C.G.J., Kich J.D., Colodel M.E., Nakazato L. & Dutra V. 2009. Prevalence of *Salmonella* sp. in swine slaughtered at Mato Grosso state, Brazil. *Ciênc Rural*, **39**, 266-268.

Silva L.E., Gotardi C.P., Vizzotto R., Kich J.D. & Cardoso M.R.I. 2006. Infecção por *Salmonella enterica* em suínos criados em um sistema integrado de produção do sul do Brasil. *Arq Bras Med Veterinária E Zootec*, **58**, 455-461.

Souza R.B., de Magnani M. & Oliveira T.C.R.M. de. 2010. Mecanismos de resistência às quinolonas em *Salmonella* spp. *Semina Ciênc Agrár*, **31**, 413-427.

Teixeira S.R. 2007. Detecção de *Salmonella* spp. em amostras de fezes, linfonodos e carcaças de suínos no momento do abate (text). Universidade de São Paulo. https://doi.org/10.11606/D.10.2007.tde-14052007-133108.

Tenover F.C. 2006. Mechanisms of antimicrobial resistance in bacteria. *Am J Infect Control*, **34**, S3-10; discussion S64-73.

Trobos M., Lester C.H., Olsen J.E., Frimodt-Møller N. & Hammerum A.M. 2009. Natural transfer of sulphonamide and ampicillin resistance between *Escherichia coli* residing in the human intestine. *J Antimicrob Chemother*, **63**, 80-86.

Vigo G.B., Cappuccio J.A., Piñeyro P.E., Salve A., Machuca M.A., Quiroga M.A., Moredo F., Giacoboni G., Cancer L.J., Caffer G.I., Binsztein N., Pichel M., Perfumo J.C. & Perfumo C.J. 2009. *Salmonella enterica* subclinical infection: bacteriological, serological, pulsed-field gel electrophoresis, and antimicrobial resistance profiles - longitudinal study in a three-site farrow-to-finish farm. *Foodborne Pathog Dis*, **6**, 965-972.

Viott A.M., Lage A.P., Cruz Junior E.C.C. & Guedes R.M.C. 2013. The prevalence of swine enteropathogens in Brazilian grower and finish herds. *Braz J Microbiol*, **44**, 145-151.

Zebral A.A., Freitas C.A. & Hofer E. 1974. Ocorrência de *Salmonella* em gânglios linfáticos de suínos aparentemente normais, abatidos no matadouro de Santa Cruz, cidade do Rio de Janeiro, Guanabara. *Mem Inst Oswaldo Cruz*, **72**, 223-235.